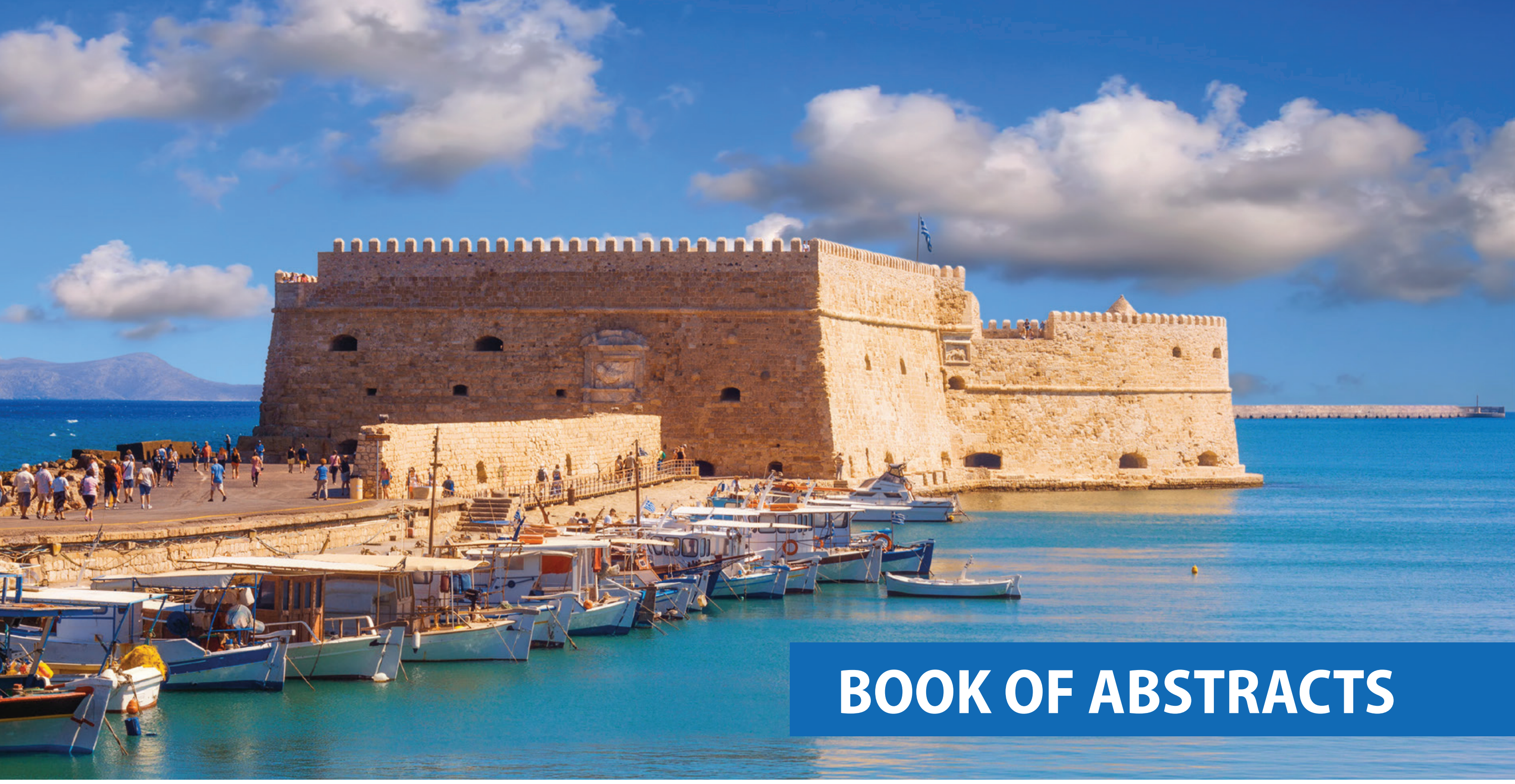

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## European Congress of Entomology

## XII European Congress of Entomology

16-20.10.2023
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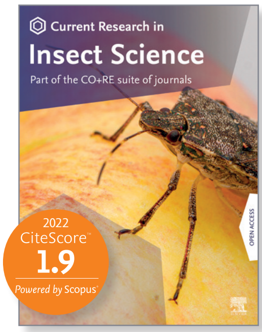
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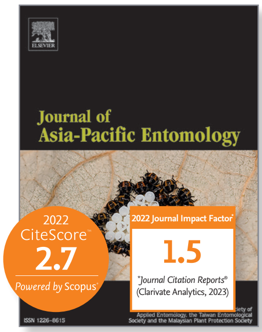
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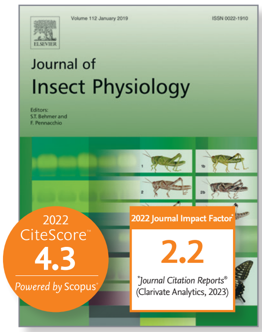
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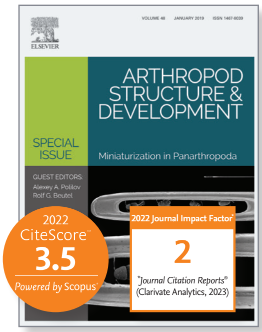
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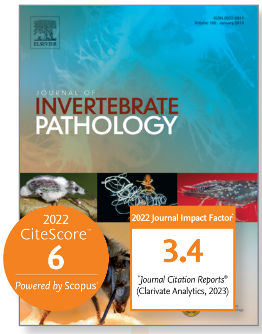
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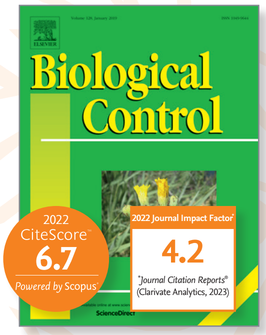
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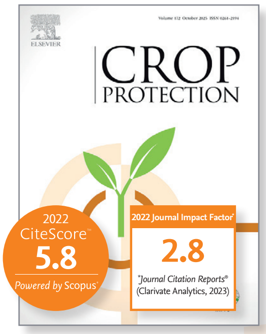
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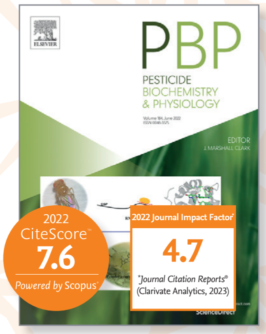
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# Welcome Letter

Dear Colleagues,

On behalf of the Organizing Committee of the ECE 2023 and the Hellenic Entomological Society, we are delighted to welcome entomologists from around the globe to the XII European Congress of Entomology (ECE 2023), in Heraklion, Crete, on 16-20 October 2023.

Forty-five years after the first European Congress of Entomology in 1978 at Reading University, UK and following a series of successful congresses around Europe, we now have the great honor to host this major European entomological meeting in our beautiful country and with it the opportunity to share knowledge, expertise and perspectives.

The world of entomology faces many problems with the increasing threat of climate change, damaging vector-borne diseases, biodiversity loss and the need to feed an expanding world population. Our goal is to gather scientists from around the globe and provide a unique opportunity to exchange information and to communicate research results on a wide range of topics. The ECE 2023 audience consists of researchers and academics from a diverse range of entomology related fields, as well as policy-makers, field agronomists, crop protection experts, medical practitioners, engineers, social scientists and artists.

The congress program includes invited lectures from international experts, symposia, oral and poster sessions, workshops and satellite seminars. In addition, the congress is undoubtedly the ideal meeting place to network and interact, establishing the starting point for future collaborations and breakthrough innovations in entomology.

The historic, as well as magnificent island of Crete sets the ideal scenery for ECE 2023. The congress is hosted in the Cultural and Conference Center of Heraklion, a contemporary venue of international standards, offering high level services in an inspiring environment.

We strongly believe that we will deliver a high-quality meeting which will turn out to be a memorable experience. The success of the congress depends on the contributions of the delegates and we thank you for being actively involved.

Welcome at ECE Congress in Heraklion in 2023!

On behalf of the Organizing Committee

**Emmanouil Roditakis, Stefanos Andreadis**
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# Plenary Sessions

## PL1. A mighty model: Unraveling the molecular genetic mechanisms of extreme polyphagy and pesticide resistance in the crop pest *Tetranychus urticae*

T. Van Leeuwen*
*Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium*

*Corresponding author: Thomas.VanLeeuwen@ugent.be

The spider mite *Tetranychus urticae* is a notorious crop pest with a remarkable evolutionary potential to adapt to toxins. Not only is this species extremely polyphagous, necessitating its adaptation to a plethora of plant defenses, it also rapidly develops resistance to pesticides used to protect our crops from attack. The mechanisms underlying this rapid adaptation have received considerable scholarly interest, not only due to their intriguing scientific complexity, but also because of their economic importance in devising adequate pest control strategies. Consequently, spider mite research has come to the forefront in the field of molecular genetics of adaptation in arthropods. I will argue that spider mites are excellent model organisms to address some of the main scientific questions in the field. Subsequently, I will provide an overview of progress made during the past 15 years. Topics will include the elucidation of pharmacokinetic and -dynamic mechanisms of pesticide resistance, the role of horizontal gene transfer in adaptation, the characterization of plant-mite interactions with the discovery of effector proteins, as well recent efforts in unravelling the gene regulatory architecture of adaptation. Finally, I will argue that highly efficient CRISPR/Cas protocols, newly designed for spider mites, open up new avenues to further develop spider mites into a convenient genetic model.

**Keywords:** *Tetranychus urticae*, adaptation, plant-mite interactions, genetics, detoxification, gene regulation, genetic mapping, pesticide resistance, genetic transformation, CRISPR/Cas

---

## PL2. Bumblebee leaf-damaging behavior and its effects on plant flowering

C. M. De Moraes
*Biocommunication Research Group, Department of Environmental Systems Science, ETH Zürich, Switzerland*

*Corresponding author: consuelo.demoraes@usys.ethz.ch

Research in my group focuses on the role of information in mediating ecological interactions among species. Our research explores communicative interactions in a range of systems, with a focus on chemically mediated interactions among plants, insects, and microbes.Recently, we documented a novel interaction in which bumblebee workers respond to pollen scarcity by damaging plant leaves in a way that accelerates plant flowering. In this talk, I discuss findings from recent behavioral and ecological studies in this system, including evidence for bee damage in natural systems, flowering time effects in different plant species, details of damaging behavior and bumblebee behavioral preferences, implications for colony development and social organization, and the taxonomic distribution of damaging behavior across bumblebee species. I will also discuss potential implications for understanding the evolutionary origins of damaging behavior, as well as its implications for ecology and the maintenance of temporal synchrony between bees and flowers.

**Keywords:** behavior, bumblebees, *bombus terrestris*, flowering time, pollinators,temporal synchrony

## PL3. A CRISPR pooled screening platform in arthropod cells

N. Perrimon
*Department of Genetics, Harvard Medical School. Howard Hughes Medical Institute. Boston, Mass. USA*

*Corresponding author: perrimon@genetics.med.harvard.edu

To characterize the functions of the ~15,000 predicted *Drosophila* genes systematically in various cell-based assays, we have established genome-wide CRISPR knockout pooled format screening in *Drosophila* cells (Viswanatha et al., 2018). The approach was challenging as lentiviruses are not efficient in fly cells, leading us to develop a novel approach for plasmid delivery based on PhiC31 recombination. The platform is a breakthrough in the field and works exceptionally well, as exemplified by the identification of: 1. a transporter of the insect hormone ecdysone (Okamoto et al., 2018) (with Naomi Yamanaka's lab), demonstrating for the first time that steroid hormones do not simply enter cells by diffusion across lipid bilayers; 2. endoplasmic reticulum stress modifiers (7) (with Clement Chow's lab); and 3. receptors and mechanism of entry of various bacterial toxins (Xu et al., 2022) (with Min Dong's lab). In addition, we have extended the platform to CRISPR activation (CRISPRa) screens (Xia et al., 2023) and to mosquitoes (Viswanatha et al., 2021). I will describe the platform and applications.

Keywords: CRISPR, pooled screen, *Drosophila*, mosquito

---

## PL4. Scaling up data production and management systems to catalogue explore and monitor the richness of arthropod biodiversity

R. M. Waterhouse*
*Environmental Bioinformatics Group, SIB Swiss Institute of Bioinformatics*

*Corresponding author: robert.waterhouse@sib.swiss

Biodiversity research is an extremely broad field as it spans so many different scales- from studying a single species to whole ecosystems or even global patterns. It also encompasses many different taxonomic groups- from charismatic megafauna to the innumerable species of arthropods or even other so-called "dark taxa" such as fungi. It also relies on many different research approaches- with rich data collection characterizing targeted studies while larger-scale efforts necessarily focus on measuring a smaller number of key variables. The unifying theme is the recognition that biodiversity is critical for the health of our planet and human activities are causing unprecedented biodiversity declines that threaten the provision of ecosystem goods and services upon which human society depends. To protect and restore biodiversity we need to understand it, this is why biodiversity research, across all its dimensions, is so important. Molecular sequencing technologies are increasingly being employed to support biodiversity research, a key reason being the power molecular technologies have in overcoming the taxonomic biases which are inherent in biodiversity observations made by traditional methods, and which are often particularly challenging amongst the megadiverse phylum of Arthropoda. Increasingly these molecular sequencing technologies are high throughput, with individual field experiments generating very high volumes of sequence data, of a variety of types. Molecular sequencing initiatives are producing reference catalogues of genetic and genomic biodiversity, which need to be connected to biodiversity research infrastructures that are aggregating knowledge from scientific collections, human-made observations, and the literature. As entomologists, we have a responsibility to contribute towards ongoing and future global efforts to understand arthropod biodiversity and help mitigate the effects of human-induced changes on one of the most important animal groups contributing to maintaining healthy ecosystems.

**Keywords:** biodiversity, genomics, genetics, arthropods, molecular data

---

## PL5. Curing disease vectors from human pathogens

G. Dimopoulos
*Dept. Molecular Microbiology and Immunology, Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA*

*Corresponding author: gdimopo1@jhu.edu

There were an estimated 247 million malaria cases in 2021, and 627,000 people died from this *Anopheles*-borne disease, despite extensive use various malaria preventive and control methods over that past hundreds of years, and intense coordinated global control efforts for more than a century. Malaria control with the aim of elimination has becomes a Sisyphean endeavor with the available control methods, requiring an endless and intense use of partially effective strategies. This situation calls for novel and innovative vector-targeted malaria control technologies that will not require costly, intense, continuous, and only partially effective efforts. Ideally, a malaria control strategy would be based on single or, at most, a few application(s), and be self-propagating and self-sustainable to achieve a long-lasting epidemiological impact. This type of transformative malaria control technology is currently being developed based on genetic modifications of mosquitoes aiming at population modification using gene-drive technology. The same concept can be applied to other mosquito-born diseases such as those caused by arboviruses. Major advantages of population modification strategies are: a. they do not involve the removal of the target mosquito from its ecological niche; and b. they can be long-lasting through by using gene drive technology and therefore not requiring repeated releases of the transgenic pathogen refractory mosquitoes. Here we will discuss approaches and challenges towards the development of pathogen refractory mosquitoes that are suitable for population modification-based disease control strategies.

**Keywords:** mosquito, vector, *Anopheles*, *Aedes*, *Plasmodium*, dengue, zika, transgenesis, population modification

---

## PL6. The Aegean bees: granted by nature, treated by humans, threatened by climate change

T. Petanidou*
*Laboratory of Biogeography and Ecology, Department of Geography, University of the Aegean, University Hill, Mytilene, Greece*

*Corresponding author: tpet@aegean.gr

Despite its low profile, the Aegean Archipelago constitutes a highly complex and admirable world. This complexity involves high species diversity vis-à-vis limited resources and time span for the activity of these species (e.g. low earth fertility, water scarcity, high temperatures, seasonality). This is exactly the case of bees in the Archipelago, with ca. 850 species that make ca. 72% of the Greek and almost 40% of the European bee fauna. This high diversity not only it has been evolved by nature, but also enhanced by the diachronic wise land management by humans. The outcome of all the above is a complex interaction network between bees and flowers, in which generalists prevail in structuring it, with the copious specialists struggling to be accommodated within the entire structure.

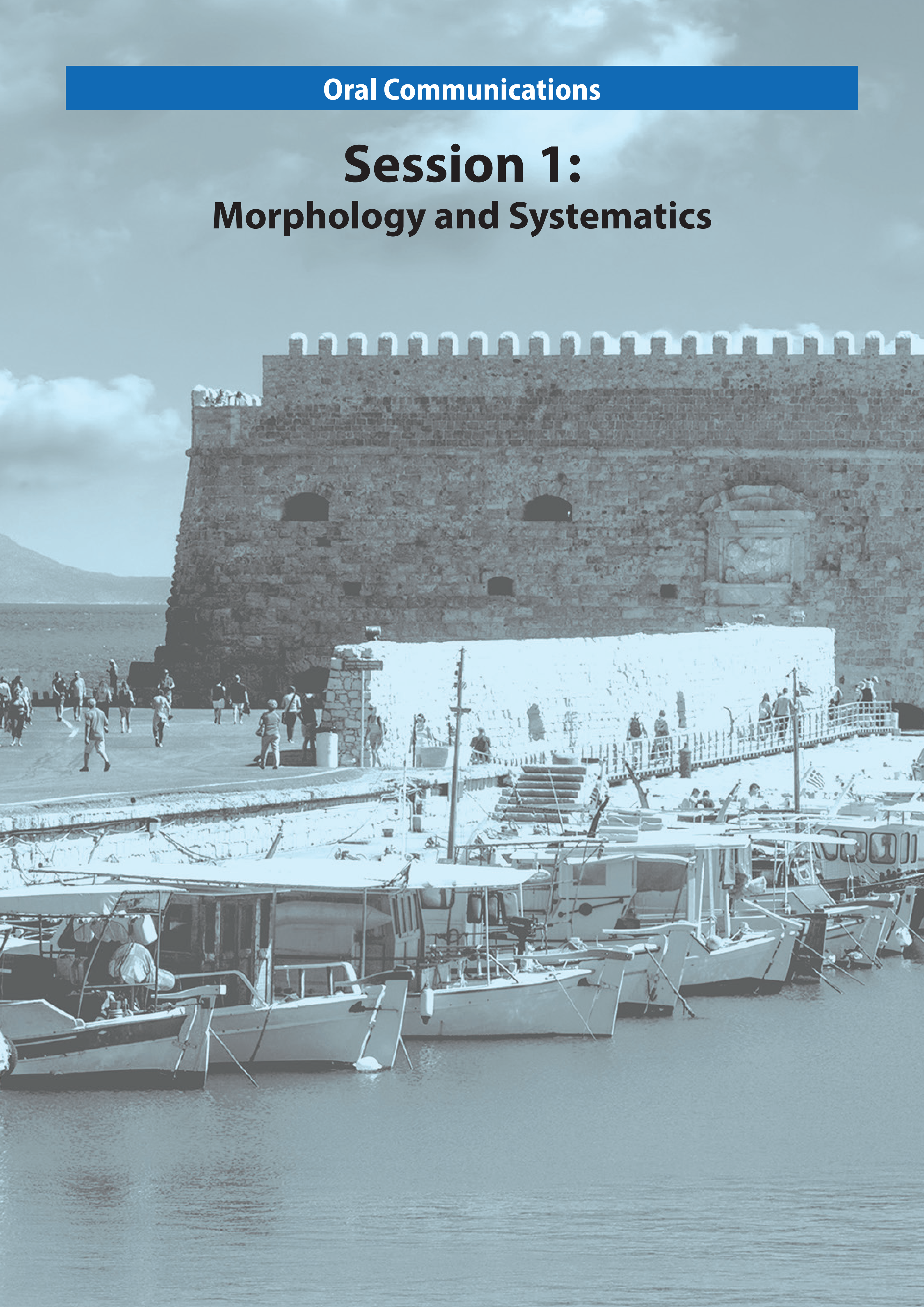
Based on data collected systematically during the last two decades in the Aegean (>250 sites located in 25 islands and a few mainland areas), and applying methodologies that include classical observation, molecular ecology, and novel holistic tools (e.g. ecological network analysis), this talk will discuss the drivers (geographical, climatic, man-induced) shaping diversity and biogeography of bees and subgroups (e.g. cleptoparasitic bees) within the Archipelago, co-considering ecological partnerships (e.g. interactions with entomophilous plants) and bee functional traits. The talk will also focus on particular threats bees and other pollinators face in the region (e.g. fires, grazing, uncontrolled beekeeping), with variable effects on bee diversity, including global warming that appears to be particularly challenging the future of bee pollinators in the Last Eden, the Mediterranean.

**Keywords:** biodiversity, biogeography, plant–pollinator interactions, bee threats, management

Oral Communications

# Session 1:

## Morphology and Systematics



## OC001. 99 million years of evolutionary stasis – first amber inclusion of the family Trichotonannidae, with an overview of the fossils of the infraorder Dipsocoromorpha (Heteroptera)

M. Roca-Cusachs[1], J. Kim[2], V. Hartung[3]*, M. Goula[1], S. Jung[4, 5]
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Dipsocoromorpha, or the minute litter bugs, comprise six families of small heteropterans with cryptic lifestyle. Only a few hundred species are known, but the true diversity of the infraorder might be much higher and include thousands of yet undescribed taxa. The fossils of the group are also quite rare to date, albeit found in several major amber lagerstätten. We present here the first known fossil of Trichotonannidae, best recognized by a rake of setae on the fore tarsi of the female. This character is common in the recent representatives of the genus *Trichotonannus* and is very similar to the state in the 99 million years old fossil, indicating that the ecological niche of these insects might not have changed very much since Late Cretaceous – an interesting example of evolutionary stasis. The fossil is used to re-evaluate the possible apomorphies of the family Trichotonannidae; also, an overview of the known fossils of Dipsocoromorpha is given.

**Keywords**: Dipsocoromorpha, amber, systematics, Trichotonannidae, stasis, apomorphy

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## OC002. Origins of the central Macaronesian psyllid lineages (Hemiptera; Psylloidea)

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Central Macaronesia has long been recognized as a natural laboratory for the study of evolutionary processes in insect diversification. The superfamily Psylloidea, or jumping plant-lice, are well represented in the Canary and Madeira Islands and shows a high level of endemism with 62 native species of which 47 are endemic. The present study provides the first comprehensive phylogenetic assessment of the origins of the psyllid fauna in this region. We employed a maximum likelihood backbone constraint analysis to place the central Macaronesian taxa within the Psylloidea mitogenome phylogeny. The native psyllid fauna in these central Macaronesian islands results from an estimated 20 independent colonization events. Post-colonization diversification varies from no further cladogenesis to modest *in situ* diversification resulting in two to four native taxa, and surprisingly given the diverse range of islands and habitats, only one substantial species radiation with more than four native species. In nearly all cases, island host plants are predicted by host plant groups of continental relatives.

Similarly, specificity to ancestral host plant genera or family is typically maintained during *in situ* diversification, both within and among islands.

Keywords: Central Macaronesia, colonization, diversification, origin, Psylloidea

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## OC003. Morphological identification and DNA barcoding of nymphs of Auchenorrhyncha species: A useful tool for species level identification

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The suborder Auchenorrhyncha is one of the most abundant and diverse insect taxa, in which species level identification is generally possible through the examination of male specimens since it is based on observation of the male genitalia. Identification of females and nymphs at the genus level is possible through the examination of external morphology, while at the species level requires associated males. In recent years an upcoming tool that can provide accurate species level identification is DNA barcoding, targeting the >500bp portion of the mitochondrial Cytochrome Oxidase I (COI) gene. The objectives of this study were to use DNA barcoding to assist in the identification of Auchenorrhyncha nymphs and to provide possible species identities correlating with the DNA barcode of the adults. Both nymphs and adults were collected using the sweeping net and the samples were stored in absolute ethanol. Total DNA extraction was performed and Polymerase Chain Reaction (PCR) was applied using BARCODE primers for the COI mt DNA gene segment, for 8 species belonging to two families, Cicadellidae and Issidae. Individual sequences were determined via automated sequencing of the above mtDNA gene segment. For data processing, the packages BioEdit 7.0.9.0, CLUSTALW2, DnaSP 5.10, MEGA 6.0.0, and GenAlEx 6.5 were applied. Results showed that DNA barcoding can be a rapid and accurate tool for nymphs' identification of Auchenorrhyncha.

Keywords: Auchenorrhyncha, sweeping net, nymphs, DNA barcoding

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## OC004. Genome skimming of sea skaters improves phylogenetic resolution of Halobatinae (Hemiptera: Heteroptera: Gerridae)

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Gerromorpha (Insecta: Hemiptera: Heteroptera) comprises some of the most ecologically diverse lineages of aquatic insects. For example, Halobatinae species have colonized surfaces of all aquatic habitats, ranging from freshwater (streams and ponds), to coastal marine (mangroves and estuaries) and even the open ocean. Their ability to adapt to numerous ecological and environmental conditions has attracted continued research interest on their behaviour, ecology and evolution. However, a recent study that analyzed four molecular markers recovered only a handful of well-supported relationships within the sub-family resulting in rather limited conclusions. Most notably, the limnic lifestyle of Metrocorini was ancestral to Halobatini and that marine lifestyle evolved only once in the common ancestor of *Asclepios* and *Halobates*. Fortunately, the emergence of high-throughput sequencing technologies has enabled recovery of more genetic markers for phylogenetic analyses. In this study, we applied genome skimming to obtain mitochondrial and nuclear genes from low-coverage shotgun sequencing reads from at least 50 specimens representing seven out of ten genera within Halobatinae. Our phylogenetic reconstruction recovered well-supported nodes across the seven genera. We confirmed that Metrocorini is paraphyletic, and *Esakia* and *Ventidius* are more closely-related to Halobatini than *Metrocoris* and *Eurymetra*. Our results show that genome skimming is a viable approach to recover genetic loci for robust phylogenetic analysis.

**Keywords**: Aquatic insects, Evolutionary relationships, Molecular phylogeny

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## OC005. Rapid species-level hemolymph color test for all life stages of *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae), an invasive and regulatory pest in the United States

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Slide-mounting is mandatory for accurate morphological identification of mealybug species (Hemiptera: Pseudococcidae). A typical slide-mounting process is performed in laboratory settings and requires several tedious and technical steps that can take up to 2 hr. Moreover, most of the taxonomic literature on mealybugs is based on adult females, whereas it can be difficult to find adult females in good condition in field samples. We tested 2,635 specimens of 57 mealybug species from 26 genera of Pseudococcidae collected from all over Florida for two consecutive years to discover an alternative to slide-mounting specimens of *Nipaecoccus viridis* (Newstead), an emerging polyphagous pest of regulatory concern in the United States, now known to occur in Florida. Recent exceptionally high infestations in commercial citrus-growing areas have activated regulatory agencies to implement quarantine and regulatory measures to halt its further spread within and outside Florida. A 5-min hemolymph color test of eggs, immatures, and adults is species-specific to *N. viridis*. This test will allow

for identifying all life stages, even when an adult female is unavailable. We discuss implementing this test in the field to help growers, researchers, and regulators make preliminary identifications.

Keywords: adults, early detection, eggs, field identification, first instar, lebbeck mealybug, pest management

## OC006. Molecular diversity among the mirid bugs infesting cotton in Indiaand DNA barcoding of undescribed species

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Miridsbugs (Hemiptera: Miridae) are major pests infesting cotton after adaptation Bt cultivars. *Creontiades pallidus* and *C.dilutes* have been key issues of management in Australia,US and China. A mirid *Creontiades biserratense and other two bugs* reported infesting Btcottons in Karnataka,India seriously during 2006. Further *Helopeltis bradyi* also appearing seriously on cotton. Colour morphs and variation in size, it becomes difficult to identify the species of mirid bugs infesting cotton. Morphologically distinct typical taxonomic keys are absent for easy identification *Creontiades* spp. \ Mosquito bugs also have species complex. In Karnataka *Creontiades biserratense* is dominant with diverse morphs. It was important to identify species of cotton miridbugs from Karnataka,Andhra Pradesh,Tamil Nadu and Maharashtra by molecular tools suitably for management. A 658 bp region mitochondrial cytochrome oxidase I (mtCOI) gene was amplified, cloned and sequenced from 17 of isolates of nine species. Sequence analysis showed variation at mitochondrial COI gene of *C. biseratense*, *Compylomma livida, Hyalopeplus similis, Heliopolis antoniii, Helopeltisbradyii, Creontiades pallidus, Taylorilygus apicialis and Deraecoris peciocola.* The phylogenetic analysis indicated high similarity between *Creontiades biserratense,Compylomma livida* and *Creontiades pallidus* and less similarity between *Hyalopeplus similis, Helopeltis antonii*, *H. bradyi*. No prior sequence information was available on *C. pallidus, C. biseratense*, *C. livida, D. Piceicola* and *H. similis* infesting Cotton from India. DNA barcodes have been generated for *Creontiades biserratense, Compylomma livida* and *Helopeltis bradyii* first time. This information will aid management strategies.

## OC007. Brindley glands in *Zelus renardii*

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*Zelus renardii* Kolenati, 1856 (Hemiptera: Reduviidae) is one of the alien insects recently acclimatised to the Mediterranean basin. The trivial name "leafhopper assassin bug" preludes the reduviid prey preference. In the Mediterranean, *Zelus renardii* adapted to preying on vicarious indigenous species, including *Philaenus spumarius* (Hemiptera: Aphrophoridae), the European vector of *Xylella fastidiosa*.
Reduviidae has several thoracic glands, pair or unpair, whose secretions may regulate adult insect behaviours, such as defence, alarm, and mating. *Zelus renardii* also possesses a pair of Brindley's glands, each consisting of about one hundred elements secerning in a reservoir with an outlet that opens at the thoracoabdominal limit. Stressful events elicit the production and secretion of a semiochemical bouquet, acting as alarm pheromones. This bouquet comprises 2-methyl-propanoic acid, 2-methyl-butanoic acid and 3-methyl-1-butanol as significant components, effectively repel conspecifics and suggest the role of Brindley's glands as alarm pheromone foci.
The predator can modulate the mixture release, reacting differently to stress or prey. *Zelus renardii* reduces the production of alarm pheromones and the chance of being detected by prey interacting with *P. spumarius*. Alternatively, the alarm pheromone could help the predator to mark its territory, avoiding interaction with a conspecific.
Evidence of the ability of *Philaenus spumarius* to perceive and react to the predator's semiochemical would provide a further means to manage transmission and infection by *Xylella fastidiosa*.

Keywords: Infochemicals, OQDS, Alien, Invasive, Quarantine Pest, Antifragility

## OC008. A description of egg collection, morphology, and hatching rates of eight Lepidopteran species of California USA

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California is a major source of dried fruits and tree nuts for the global marketplace and eight key lepidopteran species cause production and postharvest losses in this region: *Plodia Interpunctella* (Hübner), *Amylois transitella* (Walker), *Cydia pomonella* (Linnaeus), *Ephestia elutella* (Hübner), *Cadra figulilella* (Gregson), *Cadra cautella* (Walker), *Ectomyelois ceratoniae* (Zeller), and *Anarsia Lineatella* (Zeller). Understanding the egg biology of these species is critical since this life stage is typically the most difficult to control with contact insecticides and fumigants. Using scanning electron microscopy, we examined the chorionic characteristics and in most of the cases, the appearance and shape were similar with the exception of *C. pomonella*, which was the only species that lacked aeropyles. Moreover, we described the egg collection procedures as well as tracked the development of the eggs for each species in laboratory conditions, at 25°C and 65% relative humidity. Similar patterns were observed for the development time among the species ranging from three to five days. Overall, our study is to guide bioassays focused on the egg life stage of these species, and potentially others.

Keywords: eggs, moths, electron microscopy, morphology, development time

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## OC009. Discrimination of the *Anastrepha fraterculus* complex using the ITS2 sequence

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The range of *Anastrepha fraterculus* (Wiedemann) extends from Texas to South America where it is a polyphagous pest of cultivated fruits. It is a cryptic species complex with extensive morphological variation throughout its range. To date, eight morphotypes or putative species have been identified. We evaluate the use of the internal transcribed spacer 2 (ITS2) for discriminating members of the complex. Nine wild samples from Argentina, five laboratory strains representatives of the Mexican, Peruvian 1, Brazilian 1 species 1 and Brazilian 1, species 3 morphotypes, and two wild *An. schultzi* samples were considered. The ITS2 sequences were analysed by AMOVA, principal component analysis (PCA) and phylogenetically using Maximum Likelihood and Bayesian methods.

A total of 135 sequences were obtained from 56 individuals. Most variation (73%) was among morphotypes, no significant variation was present within morphotypes whereas the remaining 26% variation was present within samples. In the PCA the Brazilian 1 samples cluster together and are separated from the Mexican and Peruvian/Brazilian species 3 samples by the first axis. The Mexican and Peruvian/Brazilian species 3 clusters are separated by the second axis. The Peruvian/Brazilian species 3 and *An. schultzi* samples are partially separated by the third axis. Both the Maximum Likelihood and Bayesian trees display very similar topologies with distict morphotype clusters supported by high posterior probability and bootstrap values.

The ITS2 sequences are clearly useful for discriminating members of the complex. More extensive sampling will permit the development of ITS2-based tools for rapid discrimination.

**Keywords:** South American fruit fly; ITS2; species complex

## OC010. EURL and regulated fruit-flies: two sharper tools for a tighter list

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The number of EU-regulated fruit-flies was increased to 75 taxa in 2022. EURL for Insects and Mites therefore initiated two special projects to fulfill its mission of technical assistance to European NRLs.

The online and interactive Q-Tephrikey is dedicated to the identification of regulated Tephritidae. It includes three matrices separating 87 taxa from an artificial and inclusive "non regulated Tephritidae" grouping. The "adults" and "larvae" matrices code them for 36 morphological characters. The artificial grouping is positively encoded for most of them: the final discrimination between it and a given species is ensured by a unique combination of specific features (the "check" step). Because of limitations in

larvae knowledge, we supplemented the “larvae” matrix with a third (“pathway”) matrix describing host-plant and geographical origin, and including a “common pathways” shortcut encoded with Europhyt-reported interceptions during the 30 last years. Q-Tephrikey is currently tested within the EU-NRLs network before publishing a fully operable version.

The second project deals with the exploration of “black holes” in EU phytosanitary interception. Actually, the previous EU-regulation led to the notification of numerous barely identified “non-European Tephritidae” in interception databases. Molecular re-identification of voucher specimens kept in the French NRL revealed at least one species (*Bactrocera trilineata*) which was unreported in previous records. EURL is now extending this survey to other EU-NRLs collections, focusing primarily on minor irregular pathways. We aim to unveil other undetected entries of minor though regulated pests, to complete the current identification tools and to prevent anything from falling through the net.

## OC011. Denucleation of neurons is a key to extreme brain miniaturization

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Anucleate animal cells are a peculiar evolutionary phenomenon and a useful model for studying cellular mechanisms. Anucleate neurons were recently found in one genus of miniature parasitic wasps of the family Trichogrammatidae, but it remained unclear how widespread this phenomenon is among other insects. We studied the anatomy of miniature representatives of another parasitic wasp family (Hymenoptera: Mymaridae) using array tomography and found two more species with nearly anucleate brains at the adult stage. Thus, the lysis of the cell bodies and nuclei of neurons appears to be a more widespread means of saving space during extreme miniaturization, which independently evolved at least twice during miniaturization in different groups of insects. These results are important for understanding the evolution of the brain during miniaturization and open new areas of studying the functioning of anucleate neurons. This study was supported by the Russian Science Foundation (project no. 22-14-00028).

**Keywords:** miniaturization, anucleate neurons, Trichogrammatidae, Mymaridae

## OC012. Denucleation of neurons during metamorphosis in *Megaphragma viggianii* (Trichogrammatidae)

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During metamorphosis, the central nervous system (CNS) of holometabolous insects undergoes complex structural and allometric changes. In exceptional cases, the CNS can follow an extreme path of cell reduction, such as the denucleation of neurons in species of the parasitic wasp genus *Megaphragma* (Hymenoptera: Trichogrammatidae). Studying the metamorphosis of the CNS in one of

the smallest wasps, *Megaphragma viggianii*, we obtained the first data on the morphological and volumetric changes in its CNS during pupal development with emphasis on nucleus lysis in the neurons. We showed that the absolute and relative volumes of the brain during pupal development decrease by a factor of 5, due to the decreasing cell body rind. Our analysis of the cellular ultrastructure of the pupal brain showed that most (up to 97%) of the loss of neuron nuclei occurs at the stage of the pharate adult. Despite the cell body rind loss, the volume of the neuropilar part of the brain remains virtually unchanged during pupal development. The first signs of the lysis of neuron nuclei are observed in the red-eyed pupa. In the black-eyed pupa, the number of lysis foci increases considerably, many organelles are destroyed, and the degree of chromatin compaction noticeably increases.
Elucidation of the molecular basis of denucleation, mechanisms that provide for anucleate neurons functioning, and principles of the organization of neuronal circuits in a nearly anucleate brain is of fundamental value for neuroscience and require further comprehensive studies. This study was supported by the Russian Science Foundation (project no. 22-74-10008).

Keywords: *Megaphragma*, denucleation, microinsects, brain

## OC013. Flight mechanics of the miniature parasitoid wasp *Megaphragma vigianii*

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The study of microinsect flight is an area of modern entomology, closely related to biomechanics and aerodynamics. Some of the smallest body sizes among flying insects are found among parasitoid wasps of the family Trichogrammatidae. The body length of one of the smallest species of this family, *Megaphragma vigianii*, is only about 250 µm. As most other microinsects *M. vigianii* has bristled wings. We perform macro video recording of the flight of *M. vigianii* and reconstruct flight kinematics. Additionally, we perform CFD reconstruction of hovering flight. *M. vigianii* is capable of active maneuverable flight, the average and maximum flight speeds were 0.06 and 0.25 m/s (1000 body lengths/s), respectively. The average Reynolds number during the wing cycle is about 3, which means that viscous forces are very significant on vertical force generation, and the leakiness between the bristles must be quite low. Flight cycle of *M. vigianii* is characterized by the aerodynamic asymmetry. It consists of two power strokes, recovery stroke between upstroke and downstroke and supination between downstroke and upstroke. The most of the aerodynamic force produced during power strokes where angles of attack and wing speeds are maximal. During recovery stroke angles of attack and wing speeds are small to minimize drag. The estimated aerodynamic features revealed in our study and unusual kinematics of the operation of the microwasp wings are an important adaptation to flight at ultra-low Reynolds numbers, under conditions of prevalent viscous forces. This study was supported by the Russian Science Foundation (project no. 22-74-10010).

Keywords: flight, miniaturization, bristled wings, Hymenoptera, CFD

## OC014. Advances in the systematics and taxonomy of whitefly parasitoids (Hymenoptera: Chalcidoidea, Platygastroidea)

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Parasitic wasps (Hymenoptera: Apocrita) are perhaps the most diverse lineage of animals on the planet. They attack and develop in and on a wide variety of hosts including arachnids, insects, and nematodes, with numerous species being employed as biological control agents of specific pests. Whiteflies (Hemiptera: Aleyrodidae) are a species-rich, yet taxonomically poorly known, lineage of obligate plant feeding insects. This family is of considerable economic importance given (1) their direct mode of feeding on hundreds of different crop, fiber, and ornamental plants, (2) the ease with which they are unintentionally moved outside their native range, and (3) the ability of some species to transmit viruses. Immature whiteflies are attacked by eight families of parasitic wasps in two proctotrupomorph superfamilies: Chalcidoidea and Platygastroidea. Much taxonomic effort has been exerted in recent years to gain a better understanding of the evolutionary relationships between and among these different lineages. These studies include alpha-taxonomic revisions and molecular analyses of Sanger sequencing and phylogenomic scale datasets. Our current understanding of the diversity and relationships between different whitefly parasitoid taxa will be discussed in the context of historical and recent advancements made in these essential natural enemies of whiteflies.

Keywords: Aleyrodidae, biological control, phylogenetics

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## OC015. Fruit fly identification: it's apt to use an app

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Morphological identification of insects traditionally relies on dichotomous keys which are often problematic to be used by the non-specialist. Immature stages such as larvae are even more difficult due to the limited characteristics present. The lack of easy and readily available tools hampers a swift and timely identification, of crucial importance in detection and surveillance programmes. Modern technology offer the possibility of alternative identification tools such as digital multi-entry keys with illustrated character states. Such tools were developed for adults and larvae of tephritid (Diptera, Tephritidae) fruit flies of economic significance, mainly of the genera *Ceratitis*, *Bactrocera Dacus*. *Trirhithrum*, and *Zeugodacus*. One application includes 23 adult species of the genera that are considered of importance for the EU. A second key covers third instar larvae of thirteen species and species-groups, while a third comprises 29 adult fruit fly pest species occurring in different regions in Africa, including the islands in the western Indian Ocean. All were developed along similar lines, based

on a character state matrix scored for each taxon. Adult keys use characters from the main morphological structures (head, thorax, wing, legs, abdomen, genitalia) while the larval key mainly uses characters of the cephalopharyngeal structure in addition to the number of tubules in the front spiracles, and their position in relation to the cephalic skeleton. The keys were developed and afterwards converted into a mobile application by LUCID, for both Android and Apple devices. They are freely accessible and downloadable.

Keywords: Tephritidae, surveillance, identification, invasive species, pest species

## OC016. Automated 3D-modeling of small invertebrates

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Accurately identifying, describing, and classifying new and already described insect species based on morphology can be difficult with only one view of the specimens. Therefore, techniques have been developed for obtaining photorealistic 3D models of dried, pinned invertebrates, but no automated approach is available for small invertebrates preserved in ethanol. To address this shortcoming, we present an automated 360-degree imaging system that captures multiple images of small, ethanol-preserved invertebrates to create 3D models. The system captures image stacks from different positions, which are then processed using Helicon Focus software to obtain extended-focus images. The Agisoft Metashape software creates a textured 3D model, enabling photorealistic models of insects smaller than three millimeters preserved in ethanol. The image acquisition process optimizes the trade-off between the quality of the 3D model and processing time by taking the minimum required number of images. Automated focus stacking and 3D modeling processes can be fine-tuned to optimize model quality. In addition, we are currently developing methods to determine the surface area and volume of the specimens. We predict that researchers will use the 3D models to analyze insect morphology in collaborative teams that can view the models simultaneously on computers or virtual reality headsets. Overall, this automated approach using photogrammetry has the potential to streamline and enhance biodiversity research and taxonomic research on small insects.

Keywords: taxonomy, biodiversity, photogrammetry, automation

## OC017. Unusual flight style and bristled wings help miniature beetles to fly faster

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Flight speed generally increases with animal body size, but miniature Ptiliidae beetles can fly at speeds and accelerations of closely related Staphylinoidea beetles three times as large. We showed that this performance results from light bristled wings and previously unknown type of wingbeat cycle. We obtained three-dimensional reconstructions of morphology and kinematics in one of the smallest ptiliids, *Paratuposa placentis* with 395 µm body length, and performed flight mechanics analysis using dynamically scaled models and computational fluid dynamics.

Bristled wing of *P. placentis* is at least 5 times lighter than equivalent membranous wing but at the same time it produces only 25% less aerodynamic force. Motion of bristled wings requires little inertial power, so muscle mechanical power remains positive throughout the wing beat cycle, making elastic energy storage obsolete.

Wingbeat trajectory of *P. placentis* has the shape of a pronounced figure-eight loop that consists of subperpendicular upstroke and downstroke followed by claps at stroke reversals, above and below the body. Computational analyses suggest a functional decomposition of the flapping cycle in two power half strokes producing a large upward force and two down-dragging recovery half strokes. The wings thereby produce high aerodynamic torques that cause the high-amplitude body pitch oscillation. We found that relatively massive elytra function as inertial brakes and enhance posture stability due to unusual high-amplitude movement.

This novel flight style evolved during miniaturization may compensate for costs associated with air viscosity and helps explain how extremely small insects preserved superb aerial performance during miniaturization.

Keywords: biolocomotion, insect flight, miniaturization, bristled wing, Ptiliidae, Coleoptera

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## OC018. Tissue liquid diffusivity and chemical composition changes in *trypoxylus dichotomus* during pupal stage using Magnetic Resonance Imaging and Spectroscopy

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Tissue destruction and reconstruction process in the pupal body of holometabolous insects might be a clue for a new perspective of regenerative medicine. In this study, we used Magnetic Resonance Imaging (MRI) to observe morphochemical changes in the pupal body of *Trypoxylus dichotom*us. Metamorphosis process of the specimen (3 males and 7 females) were continuously observed from prepupal stage to just before emergency using a 9.4-T MRI system. The imaging conditions for morphological images were TR/TE, 64/4685.4ms; slice thickness, 1mm; FOV, 40×50mm; matrix, 320×400 (in-plane spatial resolution, 130µm). Those for diffusion-weighted image were TR/TE, 21.15/2500ms; b-value, 650 $mm^2/s$; number of diffusion gradient axes, 30, slice thickness, 1mm; FOV, 42×30mm; matrix, 168×120 (in-plane spatial resolution, 250µm). Spectroscopic data were taken with TR/TE, 2500/17.5ms; voxel size, $3^3/1^3/0.5^3 mm^3$; number of data points, 2048; receiver bandwidth, 5496.68Hz (13.7417ppm). During the 3 to 4 days between prepupa and pupation, the larvae's digestive tract developed thicker and constructed a liquid pool. The tract then became longer and narrower, while the surrounding tissues were constructed. Diffusion of water molecules in the fluid pool was fast and exhibited clear anisotropy until just after pupation, and then became slower and less anisotropic. These findings implied that the liquid pool was the result of the influx of collapsed tissues into the digestive tract. The morphochemical observation suggested that the content of pupal body was the collapsed tissues stored in the digestive tract to reconstruct adult tissues.

Keywords: Magnetic Resonance Imaging, Diffusion Weighted Imaging, Magnetic Resonance Spectroscopy, pupa, metamorphosis, *Trypoxylus dichotomus*

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## OC019. A study on colour complexity in *Trypocopris vernalis* (Linnaeus, 1758) (Coleoptera, Geotrupidae)

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Iridescence is a widespread remarkable phenomenon shared by a manifold of taxa, such as (e.g.) insects and birds. In Coleoptera the complex stratification of exoskeleton layers often leads to extremely variegated colourations for which the visual survey of an observer is poorly effective to define the exact hue. This is the case of *Trypocopris vernalis*, with colours ranging from black to blue, violet or various shades of green depending on light incidence. Here, we separately evaluated both pronotum and left elytron of dor beetle specimens from Germany to Italy and Greece. The colouration was quantified by a colorimeter (emitted light = full daylight). The instrument measures the absorbency of light, and defines colours within the visible spectrum of human eye (380-750 nm) according to the CIEL*a*b* System. The Euclidean distance among individuals was calculated, then the decision tree method was applied to the structures separately and together. The results depicted within this context are more articulated than previously assumed, with the pronotum usually darker and often less varying than elytron, suggesting thus that the latter could be involved in the mimetism by camouflage.

Keywords: CIEL*a*b* system, reflectance, Euclidean distance, Palearctic, Geotrupidae, dung beetles

## OC020. Phylogenomic species delimitation of the twisted-winged parasite genus *Stylops* (Strepsiptera)

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The twisted-winged parasite genus *Stylops* has a history of different species concepts with varying host specificity that have had large implications on species diversity estimates. An incorrect supergeneralist species concept, resulting in synonymization of all West Palaearctic *Stylops* species, hindered the study of the genus in Europe for decades. Underutilization of molecular data has resulted in taxonomic controversies in this morphologically conserved genus. To solve these discrepancies and obtain novel information about host associations of the genus, we apply whole-genome sequencing to 163 specimens representing a large proportion of putative European species. We evaluate the existing and conflicting species hypotheses with molecular species delimitation methods SODA and GMYC and use a phylogeny obtained from IQ-TREE2 to investigate host associations of the species. Furthermore, we evaluate the effect of number of loci used in SODA for the number of inferred species. We find justification for synonymization of multiple species and indications of undescribed species, as well as confirm new host-parasite relationships. We show that the number of inferred species in SODA is exceedingly and positively correlated with the number of loci used, urging for cautious application. The results of our study bring clarity to the West Palaearctic species diversity of *Stylops*. Furthermore, the comprehensive molecular data set generated in this study will be a valuable resource for future research not only on *Stylops*, but on evolution of parasites in general.

**Keywords:** Strepsiptera, *Stylops*, species delimitation, WGS, cryptic species, entomophagous parasites

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## OC021. Unraveling the taxonomic status of the endemic alpine dytiscid *Agabus nevadensis* by an integrative taxonomy approach

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Integrative taxonomy offers a practical approach to shed light on unresolved species complexes, contributing to our understanding on the ecological and evolutionary processes that generate biodiversity, and providing a powerful tool for conservation practice. The Sierra Nevada mountain range (Spain) is a global biodiversity hotspot hosting numerous endemic species, such as the freshwater diving beetle *Agabus nevadensis*, restricted to this massif. However, its taxonomic status in relation to its widespread congener *A. bipustulatus* has been much doubted. The *A. bipustulatus* complex is known for its large morphological variation along altitudinal gradients, showing several montane cold-adapted morphotypes across Europe. To disentangle the taxonomic status of these taxa, we combined

physiological experiments (thermal tolerance measurements), geometric morphometry (body shape variation) and genome-wide Single Nucleotide Polymorphysm (SNP) data from populations covering the full altitudinal range across the distribution range of *A. nevadensis* and the Iberian range (plus several European and Middle East populations) of *A. bipustulatus.* Preliminary genomic analyses do not support the current taxonomic delimitation between these taxa and suggest a lack of genetic structure, except from some high-altitude populations. In both sexes, elytra shape differed between the taxa, but it showed a gradual variation correlated with altitude. Upper, but not lower thermal limits differed between the taxa, and we found no clear pattern in thermal tolerance variation in relation with altitude. Overall, our preliminary results point to no clear-cut species delimitation between *A. bipustulatus* and *A. nevadensis* and suggest that altitude-driven selection maintains ecotypic differentiation within *A. bipustulatus.*

Keywords: altitudinal gradient, coleoptera, genome-wide SNP, geometric morphometry, phenotypic plasticity, thermal tolerance

## OC022. Speciation in troglophilic and parthenogenetic Amblypygi from the Levant

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Whip spiders (Amblypygi) can be found in tropical and sub-tropical regions, where they inhabit shaded humid environments. This habitat preference makes them pre-adapted to life in subterranean conditions, where in turn, additional adaptations occur and may lead to speciation. Two whip spider species are known from Israel: *Sarax ioanniticus*, a widely distributed troglophilic species found across the eastern Mediterranean, and *S. israelensis*, a troglomorphic species that is endemic to caves in Israel. The two sympatric species have superficial morphological similarities, with few diagnostic characters, and can be found in proximity within man-made caves. In recent years, we utilized morphological and molecular tools to investigate the relatedness of these species, their mode of speciation, eye development mechanisms, as well as their population genomics. We found that *S. israelensis* is genetically distinct from *S. ioanniticus*, even when inhabiting the same chamber of a cave. Within each species, we found almost no genetic variability, a result of their parthenogenetic life history. These genetic invariances hamper our ability to determine the biogeographic history of *S. israelensis*. Although these species are genetically invariant, we found considerable within-population variation in the degree of eye reduction, particularly in the troglomorphic species *S. israelensis*. We identified several RDGN genes that are involved in the formation and reduction of eyes in these species. Our results suggests that variation in eye morphology in *S. israelensis* is driven by epigenetic mechanisms. These findings are significant to the recognition and delimitation of cave-adapted species and have consequences for assessments of conservation priorities.

Keywords: eye-reduction, Levant, whip spiders

## OC023. Crawling in the deep - exceptional subterranean speciation of cryptic troglobitic spiders

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Caves have long been recognized as a window into the mechanisms of diversification and convergent evolution, due to the unique conditions of isolation and life in the dark. These lead to adaptations and reduce dispersal and gene flow, resulting in high levels of speciation and endemism. The Levantine cave fauna in general, and the Israeli cave arachnofauna in particular, are poorly known, but likely represents a rich assemblage. In a recent survey, we found troglophilic funnel-web spiders of the genus *Tegenaria*, present mostly at the cave entrance zone. In addition, we identified caves inhabited by troglobitic *Tegenaria*, which are present mostly in the twilight and dark zones. Ten of the caves are inhabited by both troglophilic and troglobitic *Tegenaria*. These spiders bear superficial phenotypic similarities but differ in the levels of eye reduction and pigmentation. To test whether these taxa constitute separate species, as well as understand their relationships to epigean counterparts, we conducted a broad geographic sampling of cave-dwelling *Tegenaria* in Israel, using morphological and molecular evidence. Counterintuitively, our results show that the troglobitic *Tegenaria* are distantly related to the troglophilic *Tegenaria* found at each of the cave entrances. Moreover, seven new troglobitic species can be identified based on genetic differences, eye reduction level, and features of the female and male genitalia. Our COI analysis suggest that the Israeli troglobitic *Tegenaria* species are more closely related to eastern- Mediterranean congeners than to the local sympatric troglophile *Tegenaria* species, suggesting a complex biogeographic history.

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## OC024. Exploring the sympatric cryptic diversity within *Colomerus vitis* (Acari: Eriophyidae), the vector of Grapevine Pinot gris virus

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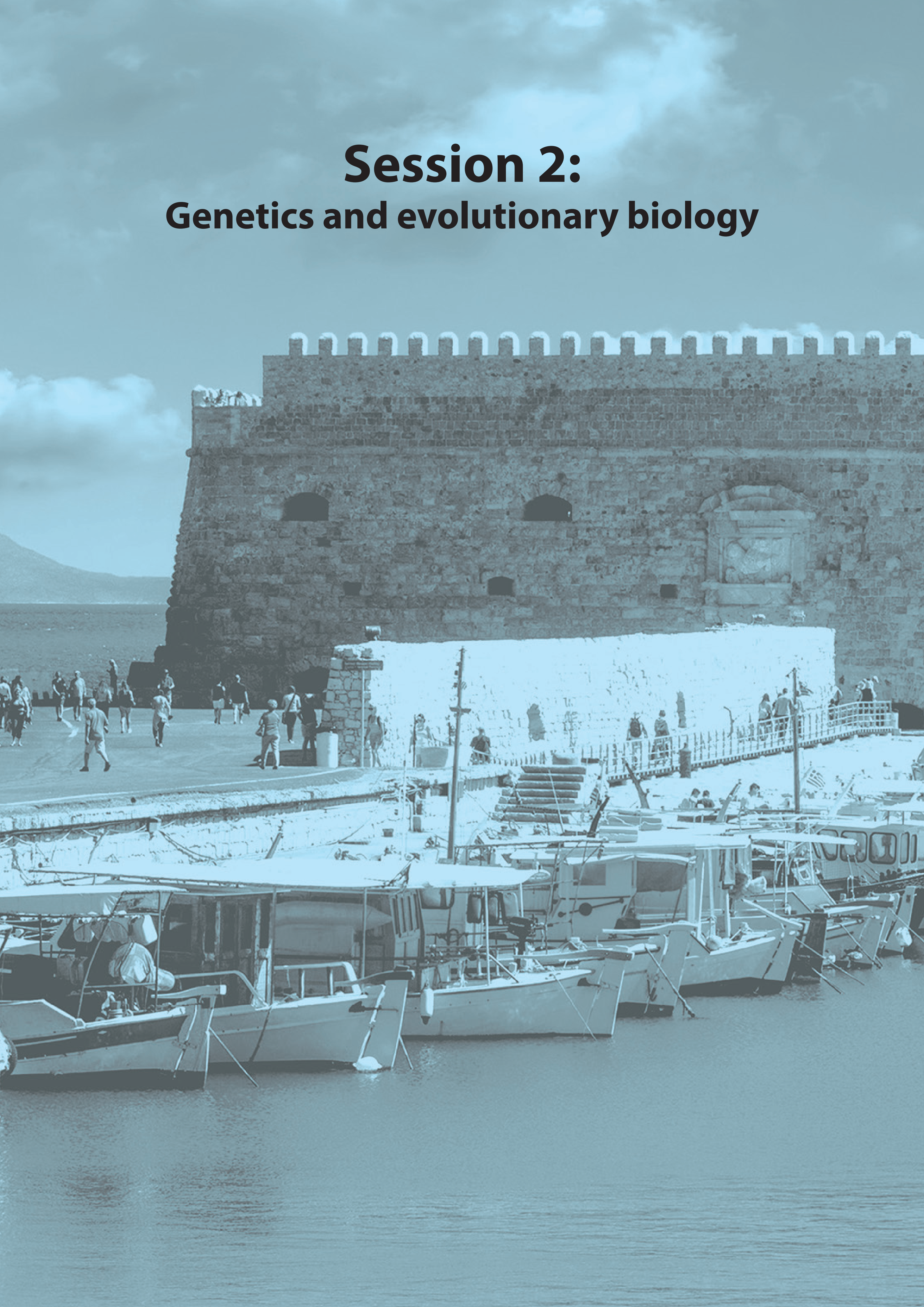
The eriophyid mite *Colomerus vitis* (Pagenstecher) is widespread in wine-growing areas around the world. In addition to causing direct symptoms on grapevine (leaf erinea, anomalies in bud development, and leaf curling), this mite has been shown to vector Grapevine Pinot gris virus (GPGV), an emergent virus in Europe (particularly in France and Italy) GPGV is associated with Grapevine Leaf Mottling and Deformation disease. Misidentification of species within cryptic complexes can make it difficult to understand pathosystems and their epidemiology, particularly as cryptic species may present differentiated transmission efficiency. Studies of *COI* DNA sequences have shown the occurrence of cryptic diversity in *C. vitis* with at least five highly diverged groups. The objective of this study was to **i)** characterize populations from five regions in France (Nouvelle-Aquitanie, Alsace, Occitanie, Provence-Alpes-Côte d'Azur and Corse) and two regions in Italy (Trentino Alto Adige and Apulia) based on *COI* sequences and compare with previously studied populations (Egypt, Iran, South Africa, Spain, USA); and **ii)** conduct the first variability analysis of nuclear markers (ITS, D2) for populations from France, Italy, and Iran. Phylogenetic analysis revealed the co-occurrence of four diverged *COI* haplotypes in France and three in Italy corresponding to previously characterized groups, and that at least two of which occur sympatrically. Nuclear markers confirmed the occurrence of at least four highly divergent groups. Genetic distances of mitochondrial and nuclear markers suggest that groups correspond to cryptic species. Further studies should investigate the efficiency of virus transmission by the different groups.

**Keywords:** Eriophyoidea, virus transmission, *Trichovirus*, molecular markers, systematics, cryptic species

# Session 2:

## Genetics and evolutionary biology



## OC025. Genetics and evolution of insect sex determination mechanisms: progress and prospects

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Insect sex determination mechanisms are diverse and evolve rapidly. In many groups sex is determined by specialised sex chromosomes, such as male heterogamy (XX-XY) or female heterogamy (ZZ-ZW). These sex chromosomes carry the primary signals, dominant male or female determiners, or counting elements. Some groups reproduce by haplodiploidy, with haploid males developing from unfertilized eggs and diploid females developing from fertilized eggs. A number of unusual systems exist, such as monogenic sex determination and paternal genome elimination. I will present recent progress in elucidating the molecular genetic basis of sex determination in insects. We start to see common patterns in the variation in underlying gene networks of sex determination in various groups. A number of primary signal genes have now been characterised, but thus far show little to no homology. There is growing knowledge on how other genetic elements, such as sex ratio distorters and intracellular bacteria can interfere with host sex determination. From an evolutionary perspective, I will discuss how changes in sex determination pathways may occur.

**Keywords:** heterogamy, haplodiploidy, homology, sex determiner, sex chromosome, reproduction

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## OC026. Endosymbionts induce parthenogenesis by manipulation of sex determination gene transcripts

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Endosymbionts gain transmission advantage by skewing their host's progeny sex ratio towards females. An extreme case of reproductive manipulation is thelytokous parthenogenesis, a form of asexual reproduction. In haplodiploid arthropods, unfertilized eggs are haploid and develop into males, but endosymbiont-infected thelytokous females produce diploid unfertilized eggs, that develop into females. To understand the underlying molecular mechanisms, we examined splicing and expression patterns of canonical sex determination genes transformer (tra), transformer-2 and doublesex in two haplodiploid wasp species that have both thelytokous populations infected by Wolbachia and geographically isolated uninfected populations that reproduce sexually. Female-specific tra mRNA is present in the ovaries of infected females, prior to diploidization of the eggs, and during early embryo stages of both species. Furthermore, overall tra transcription is upregulated in one of the infected species, suggesting similar targets yet varying degrees of manipulation. Upon removal of Wolbachia, maternal provision of tra mRNA reverts to the pattern of sexually reproducing wasps that do not contain Wolbachia. Our study indicates that endosymbionts can increase their transmission by manipulating germline regulation of host sex determination genes in these wasp species. This phenomenon may be much more widespread and implies that solely diploidization is not sufficient for feminization of host eggs and to induce thelytokous parthenogenesis.

Keywords: sex determination, reproductive parasites, *Wolbachia*, haplodiploidy, parthenogenesis

## OC027. Fertility related long non-coding RNAs as novel species-specific insect control targets

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Long non-coding RNAs (lncRNAs) have gained importance as essential regulators of cellular and metabolic processes. The way they regulate gene expression varies, ranging from guiding transcription factors, remodeling chromatin, functioning as miRNA sponges, or regulating post-transcriptional mRNA modifications. Due to the absence of coding capacity, lncRNAs show a lack of nucleotide sequence conservation even among closely related species. This characteristic, however, offers an exceptional possibility for the development of species-specific approaches for insect control.

In that regard, in an effort to target the fertility of the Asian tiger mosquito, *Aedes albopictus*, we investigated the role of tissue and development-specific lncRNAs. Through the analysis of transcriptomic data, we identified several lncRNAs that were differentially expressed upon blood feeding; we called these genes Norma (NOn-coding RNA in Mosquito ovAries). Silencing Normas resulted in 43% oviposition reduction, smaller ovaries and 53% hatching reduction of laid eggs. Moreover, a significant downregulation of two neighboring mucins was observed, indicating a potential interplay between Norma3 and the mucins. We further identified four reproduction-related lncRNAs expressed both in post-blood meal ovaries and in early embryogenesis, possibly implicating a maternal effect inherited to the offspring. Our work constitutes the first experimental proof-of-evidence connecting lncRNAs with mosquito reproduction and opens a novel path for pest management.

We are employing a similar strategy for the discovery of fertility-related lncRNAs in agriculturally important Tephritids. Since mosquito ovaries and Normas are induced upon mosquito blood feeding, we adapted our protocol to non-blood feeding insects. Differentially expressed lncRNAs are under investigation.

Keywords: long non-coding RNAs, fertility, fecundity, species-specific control

## OC028. Post-mating response in a viviparous species: the case of *Glossina morsitans*

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Tsetse flies, vectors of African trypanosomiasis, evolved a unique reproductive biology compared to other disease vectors. They reproduce by obligate viviparity with intrauterine larval development and nutrient provisioning by the mother. Consequently, they have a slow reproductive strategy that invests large amounts of nutritional resources and time into few offspring. We are investigating the changes that occur in *Glossina morsitans* female reproductive apparatus in the 72h post-mating to understand the processes that facilitate live birth. Female insemination occurs with the delivery of ejaculate within a spermatophore, an adaptive trait that ensures male reproductive success. Insemination initiates the post-mating response which, in the mated female, results in dramatic metabolic and molecular changes that block mating and initiate the reproductive processes. Indeed, the virgin female metabolic and molecular condition appears as a preparatory status with the accumulation of precursors for nutrients, antimicrobial compounds, and developmental regulators. Immediate post-mating is characterized by transfer of ecdysone precursors, rapid accumulation of signaling molecules, activation of gene expression associated with amino acid metabolism and expression of a ovary-specific odorant binding protein coding gene. These appear to play roles in the female response to male-derived compounds and may be required for initiation/regulation of the post-mating response by female tsetse flies. The long-term post-mating effects include visible changes to reproductive tract and fat body, biosynthesis and accumulation of lipids, energy production, processing, and gene expression of structural and developmental regulatory proteins. These data are relevant from the evolutive point of view and for vector control strategies.

Keywords: *Glossina morsitans*, post-mating response, vector control, viviparity

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## OC029. Spontaneous parthenogenesis and sex determinism in the parasitoid wasp *Cotesia typhae*

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Sex determination in Hymenopterans happens via the ploidy of individuals: haploids become males and diploids become females. Arrhenotoky is believed to be the ancestral reproduction mode in

Hymenopterans, with haploid males produced parthenogenetically, and diploid females produced sexually. However, a number of transitions towards thelytoky (diploid females produced parthenogenetically) have appeared in Hymenopterans, and in most cases populations or species are either totally arrhenotokous or totally thelytokous. Here we present the case of *Cotesia typhae* (Fernandez-Triana), a Braconidae that produces parthenogenetic females at a low frequency. This rare thelytokous phenomenon is observed in a wild population and laboratory strains at a variable frequency. It happens in the progeny of both virgin and mated females. The analysis of daughters of heterozygous virgin females allowed us to show that a mechanism similar to automixis with central fusion is very likely at play in *C. typhae*. In most species, the origin of thelytoky is either bacterial or genetic, and an antibiotic treatment as well as PCR experiments did not demonstrate a bacterial cause in *C. typhae*. As transition toward thelytoky may be impacted by the sex determination system, we studied the genetic components of the very first steps in sex determination. Finally, we searched for low frequency thelytoky in relative species from the *Cotesia* genus. The unusual case of low parthenogenetic frequency described constitutes another example of the fascinating diversity of sex determination systems in Arthropods.

Keywords: thelytoky, haplodiploid, sex determination, asexual reproduction

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## OC030. The role of the antagonistic pleiotropy on the genetic variability of thermal tolerance in *Drosophila melanogaster*

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The maintenance of genetic variation in natural populations is one of the main challenges in evolutionary biology. This maintenance of heritable variation is essential for populations to persist in changing and highly fluctuating environments, as occurs in the current scenario of climate change. One of the factors that allow the maintenance of genetic variation is the antagonistic pleiotropy on traits under natural selection. Particularly, antagonistic sexual pleiotropy, that is, when different alleles at any loci are favored in males and females, has been an important factor in maintaining genetic variation in traits such as fecundity and immune response. In the present work, we will show evidence of antagonistic sexual pleiotropy in tolerance to extreme temperatures in *Drosophila melanogaster*, using the *Drosophila* Genetic Reference Panel (DGRP). We found quantitative genetic and genomic evidence that shows that various loci that contribute to thermal resistance have alleles that segregate differentially in both sexes. For example, we found genotype-sex interactions and genotype-sex correlations for different components of the thermal tolerance landscape. We also found that SNPs associated with the maximum critical temperature and thermal sensitivity have different allelic variants for both males and females. In summary, we found evidence of the importance of antagonistic sexual pleiotropy in relevant traits associated with adaptation to climate change and that would contribute to the genetic variation of thermal tolerance.

Keywords: genome-wide association studies; global warming; single nucleotide polymorphism; thermal tolerance landscape

## OC031. Mitochondrial genotype decreases mitochondrial respiration in seed beetles: implications for male subfertility

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Evolutionary theory suggests that inheritance of mitochondria through the maternal lineage could drive the accumulation of male-harming mutations in mitochondrial genomes. Namely, due to uniparental mode of mitochondrial inheritance, natural selection is "blind" to mutations that are deleterious to males as long as they are beneficial or neutral to females. This evolutionary phenomenon is known as 'the Mother's Curse'. The male-specific adverse effects of mitochondrial mutations are thought to be due to the fact that males are sensitive to impairments in the function of the oxidative phosphorylation (OXPHOS) system, i.e. mitochondrial respiration. The OXPHOS system consists of five complexes whose subunits are coded by the mitochondrial and nuclear genomes. In our previous work, we identified several mitochondrial haplotypes (mitotypes) in laboratory populations of the seed beetle (*Acanthoscelides obtectus*). One of them, MG3b mitotype, significantly reduces male fertility compared to other mitotypes when expressed in the same nuclear environment, but has no negative effects on females. In this study, we tested whether the MG3b mitotype affects OXPHOS functioning in males compared with other, control mitotypes. Specifically, we expressed MG3b and two control mitotypes alongside the same nuclear background and measured mitochondrial respiration in males using high-resolution respirometry. Our results show that respiration linked to all OXPHOS complexes is significantly reduced in MG3b males compared with controls. This is particularly true for the complex IV, which shows mean reduction in activity of around 40%. This work provides evidence that the decrease in mitochondrial respiration is the cause of subfertility of MG3b males.

**Keywords:** the Mother's Curse, *Acanthoscelides obtectus*, mitochondrial respiration, mitochondrial genotype

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## OC032. Finding the Y: a step towards improving the assemblies of Y chromosomes

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While new insect genome projects are helping resolve most of the genomic content from ecologically and economically important species, reconstructing the Y/W chromosome sequence is challenging. Sex-limited chromosomes are crucial for understanding insect development, sexual conflict, reproduction and speciation-related events which depend on the full ascertainment of genome sequence including the Y. In heteromorphic sex chromosome pairs, the Y typically degenerates into a highly repetitive and heterochromatic sequence which confounds genome project pipelines. As a result, current research often relies on genome sequences where the Y is misassembled or missing. The classical Chromosome Quotient (CQ) method can help identify sex-limited regions but depends on high sequencing depth and computationally demanding whole genome alignments. Heuristics that can overcome these requirements will be needed if we are to reconstruct the Y chromosome in multiple species efficiently. Here we explore the Y chromosome content of Tephritid fruit flies after developing two Y-detection algorithms: a classical CQ and an alignment-free approach. We benchmark these against manually curated and PCR-validated Y contigs of *Bactrocera oleae*, *Ceratitis capitata* and *Drosophila melanogaster*. We find that the kmer method outperformed the CQ approach, could better identify misassembled Y regions, and required significantly fewer computational resources. We further outline why the manual curation of computational data is crucial and required while working with genome projects from non-model species.

**Keywords:** evolution, sex-chromosomes, Y-chromosome, comparative genomics, Tephritidae, computational biology

## OC033. Approaching the mechanistic basis of Disease Tolerance in *Drosophila melanogaster*

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Immune response against infections can be divided into mechanisms of Resistance that ensure active pathogen elimination, and of Disease Tolerance, which include host-tissue protection and repair processes that will return the host to physiological homeostasis. Studies aiming at understanding host responses to infection have mostly targeted mechanisms of Resistance, and consequently, these are now fairly well described. in contrast, the mechanistic basis of Disease Tolerance is poorly understood, in part because both mechanisms interact and are, often, difficult to separate. To tease apart these components, we have developed a protocol of oral exposure of *Drosophila melanogaster* to inactivated *Pseudomonas entomophila* bacteria, hence, minimizing the Resistance component of the host response. To get to the mechanisms underlying variation in host Disease Tolerance, we have applied this protocol to ~200 *D. melanogaster* isogenic lines (DGRP) and measured fitness traits (survival and reproduction). We observed considerable host genetic variation for both traits but a weak correlation with one another. Variation in these traits was also sexually dimorphic and varied with the pathogenicity of the bacterium species used. Upon GWAS, followed by functional validation of candidate genes, we sought to identify the genetic basis for Disease Tolerance. Finally, we contrasted our results to two more datasets, one where hosts were exposed to non-virulent bacterial strains, and another where host mutants were incapable of mounting immune-responses. These comparisons helped disentangle the effects and mechanisms of Disease Tolerance into its immunopathology and infection-induced damage components contributing to the understanding of this arm of the immune response.

**Keywords:** *Drosophila* immunity, disease tolerance, Immunopathology, GWAS

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## OC035. Lipid metabolism dysfunction in the Tsetse Fly following symbiont elimination is linked to altered Kennedy pathway homeostasis

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Lipid metabolism is critical for insect reproduction, especially for species that invest heavily into early developmental stages of their offspring. The role of symbiotic bacteria during this process is unknown but likely essential, especially in the case of obligate microbes that fulfill key biological functions in the host. Using a combined lipidomics, functional genomics and biochemical strategy, we examined the role of lipid metabolism in the interaction between the viviparous tsetse fly (Glossina morsitans morsitans) and its obligate endosymbiotic bacteria (Wigglesworthia glossinidia) during tsetse pregnancy. We observed increased CTP:phosphocholine cytidylyltransferase (cct1) expression during pregnancy. This gene codes for the enzyme that functions as the rate limiting step in phosphatidylcholine biosynthesis in the Kennedy pathway which is critical for stored lipid metabolism and progeny development. Experimental removal of Wigglesworthia impaired lipid metabolism via disruption of the Kennedy pathway, yielding obese mothers whose developing progeny ultimately starve. Functional validation via experimental cct1 suppression revealed a phenotype similar to females lacking obligate Wigglesworthia symbionts. These results indicate that, in Glossina, symbiont-derived factors, likely B vitamins, are critical for proper function of both lipid biosynthesis and lipolysis. Loss of the symbiosis has a dramatic impact on Glossina fecundity, and may be broadly applicable to other insect systems, particularly to species that require symbiotic partners to maximize lipolysis and reproductive output.

Keywords: tsetse, symbiosis, lipid, metabolism, viviparity

## OC036. Evolved transcriptional responses and their regulation after long-term adaptation of *Bemisia tabaci* to a marginally-suitable host

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Although generalist insect herbivores can migrate and rapidly adapt to a broad range of suitable host plants, they can face significant difficulties when accidentally migrating to novel and marginally-suitable hosts. What happens, at both the genome-wide regulatory and transcriptional levels, if these marginally-suitable hosts must be used for multiple generations before migration to a suitable host can take place, largely remains unknown. In this study, we established multigenerational colonies of the whitefly *Bemisia tabaci*, a generalist phloem-feeding species, adapted to a marginally-suitable host (habanero pepper) on which the initial survival is 5% or a suitable host (cotton). We used reciprocal host tests to identity the differences in gene expression and to examine the possible role of DNA methylation in their regulation. Our transcriptomic data revealed that most transcriptional changes in the habanero-pepper adapted population (survival increased to above 60%) were changes in two molecular functions: enhanced formation of cuticle structural components and reduced activity of cysteine-type peptidases, mainly cathepsin B proteins that activate plant defenses. Our methylation analyses of the DNA of the differentially expressed genes did not find strong correlation between the expression levels of the genes and the methylation status of their promoters. Further studies are required to identify the molecular regulatory

mechanism/s that control the distinct transcriptional signature of adaptation to habanero-pepper in *B. tabaci* and to test the possible involvement of selection for a subset combination of alleles and complex gene-regulatory networks in the regulation of the process.

**Keywords:** host-plant shifts, DNA methylation, transcriptome, molecular evolution, long-term host adaptation, *Bemisia tabaci*

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## OC037. Identification of the microRNAs involved in the regulation of aestivation in the cabbage stem flea beetle (*Psylliodes chrysocephala*)

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The microRNA (miRNA) pathway has been recognized as an important regulator of gene expression at the post-transcriptional level in many eukaryotic organisms, including insects. The pathway is initiated by the transcription of pri-miRNA, which is first processed into pre-miRNA and then miRNA duplex by RNase III enzymes. Subsequently, the mature miRNA strand is loaded into an RNA-induced silencing complex (RISC), which guides the degradation and translational repression of target mRNAs, mainly by interacting with their 3' UTR regions. Literature abounds with examples of biological processes regulated via the miRNA pathway, including embryogenesis, oogenesis, and even host-pathogen interactions. More recently, this pathway was also suggested to regulate diapause responses in insects, albeit experimental evidence remains scarce. To shed light on this issue, small RNA (18-30 bp) libraries were prepared from pre-aestivation (5-day-old), aestivation (30-day-old), and post-aestivation (55-day-old) *Psylliodes chrysocephala* adults, which is a key pest of oilseed rape plants and obligatorily enters aestivation (summer diapause) during late summer. Using a comprehensive miRNA identification pipeline, we mapped the small RNA sequences to the genome and transcriptome of *P. chrysocephala* to identify and quantify putative miRNAs in the three different adult phases of this pest. Furthermore, we correlated the differentially expressed miRNA data with the results of our previous RNA-seq study on the same stages of this beetle to complement our *in silico* miRNA target prediction. Overall, the study gave the first insights into the potential regulation of *P. chrysocephala* aestivation via the miRNA pathway and its evolutionary history.

**Keywords:** microRNA, small RNA sequencing, cabbage stem flea beetle, aestivation, gene regulation

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## OC038. Interaction between the parasitoid *Cotesia typhae* and its host *Sesamia nonagrioides* studied through transcriptomic and proteomic approaches: insights into virulence and resistance traits

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*Cotesia typhae* is a parasitoid Hymenoptera specialized on *Sesamia nonagrioides*, a lepidopteran stem borer. Both species originate from east Africa, but *S. nonagrioides* colonized the Mediterranean Basin during the last interglacial, more than hundred thousand years ago. There, *S. nonagrioides* has become a crop pest of maize. It is then considered to introduce *C. typhae* in France to regulate *S. nonagrioides* populations through biocontrol. In Kenya, two strains of *C. typhae*, named Kobodo and Makindu (after Kenyan locations sampled), differ in their parasitism success on the French *S. nonagrioides* population (SNF). While both strains score a good parasitism success on the Kenyan host population (SNK), that of Makindu is lower on SNF while that of Kobodo remains high. A QTL approach to investigate this difference of virulence between *C. typhae* strains had yielded 3 locus carrying hundreds of candidate genes, among which genes coding for venom proteins. To confirm their implication, we lead a comparative analysis on female proteomic venom content and abdomen transcriptomes, in order to identify candidate proteins and their related genes. In parallel, we investigated the difference of resistance between the *S. nonagrioides* populations with a comparative transcriptomic analysis of parasitized host larvae hemolymph RNA content, depending on their population origin, on *C. typhae* strain and on the time elapsed after oviposition. Temporal sampling of parasitized larvae was based on a histological study of capsule formation. Together, these analyzes will allow us to refine candidate genes involved in this virulence/resistance interaction between the parasitoid and its host.

**Keywords:** parasitoid wasp, virulence, host resistance, proteomic, transcriptomic

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## OC039. Complex inversion polymorphism landscape in spruce bark beetle

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Single nucleotide polymorphisms have often been markers of choice in studies investigating neutral and adaptive genome-wide variation patterns. However, growing empirical evidence, suggests that structural variants play crucial roles in species evolution and adaptation. In particular, polymorphic inversions seem to be underlying the maintenance of complex phenotypes or facilitate local adaptation in the face of gene flow in many species. Here, we examine genome-wide variation and scan for the presence of polymorphic inversions in the most aggressive European forest pest, spruce bark beetle. We sampled 244 individuals from 18 outbreaking and non-outbreaking populations across species range and identified 25 polymorphic inversions that cover approximately 25% of the species genome. The inversions differ in size, estimated age are polymorphic across species range. Interestingly, several inversions create complexes of adjacent inversions resembling supergene formations. Despite this fact, we found limited evidence of inversions' involvement in local adaptation and their association with complex phenotype (diapause) we investigated. Genotype-environmental association studies indicated no association between inversion genotypes and bioclimatic and land cover variables and only a few inversions show significant, small change in frequency across south-north gradient. Our results showed that spruce bark beetle has one of the most complex genome-wide inversions landscapes, raising questions about prevalence of such complexity in other bark beetle species and their involvement in beetles adaptations.

Keywords: spruce bark beetle, outbreaks, polymorphic inversions

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## OC040. Patterns of genome evolution of the arboviral vector *Aedes aegypti*

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Adaptation to live in close proximity with humans has been recognized as a major evolutionary shift in the arboviral vector *Aedes aegypti*, separating a generalist form, *Aedes aegypti formosus* (Aae), from *Aedes aegypti aegypti* (Aag), which tends to lay eggs in artificial containers and blood-feed on humans. These behaviors enhance the mosquito vectorial capacity. Taking advantage of the geographic distributions of the two forms with Aaf being constrained in Africa while Aaa being global, we compared the genomes of 511 African and 123 out-of Africa *Ae. aegypti* mosquitoes. We first identified a set of more than 300 million high confidence SNPs, then applied population genomics approaches to establish the population genetic structure among our samples, to infer divergence across populations and to identify genomic regions with different evolutionary signals in Aaa vs Aaf mosquitoes. After a comparative analysis, we identified a clear signal of genetic differentiation between Aaa and Aaf, circumscribed to a catalogue of candidate genes. These "*Aaa molecular signature genes*" include chemosensory and immunity genes, which are known to impact host seeking behavior, vector competence and overall response to external stimuli, and extend to genes associated with, mostly, neuronal and hormonal. Besides being usable as markers to genotype Aaa mosquitoes, these results provide the foundation to understand *Ae. aegypti* domestication process.

Keywords: *Aedes aegypti*, population differentiation, domestication, selection, local adaptation, arbovirus

## OC041. Thermal evolution during range expansions toward warmer and cooler regions in the damselfly species *Ischnura elegans*

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Species are expanding their ranges to both warmer and cooler regions, encountering novel thermal regimes to which they have to adapt. Therefore, rapid evolution of upper and lower thermal tolerance in life history and behaviour traits in edge populations can be crucial for the success of range expansions. We reconstructed the thermal performance curves of life history (survival, growth and development rates), physiology (metabolic rates), behaviour (food intake and functional response) and lower and upper thermal tolerance (chill coma recovery time and heat tolerance) during range expansions toward cooler and warmer regions in the aquatic larval stage of the damselfly *Ischnura elegans*. The range expansion to the warmer region in Spain was associated with the evolution of a greater ability to cope with high temperatures, and of faster development and growth rates associated with a transition to a greater voltinism. In the range expansion to the cooler region in northern Europe, the species evolved an improved cold tolerance and faster development and growth rates at the highest rearing temperatures, which were associated with a higher food intake and a lower metabolic rate. Detailed study of the predator-prey interactions using functional response curves revealed that the northern edge region evolved higher long-term predator-prey system stability at higher rearing temperatures. This work provides mechanistic evidence indicating the species can rapidly evolve to adapt to warmer and cooler conditions during range expansions and highlights potential ecological consequences.

**Keywords:** range expansions, rapid evolution, plasticity evolution, life history evolution, thermal evolution, trophic interactions

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## OC042. Interfering with the microRNA pathway in aestivating cabbage stem flea beetle (*Psylliodes chrysocephala*)

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MicroRNAs (miRNAs) are a class of small non-coding RNA molecules ~22 nucleotides in length that modulate gene expression at the post-transcriptional level by guiding target mRNA degradation and translational repression. Hence, the miRNA pathway has been implicated in the regulation of various biological processes, including energy homeostasis, reproduction, metamorphosis, immunity, and diapause. The latter is a dormant state that allows insects to survive anticipated unfavorable conditions, such as extreme temperatures and highly regulated at the transcriptional level. Although recent studies have suggested differential expression of miRNA transcripts in diapausing vs. non-diapausing stages of insects, functional proof of whether the miRNA pathway is playing an important role in diapause initiation and maintenance is lacking. The cabbage stem flea beetle (*Psylliodes chrysocephala*) is a key pest of oilseed rape and a potential model for studying miRNA-diapause interaction as its adult obligatorily enters summer diapause (aestivation). In this study, we first characterized the functions of aestivation and subsequently used RNA interference to reveal the putative roles of miRNA pathway enzymes, including *Drosha* and *Dicer-1*, in this stage. The results showed that the aestivating state provides several advantages over the non-aestivating state including increased resistance to water loss and survival under summer conditions, and the functional analysis of the core miRNA pathway genes opened a perspective for further research on miRNA-diapause interaction in this pest species.

Keywords: cabbage stem flea beetle, Dicer-1, miRNA pathway, summer diapause, aestivation, functional analysis

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## OC043. Novel molecular approaches to study malaria transmission and mosquito-parasite-human interplay

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It is well known that mosquito, parasite and human genetic backgrounds may importantly affect the efficiency of *Plasmodium falciparum* transmission from the human host to the *Anopheles* vector. Transmission and infection studies have been performed so far through demanding *in vivo* and *ex vivo* experiments, with significant logistical, technical and ethical obstacles. The aim of this work is to evaluate the feasibility of studying malaria transmission and vector response to infection exploiting the fact that single infected mosquitoes carry nucleic acids from the three organisms involved in malaria: the human host, the *Anopheles* vector and the parasite *Plasmodium*. To this end we extracted DNA and RNA from single *Anopheles coluzzii* mosquitoes infected with *P. falciparum* by membrane feeding and collected at 12, 18, 24 and 36 hours post infection (i.e., at different stages of ookinete development). RNA was used for RTqPCR expression analysis of 4 selected ookinete markers and confirmed the peak of transcript abundance at 24 hpi, that is during midgut invasion, allowing for the relative quantification of parasite load in individual mosquitoes. On the other side, genomic DNA was successfully used for the identification of selected human genotypes. Next generation sequencing is currently in progress to investigate, in different infection-intensity conditions, both the mosquito immune response (by RNA-seq) and the possible role of the mosquito microbiota (by metagenomics). Finally, our experimental planning involves a validation of the above-mentioned molecular approaches in field collected naturally infected mosquitoes, which is presently undergoing.

Keywords: *An. coluzzii*, *P. falciparum* ookinete markers, malaria transmission, parasite load, mosquito immune response

## OC045. Insights into comparative phylogeography patterns of *Pyrrhalta viburni,* a specialist leaf feeder of *Viburnum* plants and its associated egg parasitoid *Aprostocetus* as part of a biological control program

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A long-standing goal of comparative phylogeography is to determine whether there are common geographic and temporal patterns of genetic diversity and divergence among populations of co-distributed species that have arisen in response to common historical events and processes. By identifying common phylogeography patterns among co-distributed taxa, one can better uncover the underlying effects of geography, species (i.e. traits), and their interactions. Such is the ambition of the present comparative phylogeography of the viburnum leaf beetle (VLB) *Pyrrhalta viburni* (Paykull), (Coleoptera: Chrysomelidae) and its associated egg parasitoid *Aprostocetus* sp.. This work is part of a biological control program in North America where VLB is highly invasive. VLB is a specialist leaf-feeder that attacks shrubs and trees belonging to the genus *Viburnum*. The native range of VLB covers most of Eurasia, but nothing is known of the underlying genetic diversity. Exploration of the assemblage of natural enemies of VLB focusing on egg parasitoids including *Aprostocetus* (Hymenoptera: Eulophidae) started in 2018 in Europe. Overall the diversity of these *Aprostocetus* was poorly documented and in particular of one dominant species *A. suevius /A. celtidis* for which the taxonomy status has also been clarified in the present study. The comparison of multigene phylogeography patterns of the dominant *Aprostocetus* species and of the host VLB across a wide region in Europe is presented here for the first time, emphasizing how the phylogeographic findings could influence the outcomes of a biological control program.

**Keywords:** invasive species, vicariance, specialization, Hymenopteran, Eulophidae

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## OC046. Intraspecific variation and molecular phylogeny of *Scaphoideus titanus* using complete mitogenomes

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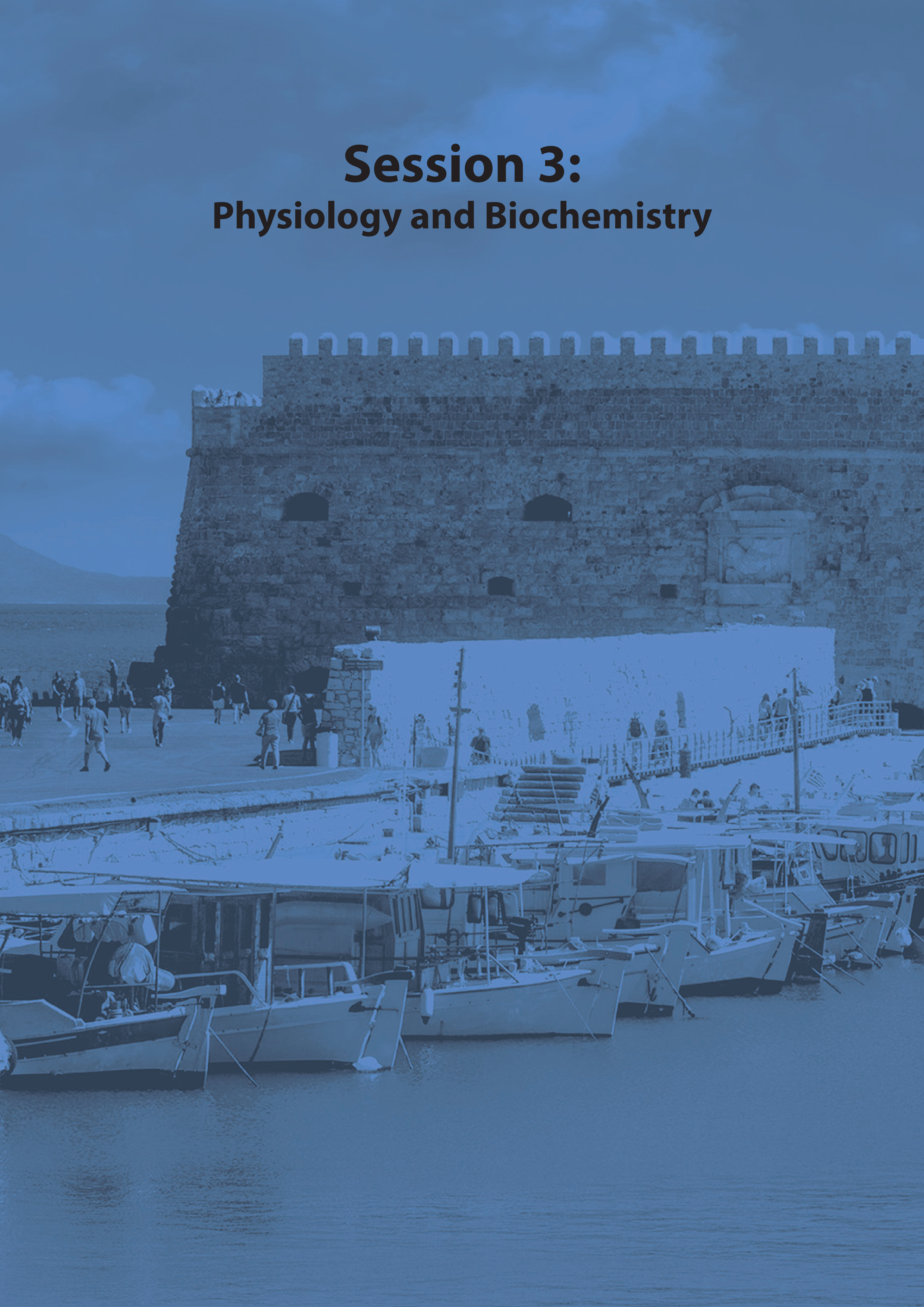
The American grapevine leafhopper *Scaphoideus titanus* (Hemiptera: Cicadellidae) is the main vector of *Candidatus* Phytoplasma vitis the aetiological agent of Flavescence dorée in Europe. This disease belongs to one of the most dangerous grapevine yellows that cause economical losses for grapevine producers. The first occurrence of *S. titanus* in Europe was reported in France in 1960. Afterwards, several populations spread across many European regions with long-lasting viticultural traditions such as Italy, Spain, Portugal and Serbia. Previous studies suggested that *S. titanus* arrived in Europe with a single introduction. However, the use of new marker genes show higher rates of nucleotide substitution that can provide different resolution in evolutionary models. Here, we present the first complete mitochondrial genome of *S. titanus* and related *Scaphoideus* species from Europe and North America, using a hybrid genome assembly approach using short (Illumina) and long (Oxford Nanopore) read technologies. Also, we reconstruct the phylogenetic relationships of these populations and assess the intraspecific variation of mitochondrial genes. Our preliminary results suggest a low variability in genetic subunits like COXI and COXII and a higher variability involving different mitochondrial genes. Moreover, the phylogenetic reconstructions will present a broader understanding of the spatial distribution of the American grapevine leafhopper in the European continent.

Keywords: Phylogenetics, Mitochondrial Genome, leafhopper, *Scaphoideus titanus*

# Session 3:

## Physiology and Biochemistry



## OC047. Silencing FMRFamide-like peptide (FLP) and short neuropeptide F (sNPF) leads to alter lipid metabolism in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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FMRFamide-like peptide (FLP) and short neuropeptide F (sNPF) are FMRFamide-related peptides (FaRPs) with different evolutionary origins. FMRFamide-like peptides are recognized as one of the largest families of neuropeptides taking part in various processes, such as managing reproductive tracts, heart, and other visceral muscle contractility. The short neuropeptide F, an orthologue of the prolactin-releasing peptide of invertebrates, leads to different biological activities, such as feeding, olfaction, locomotion, and sleep homeostasis in insects. In the current study, we focused on the potential physiological role of sNPF (LdsNPF) and FLP (LdFLP) in the lipid metabolism of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), a major defoliator of potatoes (*Solanum tuberosum*). Silencing both *LdFLP* and *LdsNPF* by RNA interference (RNAi) led to noticeable decreases in the total lipid levels of the larvae with disrupted development without weight loss. Use of RNAi, in particular targeting insect neuropeptides, have promising potential in the control of *L. decemlineata*. This research is funded by Oyak Biyoteknoloji.

**Keywords:** FLP, sNPF, Colorado potato beetle, RNAi

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## OC048. Short neuropeptides F regulate feeding related processes in *Tenebrio molitor* beetle

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Short neuropeptides F (sNPF) are pleiotropic neurohormones which are involved in the regulation of various physiological processes. As orthologues of mammalian prolactin releasing peptides (PrP) the major role of sNPF seems to be the control of feeding and energy metabolism. However, thus far the role of these neuropeptides are contradictory where they stimulate or inhibit feeding. In beetles – the biggest insect order, sNPF role in feeding needs to be unraveled. Here, we investigate the effects of sNPF (Trica-sNPF, SGRSPSLRLRFa) injections on different feeding related processes (digestive enzyme activity, food intake) as well as metabolic parameters (free sugar haemolymph) in *Tenebrio molitor* beetle. We searched also for sNPF receptor in the digestive tract of this beetle and measure the myotropic activity of gut muscles after application of synthetic sNPF. Moreover, we measured the sNPF gene expression level in the nervous system of beetles during starvation conditions. We identified the sNPF receptor in the gut of the adult *T. molitor* and showed that synthetic Trica-sNPF increases the food intake and slightly stimulates the gut muscle contractions as well as regulates the activity of various digestive enzymes. The free sugar in haemolymph also increased after injections of synthetic sNPF.

Moreover, the expression level of *snpf* increase during starvation. Overall, we showed that sNPF are involved in the regulation of different feeding related processes.

**Keywords:** short neuropeptide F, feeding, metabolism, beetles, *Tenebrio molitor*

## OC049. Unveiling the neuropeptidome and functions of adipokinetic hormones in Blattodea

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Investigating the neuropeptidome has become a growing field in the realm of insect physiology and behavior. The order Blattodea, comprising the cockroaches and termites, is no exception. Despite the growing interest in this field, only three neuropeptidomes have been characterized in Blattodea to date. Here, we aimed to carry out a comparative analysis of neuropeptides across the major termite lineages, including solitary cockroaches and subsocial wood roaches, focusing particularly on a comparative molecular analysis of adipokinetic hormones (AKH) and their receptors (AKHR). We then investigated the impact of two AKH decapeptides on physiological metabolism of carbohydrates and lipids in the hemolymph of *Blattella germanica*, a widespread pest that poses a serious threat to both public health and the economy in numerous regions globally. Furthermore, the potential role of AKH peptides in mediating immunity, by co-injection peptides together with the Gram-negative bacterial pathogen *Pseudomonas entomophila* was also investigated. Our study represents the first to investigate the evolution of termite neuropeptides and their receptors as well as the metabolic role of AKH and its intersection with blattid host immune function. In addition, our study, provides a fresh perspective on the feasibility of using neuropeptides as potential candidates for biorational pest control agents against invasive termites and cockroaches.

**Keywords:** neuropeptidome, adipokinetic hormone, *Blattella germanica*, adipokinetic hormone receptor, “green” pesticide

## OC050. Tachykinin-related peptides modulate immune system activity of mealworm beetle, *Tenebrio molitor* L

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Tachykinin-related peptides (TRPs) are one of the most important insect neuropeptides. These hormones participate in the regulation of a wide spectrum of physiological processes, such as nociception and lipid metabolism. Our current research also showed that TRPs can be important regulators of insect immune system. Application of Tenmo-TRP-7 (RPKPQQFFGLMa, one of the TRPs identified in *T. molitor*) very often elicits time- and dose-dependent changes in the basic immune parameters of the mealworm beetles. For example, tested TRP influences the adhesion ability of haemocytes and expression level of genes participating in the regulation of cellular response, like gene for cathepsin L-like proteinase. Moreover, performed experiments also suggested anti-apoptotic action of Tenmo-TRP-7. Interestingly, these effects seem to be direct, because transcript characteristic for TRP receptor was found in the *T. molitor* haemocytes. Injection of Tenmo-TRP-7 also strongly affects the humoral response, especially related to phenoloxidase activity, lysozyme-like activity, and expression level of different set of genes involved in the antimicrobial peptide synthesis. Especially at the higher concentration ($10^{-6}$ M), the strong immunostatic effect of Tenmo-TRP-7 was observed. The direct effect of Tenmo-TRP-7 on the humoral response was partially confirmed by usage of potent antagonist of TRP receptor, spantide II. Similarity of the action of insect TRPs and vertebrate tachykinins (TKs) results shed new light on the regulation of the insect immune system by neuropeptides and the functional homology of TK signalling across different animal phyla. This work was partially supported by the grant No. 2021/41/B/NZ9/01054 from the National Science Centre (Poland).

**Keywords:** insect neuropeptides, cellular response, humoral response

## OC051. “Am I overweight?” New insights into Adipokinetic hormone (AKH) pathway in Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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Adipokinetic hormone (AKH) is an insect neuropeptide produced by the corpora cardiaca, and responsible for mobilizing carbohydrates and lipids from the insect fat body. Alteration of the adipokinetic hormone levels leads to negative consequences in the insect body and functions during developmental stages. In the current study, we focused on the role of AKH in insect metabolism, using Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), one of the most harmful pests of Solanaceae plants worldwide. In particular, we first investigated the expression patterns of *L. decemlineata* AKH (LdAKH) in relation to development and diapause, followed by the outcome(s) of synthetic AKH-application (either by injection or feeding) in the beetle lifespan, development, reproduction, vitality; as well as energy metabolism (total triglyceride and trehalose levels). The AKH expression levels increased during diapause and pupation and decreased by molting. The *per os* feeding of the larvae with synthetic AKH led to larval weight loss, retardation in pupal developmental, lowered fecundity, and decrease in trehalose levels. Delivery method (injection or feeding) led to different outcome in triglyceride levels. Overall, strategies targeting AKH pathway might

have great potential in the control of *L. decemlineata*; as well as in the modeling of the human eating disorders such as Anorexia. This research is funded by Oyak Biyoteknoloji.

**Keywords:** AKH, Colorado potato beetle, diapause

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## OC052. Effect of the rearing diet on the gene expression of antimicrobial peptides in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

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The farming of edible insects is an alternative strategy for the production of protein-rich food and feed with a low ecological footprint. In order to preserve the mass rearing systems against the attack of entomopathogens while always ensuring a high quality product for the consumer, it is necessary to implement new strategies to safeguard the health status of insects. The modulation of immune responses through the diet is currently a topic of great interest from an application point of view.

We studied the impact of different agro-industrial by-products (brewers' spent grains, roasted cocoa husks and roasted hazelnut husks) included in the feeding medium on the gene expression level of three antimicrobial peptides (AMPs) (Coleoptericin-1, Cecropin-2 and Tenecin-3) in *Tenebrio molitor* L. larvae. The brewers' spent grains proved to be the most interesting inclusion having resulted in faster larval development, increased larval weight and an upregulation of the three AMPs. Moreover, a higher fold change of the antifungal AMP Tenecin-3 was observed in larvae experimentally infected with *Beauveria bassiana*, when reared on a brewers' grain enriched substrate since the egg eclosion. An increased antimicrobial activity was observed also by diffusion assays in solid medium using hemolymph extracted from the tested larvae.

The possibility of modulating the transcription of AMPs through the diet, and in particular through the inclusion of agro-industrial by-products, is not only a valuable opportunity in order to optimize health status in insect mass rearing, but opens also interesting new perspectives within a circular economy scenario.

**Keywords:** coleoptericin, cecropin, tenecin, brewers' spent grain, immune responses, entomopathogens

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## OC053. Comparative efficacy of a recombinant and chemically-synthesized spider toxin peptide against *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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Peptide-based insecticides, in particular those based on spider toxins, have shown great promise in the control of agricultural pests. Spiders have evolved as natural predators and prey, and have developed certain mechanisms for hunting insects, including use of their toxins that target the nervous system of insects, leading to paralysis and eventual death. Some species of spiders can produce over a thousand unique peptides, which are categorized into different groups, such as salts and small organic compounds, linear cytolytic peptides, disulfide-rich peptide neurotoxins, enzymes, and large presynaptic neurotoxins. Nevertheless, the majority of spider-venom peptides have a mass of 3.0 to 4.5 kDa. In the current study, a 27-amino acid with an approximate molecular weight of 3.0 kDa-neuropeptide belonging to *Oligoctenus ornatus* was chemically synthesized. Subsequently, a recombinant version of this peptide was generated and was purified using His tails with Ni-NTA resin. Both products were tested in terms of their efficacy on *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Our preliminary data indicates that the peptide has promising potential in the control of the beetle and possibly other pests. This research is funded by Oyak Biyoteknoloji.

**Keywords:** recombinant, bioinsecticide, spider peptide toxin

## OC054. Launching engineered prototypes to better understand the factors that influence the click beetle jump

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In nature, click beetles (Coleoptera: Elateridae) use a unique hinge structure between their prothorax and mesothorax that acts as a latch-mediated spring actuation system to produce a high acceleration that can result in a jump. This mechanism enables them to jump a height of several times their body length without using their legs when the beetle is unconstrained. To study the beetle jump trajectory, we designed simplified beetle-inspired prototypes and a launching platform. The simplified prototypes are fundamentally two masses connected by a spring. The masses simulate the portion of a click beetle's body located anteriorly (M1) and posteriorly (M2) to the clicking mechanism, and the spring simulates the elastic energy storage element. The launcher uses a quick-reaction release mechanism and magnetic actuator to simulate the unlatching process. In trajectory analysis, the parameters that are most important are initial velocity at take-off and the take-off angle since both the click beetles and the prototypes are governed by ballistic motion. We determined that morphological features such as elytra (body) curvature and the ratio of the two body masses affect these two dynamic parameters. Our findings provide further insight into the design and fabrication of legless jumping robotic mechanisms and apply engineering models and experimental tools to answer key biological questions.

**Keywords:** bioinspiration, biomimicry, power amplification, biomechanic

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## OC055. Obligate diapause and its termination in a frozen state are essential for the seasonal life cycle of the Antarctic midge

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The Antarctic midge *Belgica antarctica* is the only insect endemic to the Antarctic Peninsula and its offshore islands. This species shows a clear seasonal life cycle. Adults emerge and lay eggs in the early summer, and larvae develop during summer and overwinter twice. Pupation and adult emergence occur at the beginning of the third summer. However, the physiological mechanisms underlying this seasonal life cycle are still veiled. A laboratory experiment revealed that the 1st instar larvae developed to the 4th (final) instar stage. However, 4th instar larvae never pupated, i.e., obligate diapause. Simulated Antarctic winter conditions (-5 °C) successfully broke diapause and larvae resumed development under subsequent warm spring conditions. A long winter exposure is more effective for breaking diapause than a short one. We further investigated the effects of winter larval statuses

(freezing and cryoprotective dehydration [CD]) and winter temperature conditions (-3 and -5 °C) on diapause termination. It has been shown that larvae take either of two strategies for overwintering in Antarctica, i.e., freezing vs. CD. CD is one of the freeze avoidance strategies employed by polar invertebrates. Survival was higher in a group that experienced freezing than in a group that experienced CD at both temperatures, suggesting that the CD is an inadequate strategy for overwintering. We further observed that freezing at -5 °C was most effective for diapause termination. Obligate diapause and its termination in a frozen state are essential for the seasonal life cycle of the Antarctic midge.

Keywords: Antarctic midge, cryoprotective dehydration, freezing, obligate diapause, seasonality

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## OC056. Regulation of *corpora allata* activity: allatoregulary factors and feedbacks

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Juvenile hormones (JHs) are sesquiterpenoids synthesized by the *corpora allata* (CA). They play critical roles during insect development and reproduction. The biosynthetic activity of the CA is regulated by developmental transitions, mating, nutrition, circadian rhythms and additional environmental and physiological factors. In this talk, I will describe new advances in our understanding of the allatoregulatory molecules that mediate the effects of these factors, as well as the existence of a feedback of the circulating JH titer on the biosynthetic activity of the CA. This research was funded by project 22-21244S from the Czech Science Foundation, Czech Republic to MN.

Keywords: juvenile hormone, mosquitoes, neuropeptides, *Aedes aegypti*, *corpora allata*

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## OC057. MicroRNAs from mosquito saliva may contribute to vertebrate host manipulation with potential implications for pathogen transmission

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Mosquito saliva is a complex cocktail of salivary proteins whose main role is to counteract vertebrate physiological responses to tissue injury, namely hemostasis, inflammation and immunity. Genomic, transcriptomic and proteomic studies on mosquito salivary proteins highlighted their fast evolutionary rate and involvement in host manipulation and pathogen transmission. Since metazoan biological fluids are known to carry microRNAs, we analyzed small RNAs from saliva and salivary glands of *Anopheles coluzzii* and *Aedes aegypti* (either uninfected or infected with the chikungunya virus). We found that a specific subset of miRNAs is selectively enriched in mosquito saliva and conserved in the evolutionary distant tick *Ixodes ricinus*, suggesting some underlying non-randomly sorting mechanism. Intriguingly, eleven of the most abundant miRNAs in mosquito saliva mimicked human miRNAs targeting genes

involved in immune and inflammatory pathways, as is the case for miR-7-5p, miR-100-5p, miR-92a-3p or miR-200b-5p, which are known to target the NF-kB, mTOR and TLRs signaling pathways. These observations suggest that miRNAs from mosquito saliva, perhaps enclosed within exosome-like microvesicles and in concerted action with salivary proteins, may contribute to vertebrate host manipulation, with potential implications for pathogen transmission. Using artificial feeding for massive saliva collection we could show by electron microscopy and nanoparticle tracking analysis that mosquito saliva carries exosome-like microvesicles. To get further insights on their composition and function we are currently collecting salivary exosome-like enriched fractions which we plan to use for RNA-seq and proteomic analyses, as well as for functional studies in suitable cultured cells.

Keywords: mosquito saliva, miRNAs, host manipulation, *Anopheles coluzzii*, *Aedes aegypti*

## OC058. A novel olfactory protein of *Anopheles gambiae* involved in the recognition of plant-derived repellents

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In the last decade, OBP-based reverse chemical ecology has emerged as a promising approach for the discovery of novel efficient repellent/attractant molecules. In insects, a large family of small soluble olfactory proteins, the Odorant Binding Proteins (OBPs), mediate the selective transportation of hydrophobic semiochemicals to the olfactory receptors. Interaction of synthetic or natural repellent molecules with selected mosquito OBPs may disrupt their host detection process.

Among the OBPs encoded in the genome of the African malaria vector *Anopheles gambiae*, AgamOBP9 has been reported as the most abundant protein in all chemosensory tissues and developmental stages of both female and male mosquitoes. In contrast to other OBP targets, its expression levels increase dramatically in female antennae after a blood meal, suggesting its possible involvement in detecting energy sources or oviposition sites.

To gain insight into the physiological function of AgamOBP9 and explore its potential as a target for the discovery of mosquito behavioral disruptors, we determined its structure in apo and liganded-forms. Herein, we present the 1.2 Å resolution crystal structures of AgamOBP9 in complex with the plant-based mosquito repellents p-menthane-3,8-diol (PMD) and N-butyl cinnamate, as well as methyl-eugenol which also displays mosquitocidal and attractant activity on herbivores. Furthermore, the binding of these bioactive molecules to the protein was characterized by fluorescence assays and Isothermal Titration Calorimetry. The detailed knowledge of the AgamOBP9-ligand interactions and protein specificity provide valuable information for the structure-based discovery of novel active compounds targeting AgamOBP9 to be used in integrated mosquito control strategies.

Keywords: mosquito, *Anopheles gambiae*, olfaction, OBP9, natural products

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## OC059. Deciphering the regulation of nitrogen and carbon metabolism in *Aedes aegypti* mosquitoes

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Our recent mass spectrometry-based stable-isotope tracer studies demonstrated that glucose contributes to nitrogen waste clearance in *Aedes aegypti* females, vectors of viruses causing serious threats to public health. Our *in vitro* characterization of recombinant *Ae. aegypti* pyruvate kinase (AaPK) revealed that specific amino acids and sugars regulate AaPK conformational dynamics and catalytical changes. Moreover, AaPK protein expression and activity increase in fat body of starved females. To determine whether AaPK could be deacetylated by an $NAD^+$-dependent deacetylase Sirtuin 2 (AaSirt2), we conducted several biochemical and genetic analyses. Transcriptional and protein profiles of AaSirt2 in mosquito tissues indicate that AaSirt2 is regulated in response to sugar or blood feeding. To determine the cellular localization of AaSirt2, we isolated mitochondrial and cytosolic proteins from mosquito tissues and conducted western blotting using an anti-AaSirt2 antibody. We found that AaSirt2 is localized in both mitochondrial and cytosolic fractions. To assess the acetylation status of proteins during nutrient deprivation, western blotting using an anti-acetyl lysine antibody was performed. Several acetylated proteins in mitochondrial and cytosolic fractions of tissues from non-starved and starved mosquitoes were detected. To identify mitochondrial and cytosolic proteins acetylated on lysine residues, we are currently analyzing protein acetylation of immunoprecipitated lysine-acetylated proteins from mitochondrial and cytosolic fractions isolated from non-starved and starved mosquitoes by LC-MS/MS. We have observed that AaPK is less acetylated in starved females and that RNAi-mediated knockdown of AaSirt2 in the fat body of starved females decreases AaPK protein expression indicating that AaPK is a target of AaSirt2.

Keywords: lysine acetylation, protein deacetylation, enzyme regulation, starvation, posttranslational modification, insects

## OC060. Challenging the popular belief, mosquito larvae breath underwater (but pupae do not)

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It is often taken for granted that mosquito larvae only breathe through their siphons when they rest attached to the water surface. In order to establish whether or not immature mosquitoes can survive without contact with the surface and to obtain physiologically relevant amounts of oxygen from water, we analyzed the survival of the last instar-larvae of *Aedes aegypti* fully submerged at different temperatures. We also measured the consumption of oxygen from the air and dissolved in the water, of larvae and pupae of this species under different conditions. When kept submerged in water, larvae survived much longer than expected, some individuals, more than 2 months at 15°C. Individuals kept submerged rarely molted. When remained at the water surface, larvae obtained 12.72% of the oxygen from the water, while pupae obtained only 5.32%. When they were completely immersed, larvae were able to gather 100% of the needed oxygen for surviving in the water while pupae did not. Temperature affected larvae respiration rate both from the air and from the water, with relatively close $Q_{10}$ values. Additionally, while oxygen consumption was not a limiting factor for surviving, molting constituted a critical factor responsible for the death of submerged *A. aegypti* larvae. Our study presents the first quantitative analysis of mosquito larvae respiration in two media, air, and water, unraveling their capacity of breathing underwater. These findings not only challenge the classical idea that mosquito larvae only breathe air, but also the potential effectiveness of control methods based on asphyxiating larvae.

**Keywords:** mosquito larvae, respiration, $Q_{10}$, metabolism

## OC061. Current knowledge and future perspectives on the immune system of black soldier fly

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An emerging technology for the valorization of agri-food waste is the use of the black soldier fly (BSF), *Hermetia illucens*, whose saprophagous larvae can feed on a variety of organic waste and side streams. Since BSF larvae generally grow on decaying organic substrates, they have likely evolved an efficient immune system that provides a robust line of defense against pathogenic microbes and parasites. In this study, we characterized the hemocytes of *H. illucens* larvae and investigated the processes underpinning the immune response to bacterial infection. Moreover, we explored the possibility to manipulate the larval immune response through the diet.

Our data demonstrate that five hemocyte types collaborate in the cell-mediated response in larvae challenged with bacteria. Phagocytosis and encapsulation are rapidly activated after the immune challenge, while humoral components intervene later. The cooperative action of the cellular and humoral branches of the immune system rapidly, and completely, removes Gram-positive and Gram-negative bacteria from the insect, although Gram-positive bacteria persist longer in the hemolymph. Interestingly, the dynamics of these processes is affected by feeding substrates with different nutritional value.

These results provide knowledge on the immune system of *H. illucens*, but also represent a starting point for future studies aimed at modulating the larval immune response by nutritional factors to increase insect resistance to pathogens, thus reducing their negative effects on BSF mass rearing in production plants.

Keywords: *Hermetia illucens*, immune system, hemocytes, waste management

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## OC062. Black soldier fly larvae can efficiently grow on the organic fraction of municipal solid waste thanks to the physiological plasticity of the midgut

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Insect-mediated bioconversion of organic waste and by-products from agri-food supply chains can represent an innovative strategy to valorize low-value biomass and obtain sustainable bioproducts according to circular economy criteria. To this purpose, one of the most promising agents for waste bioconversion is the Black Soldier Fly (BSF) (*Hermetia illucens*; Diptera: Stratiomyidae), whose saprophagous larvae can grow on a wide variety of low-quality organic matter. We are investigating the biotransformation efficiency of the Organic Fraction of Municipal Solid Waste (OFMSW) by BSF larvae with the final aim to obtain biobased materials, such as bioplastics and biodiesel from insect proteins and lipids, respectively. In particular, in this study we evaluated the growth performance of BSF larvae and their efficiency in reducing two substrates with different nutritional content that mimic the composition of OFMSW. Although the growth rate of larvae reared on both substrates was comparable, differences in the reduction of waste and the conversion efficiency into insect body mass were observed. As the midgut plays a fundamental role in the insect-mediated bioconversion processes, we evaluated the digestion capability of BSF larvae reared on the two substrates. Our results showed that the larvae can compensate variations in nutrient composition of the substrate by post-ingestion responses, through the regulation of gene expression and activity of digestive enzymes. Indeed, despite differences in diet composition, we observed similar growth performance and chemical composition of the insect.

**Keywords**: *Hermetia illucens* larvae, waste bioconversion indexes, midgut physiology, digestive enzymes

## OC063. Investigating the role of amylases from *Hermetia illucens* larvae and their substrate in starch digestion

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Black soldier fly larvae (BSFL; *Hermetia illucens*) are increasingly studied for their ability to transform organic substrates into body proteins and lipids that can be used for animal or human nutrition. Carbohydrate content of their diet has been associated with larval growth and fat body content, and it has recently been reported that BSFL had high starch digestion efficiency and that their midgut presented high amylase activity. This study investigated the effect of starch content and type on BSFL amylase activity and starch Estimated Digestibility (ED). BSFL were fed various plant-based diets with different starch content. Both larvae and substrates were sampled after feeding periods of 4, 7 and 11 days, along with initial BSFL and diets. Each sample was ground in phosphate-buffered saline with protease inhibitor and centrifuged to collect water phase. Amylase activity was assessed in each solution via spectrophotometry using soluble starch at pH 6.5 and dinitrosalicylic acid. Values were reported as enzymatic activity units per gram of total soluble protein measured according to Bradford. Western-blot was used to detect BSFL amylases in substrate samples that could contain enzymes from plant material, microbes, or larvae. This approach provides insight into larval amylase regulation mechanisms and the role of extra-oral digestion of starch in BSFL conversion systems.

**Keywords:** insect nutrition, extra-oral digestion, alternative protein, insects as feed, Diptera, digestive enzymes

## OC064. Characterization of a phospholipase A (1)-like protein and its potential role in the lipid metabolism of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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The Colorado potato beetle, *Leptinotarsa decemlineata* is a major pest of Solanacea crops worldwide, causing significant losses by feeding particularly on potato leaves in both larval and adult stages. Hibernation is a key biological feature that enables the insect to survive for up to 5-6 months underground in severe winter conditions. Most of the diapause destined insects accumulate a lot of lipid reserves prior to diapause and sequester most of them during the diapause. One key actor in this process is lipases that are involved in the hydrolysis of the lipids. In the current study, we characterized a gene encoding a phospholipase A(1)-like protein, denoted *L. decemlineata* PLA1 (LdPLA1), and examined its site-specific, developmental and diapause-related expression. *LdPLA1* was expressed in comparable levels in midgut and fat body in larvae, while it was expressed in higher levels in the fat body in adults. The gene was upregulated by starvation and molting compared to feeding in the larvae. Although *LdPLA1* mRNA levels were higher in feeding adults than that of larvae. The gene was upregulated by emergence from diapause only in males. These findings provide new insights into regulation of lipid metabolism through lipases in the Colorado potato beetle.

Keywords: *Leptinotarsa decemlineata*, lipid metabolism, lipase

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## OC065. Environmental rearing condition influence essential fatty acid use in the migratory moth, *Mythimna unipuncta*

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Certain fatty acids are referred to as essential (EFAs), when adult insects cannot synthesise them must be acquired from host plants during larval development. Thus, in species where adult food sources contain little or no EFAs, such as nectivorous Lepidoptera, adult allocation of this limited resource could result in important trade-offs. Migratory lepidopteran species generally do as sexually immature adults, so any EFAs used to sustain long distance flight would not be available for subsequent reproduction. Using the true armyworm, *Mythimna unipuncta,* we tested the hypothesis that environmental cues influence the use of ESAs during flight. When sugar water was available daily there was no change in the amount of EFAs in the fat bodies of moths reared under fall (migratory) conditions when force flown 8h for five consecutive days, while there was a significant decline in those reared under summer (non-migratory) conditions. However, when fall moths were not provided food on a regular basis EFAs were not conserved. Our findings support the hypothesis that environmental cues responsible for the onset of migratory flight result in physiological changes that modifies lipid use to conserve EFAs, while underlining the importance of nectar availability at stopover sites during migration.

**Keywords:** migratory flight, abiotic conditions

## OC066. New Target Sites in the Control of *Myzus persicae* (Hemiptera: Aphididae) by RNAi: Facilitated Glucose Transporter Member 1-Like (FTG1) and Protein Transport Protein (Sec23A)

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Aphids are serious pests of many vegetables as they deliver the phytotoxic compounds in their saliva to the plants and are also responsible for the transmission of plant viruses. They have been controlled for decades using broad-spectrum insecticides, but these chemicals have resulted in increased resistance and adverse impacts on non-target species. RNA interference (RNAi) technology offers species-specific control of pests by double-stranded RNA (dsRNA)-mediated knockdown of targeted essential genes. In this study, two different genes, one encoding a facilitated glucose transporter member 1-like (FTG1), another one encoding Protein Transport Protein (Sec23A) were evaluated in terms of their silencing potential and related outcome. FTG1 is a key gene that enables D-glucose transmembrane transporter activity and dehydroascorbic acid transmembrane transporter activity. In addition, the Coat protein complex II (COPII) is a multi-subunit protein complex responsible for the formation of membrane vesicles at the endoplasmic reticulum. This protein is encoded by a member of the SEC23A. Target genes were isolated by PCR, cloned, and related-dsRNAs were prepared using *in vitro* transcription. The dsRNAs were then delivered topically to knock down target genes and the silencing effects were evaluated in terms of aphid development and survival. Our preliminary data indicates that silencing both genes have lethal outcomes, therefore reveal great potential as a futuristic control method against this pest. This research is funded by Hektaş.

**Keywords:** aphid, RNAi, FTG1, COPII, SEC23A

## OC067. Role of muscarinic receptor in regulation of metabolic activity of insect fat body

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Muscarinic cholinergic receptors (mAChRs) belong to wide group of metabotropic receptors and play important role in cholinergic transmission in the central and peripheral nervous system. They are involved in regulation of many physiological processes, such as the contractile activity of smooth muscles, activity of the heart, secretion of digestive enzymes, metabolism and functioning of the immune system. Their physiological role is quite well known in vertebrates.In insects, three types of mAChRs were identified. The available data suggest that they take part in regulation of flight and visceral muscles contractility, behaviour, memory, and learning. There are also some suggestions, that they tune metabolism. Nevertheless, the information is very scanty.The aim of the studies was to determine the role of muscarinic receptors in the regulation of the activity of the main metabolic pathways in the fat body of *Tenebrio molitor* beetle on the enzyme activity and the level of metabolites. The activity of mAChRs was modulated by application of agonist (carbachol and pilocarpine) and antagonist (atropine and scopolamine). We analysed the activity after 2 and 24 h of incubation.We observed changes in activity of enzymes such phosphofructokinase, 3-hydroxyacyl-CoA dehydrogenase, citrate synthase, and lactic dehydrogenase. Moreover, we noticed changes in the level of free carbohydrates and proline in the haemolymph. Obtained data indicate that muscarinic signalization is an important player in regulation of insect metabolism.The studies were supported by Excellence Initiative - Research University, project N$^o$ 037/02/POB2/0001.

Keywords: muscarinic receptors, insects, metabolism, carbohydrates

# Session 4:

## Ecology and Behavior

## OC068. Molecular basis of olfactory-driven behaviours of *Culicoides imicola*

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*Culicoides imicola* (Diptera: Ceratopogonidae) is a successful biting midge, infamous for its role as a biological vector for numerous pathogens of veterinary importance. Like in most insects, olfaction is likely to play a central role in the long-range attraction of *Culicoides* towards hosts and oviposition sites. However, studies of their sensory apparatus are scarce, and the vast majority of semiochemicals used by *Culicoides* are unknown. Our tanscriptome analysis shows that *C. imicola* exhibits many receptor gene families and provided for the first time evidence for molecular mechanisms mediating the detection of odors such as CO2, 1-octen-3-ol, Indoles, and lactic acid. Using behavioral experiments, we established *C. imicola's* attractiveness to 1-octen-3-ol and lactic acid, for which we have identified corresponding olfactory receptors.

We also investigated *C. imicola's* preference for oviposition sites. Comparing *C. imicola's* attractiveness to three different mammal fecal sources indicated that *C. imicola* prefers to lay its eggs in ovine and equine feces over bovine. Moreover, we have identified candidate bacterial species that emit microbial volatile organic compounds that may signal "suitable oviposition sites" to *C. imicola*.

This novel, basic information on the mechanisms governing *C. imicola* attraction to nutritional sources and oviposition sites may find its way into several applications, such as new monitoring and/or bio-control strategies to destabilize these notorious veterinary pests.

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## OC069. Function and regulation of insect odorant receptors

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The majority of insect olfactory receptors belong to two distinct protein families, the ionotropic receptors (IRs) which are related to the ionotropic glutamate receptor family, and the odorant receptors (ORs) which evolved from the gustatory receptor family. This talk is focused on the functional properties of ORs, and how their performance can adapt to meet the environmental and ecological challenges that insects face while exploring their odor worlds. ORs form heteromeric ligand-gated cation channels composed of odor-specific OR proteins and a co-receptor protein (Orco). OR proteins display a 7-transmembrane topology as G protein coupled receptors, but they are inversely inserted into the membrane. ORs are evolutionarily young receptors, they first appear in winged insects and seem to be evolved to allow an insect to follow sparse odor tracks during flight. In contrast to IRs, the ORs can be sensitized by repeated subthreshold odor stimulation. This talk presents a current view on regulatory processes affecting the performance of ORs and proposes a model of mechanisms contributing to OR sensitization.

**Keywords:** chemoreception, olfaction, odorant receptor, intracellular signaling

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## OC072. Reverse chemical ecology in a moth: identification of new behaviorally active semiochemicals in the cotton leafworm

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Odorant receptors (ORs) are transmembrane proteins expressed in animal olfactory sensory neurons. They are at the core of odorant detection since they recognize odorants and trigger a neuronal response that will be transmitted to the central nervous system. However, most of these ORs are still orphans, which means the odorants that activate them are unknown. The so-called "reverse chemical ecology" or "molecular chemical ecology" approaches propose to use OR-ligand and/or OR-sequence characteristics to identify potential new ligands via a combination of modelling and experimentation, which have the potential to accelerate the discovery of new ligands. Using the crop pest moth *Spodoptera littoralis* (Lepidoptera; Noctuidae), we used such approaches for the study of insect ORs. Ligand-based virtual screening coupled to experimental validation led us to extend the range of semiochemicals active at the receptor and the behavioural levels.
Our work opens new routes for i) odorant receptor function analysis, ii) a better understanding of this species odor space, and iii) the development of novel insect pest control strategies targeting chemosensory receptors.

Keywords: odorant receptor, reverse chemical ecology, machine learning, insect behaviour, crop protection, *Spodoptera littoralis*

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## OC073. A biosensor powered by a mosquito odorant receptor

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In the past decade, the growing need of high-throughput, cost-effective and user-friendly chemical sensing devices aided by the advances in polymer development have fueled the demand for biosensors. A biosensor consists of a biorecognition element (e.g., enzyme, antibody, DNA, and aptamers) for the recognition and binding of a target analyte, and an associated transducer that converts a binding event to a measurable electric signal. The ideal biorecognition element exhibits high sensitivity and selectivity towards any analyte of interest which has a practical use in industry or as a consumer product in the medical, agricultural and food industries . The structure and molecular mode of action of insect odorant receptors (ORs) position these proteins as promising biorecognition elements to be integrated in a biosensor, mainly due the direct link between a specific and sensitive binding event and the immediate ion flow through the ORco channel. The sensitivity and selectivity of insect ORs is best exemplified by the mosquito OR9, which is activated by 3-methylindole in the parts per trillion range. Carbon nanotubes (CNT) can easily form a conducting channel in a field effect transistor (FET). Moreover, their

biocompatible all carbon structure allows for covalent tethering of biological elements and due to their remarkable electrical conductivity and dimensions, CNTs can measure variation of the electric field induced by odorant binding events. These CNT-FET coupled with insect ORs represent the next-generation transducers for super sensitive and selective biosensors.

**Keywords:** biosensor; carbon nanotube field effect transistor (CNT-FET), odorant receptor (OR)

## OC074. Effects of two herbicides and a medical drug, alone or in mixtures, on learning in *Aedes aegypti* larvae

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Chemical micropollutants can affect cognitive and behavioural abilities in aquatic organisms. We investigated the effect of two herbicides (glyphosate and atrazine) and a medical drug (paracetamol) on mosquito larvae (*Aedes aegypti*) locomotor, sensory and learning abilities. A shadow projected on the water surface induces naïve larvae diving to escape from a potential danger. After repeated presentations of the shadow (an innocuous visual stimulus) the escape response decreases due to habituation, a non-associative form of learning.

Mosquito larvae were reared in different concentrations of glyphosate, atrazine or paracetamol (acetaminophen). Chemicals were dissolved either alone or in a mixture. Using an original fully automated apparatus, we recorded individual responses over the course of 10 presentations (trials) of a visual stimulus (a card-board square projecting its shadow); inter-trial interval was 2 min. Each animal's response was video-recorded and analysed by means of a tracking algorithm.

We observed deleterious effects on habituation in larvae reared with glyphosate (min. 50 µg/l), and atrazine (min. 500 µg/l). No effects were observed in larvae reared with paracetamol. Deleterious *cocktail effects* were observed in larvae reared in a mixture of paracetamol (1 mg/l) and atrazine (200 mg/l).

Thanks to an easy-to-use non-invasive bioassay, we could accurately quantify the behavioural responses. This approach allowed evincing *cocktail effects*, not observable if only chemical analyses had been applied. We propose observing changes in the behaviour of mosquito larvae as a proxy to evaluate the quality of aquatic environments submitted to anthropic influence.

**Keywords:** cognition, paracetamol, mosquito, glyphosate, atrazine, cocktail effect

## OC075. Why are insects virtually absent from the open sea?

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Insects are considered the most ecologically and evolutionarily successful group of animals, surpassing the rest in terms of biomass, diversity, and ability to colonize almost any environment. However, while these statements are appropriate as far as the mainland environment is concerned, insects are virtually absent from the oceans, in other words, excluded from 99% of our planet's biosphere. This fact has intrigued biologists for a long time and a variety of hypotheses have been proposed to try to explain it. Only a few species are present in the sea, most of them remaining at the surface, salt marshes, estuaries, or shallow waters. Remarkably, a group of sucking lice, ectoparasites of amphibious hosts, such as pinnipeds and otters, endure extreme oceanic conditions during long periods of their life. During the evolutionary transition of pinnipeds from land to the ocean, seal lice had adapted to manage the amphibian biology of their hosts, some of which may spend more than 80% of the time in the open sea, performing extreme dives, reaching depths beyond 2000m under the surface. These obligated and permanent ectoparasites have adapted to tolerate hypoxia, high salinity, low temperature, and, in particular, to endure extremely high hydrostatic pressure. We will discuss how understanding the biology of these species can teach us something about the potential capacity of insects to survive in the oceans, challenging some of the popular hypotheses that have been proposed to explain the absence of insects in the open sea.

**Keywords:** marine insects, co-evolution, adaptation, extreme environment

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## OC076. What is the secret of success of the invasive brown widow spider *Latrodectus geometricus*?

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Invasive species are those that invade new regions, often as a result of human action, and cause damage or threaten the ecological system. Invasive species may possess traits that promote their invasiveness, such as high productivity, rapid development, and high dispersal ability. In addition, they may be less susceptible to natural enemies such as predators and parasites. The brown widow spider *Latrodectus geometricus*, is a highly invasive species globally, including in Israel. One of the main natural enemies of the brown widow spider, as well as of other widow spider species, is the parasitoid wasp *Philolema latrodecti*, which lays its eggs inside the egg sac of the spider. The wasp larvae feed on the spider eggs, pupate inside the egg sac, and emerge as adults. In the current study, we found that the brown widow spider is less susceptible to parasitism by this wasp than the white widow spider, *L. pallidus*, a native species in Israel, under both field and lab conditions. Possible explanations include the preference of *L. geometricus* for human habitats where there might be fewer parasitoids, and better egg sac defenses by the female spider. Specifically, females of the brown widow spider construct spike-like silk structures that may function as a defensive layer around their egg sacs. In addition, they defend their nest more actively against the parasitoid. I will present results from several field and lab experiments supporting

these hypotheses. Such knowledge can aid in combating this, as well as other invasive species, around the world.

**Keywords:** invasive species, *Latrodectus geometricus*, *Latrodectus pallidus,* egg sac parasitoid, *Philolema latrodecti,* egg sac defenses

## OC077. Larval behavior as an adaptive strategy to agricultural practices in European and Asian corn borers: adaptation or pre-adaptation?

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Phytophagous moths in genus *Ostrinia* have produced, following the introduction of corn in Europe and Asia, two of the most important pests affecting maize production: the European corn borer (*O. nubilalis*) and the Asian corn borer (*O. furnacalis*). Compared to their close relatives that feed mostly on wild Dicot plants, these two species present a set of traits that provide them with superior performance on maize plants. After quickly reviewing the physiological and behavioral bases of this adaptation, we will focus on one original adaptive trait: positive geotaxis of late larval instars. We will show how this larval trait, absent from the closely related species *O. scapulalis*, provides the two pest species a major selective advantage in agricultural crops. Indeed, positive geotaxis strongly reduces fall mortality caused by maize harvesting. I will discuss whether this adaptive trait is best seen as an adaptation to human agricultural practices that evolved following the host-shift, or as a preadaptation initially driven by other selective forces, unrelated to human harvesting. We will focus in particular on the potential role of geotactic behavior on pathogen and parasitoid-related mortality. We will conclude by presenting our current research aiming at elucidating the genetic basis of this adaptation.

**Keywords:** behavior, corn borers, pests, adaptation, human-driven evolution

## OC078. Population dynamics of a socially behaving pest insect facing environmental changes

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Climate change is expected to increase the abundance and frequency of outbreaks among forest pest insects especially in the Northern Hemisphere. For example, higher temperatures and longer growth seasons may result in changes in species distribution ranges, phenology and abundances. This can further shape the local biotic interactions under which forest pest insect evolve. To reliably predict how the probability of pest outbreaks changes under varying environmental conditions, we need to construct realistic population dynamic models that account for the ecology of forest insects such as their social behaviour or antipredator defence strategy. For example, many pest insects have chemical defence strategies and are social in their behaviour, but these aspects are rarely considered in population models. As a first step to investigate how ecology of the forest pest insect is considered, we performed a literature survey, where we went through 44 population dynamic models for pest insect species in forest and agricultural systems, as well as invasive potential. We found that only small proportion of these models considered the sociality, sex ratios, antipredator defence strategies and heritability of adaptive traits in their respective study systems. We are currently running the simulations where we test how variation in sex-ratio of populations or antipredator defence strategies change the outcomes of forest pest insect dynamic models. We will discuss the outcomes of these models and how information about biotic interactions affects their explanatory power.

**Keywords:** modelling, hymenoptera, outbreaks, environment, anti-predator

## OC079. Behavioural and neural variability: do invasive ants need it to solve problems?

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Biological invasions currently pose one of the major threats to ecology and economics, but they also offer valuable insights into evolutionary processes in a short time because species must adapt to solve unfamiliar problems in the introduced environment. Problem-solving is usually addressed by behavioural plasticity, but it was proposed that in organisms such as ants, the costs of individual plasticity might outweigh its benefits. We analysed behavioural and neuroanatomical variability in one of the most widespread ant species, the Argentine ant (*Linepithema humile*), to unravel if the colonies cope with environmental problems by behavioural differences among individuals or/and because of within individual behavioural and neuronal plasticity. We exposed 173 age-controlled workers from five different colonies to sets of three behavioural tests conducted daily over five days. We tested ants' exploration, neophobia (encounter with an unfamiliar object) and maze-solving under laboratory conditions to analyse the mechanisms underlying problem-solving capability in a novel environment. Repeated behavioural testing allowed us to analyse personality, predictability, repeatability, memory, and learning. We analysed the correlation among behavioural variability, cognitive development and micro-glomerular connections of mushroom bodies, the brain areas in charge of processing visual and mechanosensory information. The results we will present are a first step towards unravelling the basis of how introduced social insects cope with new environments, the key to understanding how they succeed to become invaders and how they will respond to the effects of climate change.

**Keywords**: decision-making, experience, *Linepithema humile*, behavioural patterns, personality, supercolony

## OC080. Divergence and aggressiveness within a supercolony

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The invasive potential of the Argentine ant (*Linepithema humile*) has likely been increasing because they show low intraspecific competition in the introduced areas. However, multiple introductions over time or genetic divergence could increase intraspecific competition. We studied the genetic and behavioural variability of *L. humile* workers collected at six locations on the NW coast of the Iberian Peninsula, a possible scenario for multiple introductions and population divergence, due to its high level of maritime traffic and complex coastal geography. We analysed behaviours related to spatial navigation (exploration, wall-following), resource acquisition, and competition (inter and intraspecific aggressiveness) in spring and autumn. Genetic analyses using microsatellites indicated that the nests studied belonged to the most spread supercolony in South Europe. However, we identified the existence of two genetically differentiated clusters. Lethal interactions were found between workers from different and similar genetic clusters, but a trend suggests higher agonistic behaviours between the two genetic groups. Genetic differences were positively correlated with geographical distance, but aggressiveness was not correlated with any of them. Ants from all nests showed more exploration and aggressiveness, less wall-following and faster detection of food in autumn than in spring, with no intraspecific aggressiveness observed in spring. Our findings suggest competition between nests of the same supercolony and behavioural seasonal variability, supporting the hypothesis of divergent evolutionary processes. The results of our work question the assumed unity of supercolonies of this species and offer insights for understanding the future adaptation of *L. humile* in the introduced areas.

Keywords: intraspecific competition, population genetics, subcolony, unicoloniality, variability

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## OC081. Sib-mating enhances fitness in a haplodiploid beetle

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Inbreeding is generally avoided in animals due to the risk of inbreeding depression resulting from an increase in homozygous deleterious alleles and loss of heterozygosity. Species that regularly inbreed pose a challenge to understanding the effects on fitness of these risks. We investigated fitness consequences of extended inbreeding in the haplodiploid date-stone beetle, *Coccotrypes dactyliperda*. We hypothesized that persistent inbreeding would result in inbreeding depression while outbreeding would either increase fitness due to heterosis or lower fitness if co-adapted gene complexes are disrupted. We established three breeding treatments with beetles from two geographically separated populations: Sib-mating, outbreeding within the local population, and between-population outbreeding. Between-population outbreeding groups of both populations had reduced fecundity and collapsed before the experiment ended, while sib-mated groups persisted for ten generations and the females had higher fecundity than both outbreeding groups. Average inbreeding coefficients of sib-mated groups were higher than the other treatment groups, yet sib-mated beetles remained genetically polymorphic. Thus, there was no evidence of inbreeding depression, while outbreeding between distant populations led to outbreeding depression. Our findings are consistent with the life history of *C. dactyliperda*, in which sib-mating predominates within the date seed, but occasional within-population outbreeding likely occurs following local dispersal.

Keywords: date-stone beetle, inbreeding, fecundity

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## OC082. Mate-finding and mating disruption in the invasive fruit fly *Drosophila suzukii*

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Individuals may fail to find and attract mates when population density is low. This demographic phenomenon known as the “mate-finding Allee effect” has repercussions on reproductive fitness and may doom the population to extinction. Furthermore, the disruption of the signals used for mate search can strengthen this Allee effect. In this work, we investigated the Allee effect on the reproductive success of an agricultural pest, *Drosophila suzukii*. In a preliminary experiment, we first verified that the density of adult flies around fruits can vary from low to high. Then, we experimentally tested the effect of different male densities on mating success with constant and variable sex-ratio. The constant sex-ratio allows mimicking a constant intensity of male-male competition irrespective of the density, while variable sex-ratio simulate variable intensities of competition. We quantified mating success through different metrics: latency before mating, copulation time and mating occurrences. At low male densities, we found a decrease in mating success and a longer latency time, but copulation duration was not affected. In another set of experiments, we determined what social information or cues were used to estimate density. We recorded the frequency of male fighting (physical interactions to access females) and tested whether males used visual, chemicals (or both) cues to estimate the density. As expected, we observed that males’ competition through physical interactions was stronger when the density increased. We clearly showed that chemicals and visual from high density of flies can disrupt the mating probability.

Keywords: Allee-effects, males’ density, *Drosophila suzukii*, mating-failure

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## OC083. Is polyandry an important adaptive trait of *Drosophila suzukii?*

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*Drosophila suzukii* Matsumura, the Spotted Wing *Drosophila*, is a highly invasive fly that recently entered the fruit fly scene as a very dangerous pest. It is a destructive fly of most small fruits including cherries. Originating in Southern Asia it spread across most regions of Asia and invaded Europe and the Americas during the last thirteen years. Within the *melanogaster* group it represents a striking case of peculiar ecological, physiological, behavioural adaptations and genetic innovations which have resulted in a plasticity that is one of the keys of its success. From the evolutionary/ecological points of view *D. suzukii* speciated by adapting to cooler, mountainous and forest environments in temperate regions. It evolved seasonal polyphenism traits that enhance its survival under stressful conditions. Consequently, the populations undergo seasonal reproductive dynamics. Whether *D. suzukii* displays polyandrous behaviour and whether such a reproductive strategy helps it to overcome the seasonal bottleneck is an open question. We explored this hypothesis in a *D. suzukii* population established in a Northern Italian region during its post winter demographic boost leading to the abundant summer population. We found that the demographic explosion of the post-winter population is associated with a high degree of polyandry and shared paternity. We suggest that polyandry may be an additional evolutionary adaptive trait of *D. suzukii*. Indeed, it reinforces its physiological and behavioural plasticity.

Keywords: *Drosophila suzukii*, invasive species, polyandry, sperm use

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## OC084. Weather based prediction module: A promising tool for *Spodoptera litura* anticipation in groundnut?

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*Spodoptera litura* has been causing considerable damage to groundnut and other crops across India. An anticipation of the pest incidence using prediction module under changing climate is need of the hour. The *Spodoptera litura* male moth catches by sex pheromone trap from 1991-20 (30 years) during *kharif* season (rainy season from June to October) is considered for the study. The 30 years data (Main Agricultural Research Station Dharwad) clearly indicated that moths trapped were concentrated in 34th and 35th standard meteorological week(SMW), which falls during second fortnight of August and first week of September. There was steep decline in the number of moths trapped from 2015 onwards indicating consistent decrease in *Spodoptera* incidence during *kharif* at Dharwad despite of normal rainfall and other weather factors. The multiple regression model fitted was having multicollinearity of independent variables. Stepwise regression, Morning relative humidity(RH) was found to be the major deciding factor of *Spodoptera* population. Finally, simple linear regression model was fitted and the predicted trap catch data was validated with actuals for four consecutive *kharif* seasons starting from 2017. However, predicted was found to be far higher than actual number of moths trapped during respective years. Present analysis clearly indicated need for better understanding the influence of biotic and abiotic factors on *Spodoptera* under field condition.

## OC086. Fitness costs in the presence and absence of insecticide use explains abundance of two common *Aedes aegypti* knockdown resistance alleles found in the Americas

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*Aedes aegypti* is the vector of viruses that have a critical impact in human health. Control of adult mosquitoes is widely done using pyrethroids, but resistance has reduced the effectiveness of this class of insecticides. Resistance to pyrethroids in mosquitoes is commonly due to mutations in the *voltage-gated sodium channel* (*Vgsc)* gene (these mutations are known as *knockdown resistance*, *kdr*). In the Americas and the Caribbean, the most common *kdr* alleles are *410L+1016I+1534C* and *1534C*. We conducted a population cage experiment to evaluate the changes in the allele and genotype frequencies of the *410L+1016I+1534C* allele by crossing two pyrethroid-susceptible congenic strain but carrying the *410L+1016I+1534C* and 1534C alleles. Changes in allele frequencies were measured over 10 generations in the absence of deltamethrin exposure. We also applied a cycle of selection with deltamethrin at $F_9$ to evaluate the changes in allele and genotype frequencies. We also evaluated if the changes in genotype frequencies were due to fitness costs (out of Hardy-Weinberg Equilibrium) or genetic drift. Our findings suggest that fitness costs were higher with the *410L+1016I+1534C* allele, relative to the *1534C* allele, in the absence of deltamethrin exposure, but this allele provides a stronger advantage when exposed to deltamethrin relative to the *1534C* allele. Changes in genotype frequencies were not in Hardy-Weinberg equilibrium nor genetic drift which indicates the mosquito populations tested did not follow the assumptions of the Hardy-Weinberg Equilibrium. Our results suggest a likely reason for the variations in frequencies between the *410L+1016I+1534C* and *1534C* alleles in field populations.

Keywords: *Aedes aegypti*, fitness cost, knockdown resistance, Hardy-Weinberg equilibrium

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## OC087. Characterization of swarming behaviour in *Anopheles coluzzii* mosquitoes using 3D videotracking

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Malaria is the vector-borne disease with the highest burden worldwide, responsible for causing the death of over 600.000 people in 2021 (World Health Organization, 2020). Mosquitoes of the Anopheles genus are the primary disease vectors and mate in large complex swarms consisting of several male and female mosquitoes aggregating over a marker structure. It is throughout the extent of a swarming event that males and females form a copula and fly out of the swarm complex for mating. Furthermore, mating mechanisms and sex-specific performance comprise key information for understanding the behaviour of mosquitoes. However, the characterization of swarms has been performed based primarily on visual observations of field events and thus potentially subjected to an observer bias. In our study, we incorporate the use of the Photonic Fence Monitoring Device (Photonic Sentry, USA) to record laboratory simulated swarm events. Through acquiring positional data, we were able to describe various spatio-temporal aspects of male, female, and mixed mosquito swarms. With our results we are able to characterize the different structural layers of a swarm, their average height from floor and various activity peaks present throughout a swarm event. Moreover, our results suggest that contrary to the general idea of female mosquitoes acting as reactive individuals to male activity, they can form stable and comparable swarms to those of only males or mixed sex. This unique discovery poses several questions regarding the purpose of female swarming activity, opening the doors for a wide range of studies of the male/female mating interplay.

Keywords: *Anopheles*, swarming, mating, malaria, behaviour, mechanisms

## OC088. Some co-evolutionary process at work between egg parasitoids and their host(s): case of the genus *Trissolcus*

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The case study "Pentatomidae – Egg Parasitoids" has become an important issue in agriculture due to the re-emergence of species *e.g. Nezara viridula*, as well as the arrival of exotic species *e.g. Halyomorpha halys*, which are formidable pests of many crops. Consequently, a large consortium comprising academic researchers, R&D professionals, and agronomic partners has been formed to develop strategies for biocontrol using egg parasitoids (Hymenoptera) with a multi-annual perspective. These parasitoids could be local or imported from the area of origin of the pests. From an academic perspective, research on the ecology of the different protagonists is ongoing. The research aims to address the study of the variability of virulence at the inter- and intra-specific scales and the causes of this variability (genetic or plastic) in parasitoids. There are very few studies on the origin of this variability and almost none on the physiological mechanisms and virulence factors involved during parasitism by these oophagous parasitoids. Therefore, in parallel with experimental evolution studies, we started the transcriptome from the reproductive tract of three main species: *Trissolcus basalis*, *T. cultratus*, and *T. mitsukurii*. This study aims to present the first progress in understanding the co-evolutionary processes at work within these host-parasitoid interactions. This work is within the perspective of developing biocontrol solutions which will necessarily involve massive production of BCAs. During this, counter-productive processes (drift, inbreeding, and artificial selection) can take place. Therefore, the ability to characterize virulence factors in a relevant way can constitute an innovative form of quality control.

Keywords: biocontrol, egg parasitoid, Scelionidae, *Trissolcus*, co-evolution process, venom

## OC089. Flash behaviour increases prey survival against avian predators

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Flash behaviour is a widespread anti-predator defence of insects in which otherwise cryptic prey show conspicuous colouration only transiently when fleeing from potential predators. This transient conspicuous signal sets up the expectation of predators that the prey is conspicuously coloured and leads the predators into looking for the conspicuous colour when in reality the prey settles with its camouflaged appearance. The efficacy and protective mechanisms of flash behaviour have been explored recently using humans as predators, but the adaptive significance of this deception against natural predators has not yet been demonstrated yet. In this study, we replicated some experiments conducted in humans to examine whether the protective mechanisms revealed against human predators also work against avian predators using chicks (*Gallus gallus domesticus*) and virtual prey as a model system. Our main findings include that (i) flash behaviour increased prey survival against avian predators, (ii) non-conspicuous flash signals also benefited escaping prey, and (iii) the survival benefits were higher for larger than smaller prey. Our main results are analogous to those findings using humans. Our study highlights that flash behaviour is an effective anti-predator defence against avian predators which explains the widespread occurrence of this deceptive signal.

**Keywords:** insert, anti-predator defence, colouration, deception, behaviour

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## OC090. Behavioural adaption of the pink bollworm moth in response to the mating disruptive technique used in cotton fields

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The pink-bollworm moth *(Pectinophora gossypiella)* is one of the most destructive cotton pests. Mating disruptive technique is an environmentally friendly control tactic, used worldwide for many years to control its population. In this tactic, the target field is permeated with the synthetic sex pheromone of the pest, to prevent males from finding females and mating. Like other anthropogenic interference, this control tactic may induce relatively rapid environmental change for the organisms it is applied against. It often alters the interactions of the organisms in the changed environment, causing population decline or driving evolutionary changes, including adaptive evolutionary responses. We hypothesized that due to the intense selection pressure on the moths to mate when treated with mating disruption, a change in the moths' mating behaviour might evolve.

We compared the mating behaviour of naïve populations (who did not experience mating disruption) to that of field moths (collected from a field treated with mating disruption for many generations) and found two significant behavioural differences:

1. Males from the field increasingly interfere with mating couples in attempt to mate the receptive (copulating) female. When the synthetic pheromone was added, a negative interaction was detected: naïve moths reduced disturbing couples, and field moths increased this strategy.
2. Naïve males delay mating with small, inferior females, awaiting the receptivity of larger females. Field-collected male moths are less choosy and readily mate with smaller inferior females.
We conclude that field-collected male moths have developed adaptive mating strategies to overcome the masking effect of the synthetic pheromone.

**Keywords:** mating-disruption, anthropogenic-interference, behaviour, sexual-selection

## OC091. Moth pheromones produced in cell and plant factories for control of orchard and row crop pests

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Most current pheromone-based pest control products target lepidopteran pests of high-value crops, as today's manufacturing processes cannot yet produce pheromones at low enough costs to enable their use for lower-value crops, especially commodity crops. Biological production of insect pheromones has evolved rapidly as an alternative to conventional synthetic pheromone production. This strategy is environmentally friendly and has the potential to become a cost-effective way of producing large quantities of pheromones with high purity and a minimum of waste. Elucidation of pheromone production pathways and characterization of genes and enzymes paved the way for biological production of moth pheromones. Molecular tools and metabolic engineering made it possible. The oleaginous yeast *Yarrowia lipolytica* and the oilseed crop *Camelina sativa* have turned out to be ideal platforms to produce $C_{16}$ and $C_{14}$ monounsaturated fatty acyl precursors and volatile pheromone components for both trapping and mating disruption of row-crop pests**.** The technology for fermentation of moth pheromone compounds in *Yarrowia lipolytica* was developed as part of the research project *OLEFINE* and resulted in the industrial biotech start-up company BioPhero Aps. The research project *Oil Crops for the Future* gave rise to SemioPlant AB, which in partnership with ISCA Inc. aims for production of pheromones from precursors produced in the oilseed crop Camelina. Biological production of insect pheromones works, the rest is a matter of upscaling, investments, and market penetration.

**Keywords:** moth pheromone biosynthesis, biological production, pest control, *Yarrowia lipolytica*, *Camelina sativa*

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## OC092. Expanding the toolbox for detection and monitoring of cerambycid beetles: Identification and testing of a variety of new pheromone structures

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Wood-boring beetles in the family Cerambycidae provide crucial ecosystem services by starting the recycling of woody biomass. However, some species are severe economic pests, either through direct damage, or indirectly as vectors of plant pathogens. Cerambycids are particularly important as invasive species because they are readily transported to new continents by global commerce. Recent examples of species which have invaded Europe include the Asian longhorn borer *Anoplophora glabripennis* (Mots.) and the rednecked longhorn borer *Aromia bungii* Faldermann. Since 2004, increasing problems with invasive cerambycids have stimulated efforts to identify semiochemical attractants for detection and monitoring, and pheromones or likely pheromones have now been identified for several hundred

species worldwide. The pheromones run the gamut from being widely conserved within related taxa in some cases, to apparently species specific in others. The chemistry is also very diverse, including compounds containing nitrogen and sulfur, and a variety of cyclic and acyclic structures. Here, we will describe recent work elucidating new structures, including chiral alcohols and aldehydes, a series of lactone stereoisomers, and tetrahydropyrans and tetrahydrofurans. The identifications of these compounds have provided new tools for surveillance of invasive species, as well as expanding the "chemical space" occupied by cerambycid pheromones. Furthermore, because of the widespread sharing of pheromone structures within genera and subfamilies, it is virtually certain that some of these structures will prove to be pheromone components of related cerambycids on other continents.

**Keywords:** invasive species, surveillance, monitoring, pheromone, Cerambycidae

## OC094. Male sex pheromones in parasitic wasps of the *Nasonia* group

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The genus *Nasonia* has been used during the past decades as a model system to investigate all aspects of parasitic wasp biology. The genus consists of four species: *N. vitripennis, N. giraulti, N. longicornis,* and *N. oneida.* The closest relatives belong to the genera *Trichomalopsis* and *Urolepis* and together these three genera form the so-called "*Nasonia* group". Males of all species from this group studied so far produce abdominal sex pheromones that attract only virgin females. Males of all *Nasonia* species produce the chiral, fatty acid-derived (4*R*,5*S*)-5-hydroxy-4-decanolide which is synergized by the trace component 4-methylquinazoline. The cosmopolitan species *N. vitripennis* has evolved the additional pheromone component (4*R*,5*R*)-5-hydroxy-4-decanolide (RR), which is lacking in the other species of the genus and allows *Nasonia* females to respond to the male sex pheromones in a species-specific manner. *N. vitripennis* females prefer the conspecific three-component blend, *N. giraulti* females emerge from their hosts already mated and ignore male pheromones, while *N. longicornis* females discriminate against the newly evolved pheromone component RR to avoid costly sexual interactions with sympatric *N. vitripennis* males. The recent identification of the sex pheromone in *Urolepis rufipes* revealed a biosynthetic switch in the *Nasonia* group: Males of this species produce (2*S*,6*S*)-2,6-dimethyl-7-octene-1,6-diol as a sex pheromone which, although produced in the same gland and released in a similar manner as in *Nasonia*, is derived from the mevalonate pathway.

**Keywords:** *Nasonia*, parasitoid, prezygotic isolation, sex pheromones, *Urolepis*

## OC095. Differences in responses of green lacewings to methyl salicylate (Neuroptera: Chrysopidae)

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Methyl salicylate is an herbivore-induced plant volatile (HIPV), that provides information on the activity of phytophagous insects, thereby helping orientation of their natural enemies as a synomone. Several studies dealt with attraction of predatory and parasitoid insects to this compound, including green lacewings (Chrysopidae). Nevertheless, there are contradicting results; for instance, some studies reported remarkable attraction of green lacewings to methyl salicylate, whereas other studies found moderate or no attraction. We conducted field experiments in Hungary with methyl salicylate on its own and in combination with known attractants for *Chrysopa* spp. and *Chrysoperla* spp. lacewings. *Chrysoperla carnea* species complex showed weak attraction to methyl salicylate, outperformed by phenylacetaldehyde, a known floral attractant for these species. When presented in combination, methyl salicylate showed a synergistic effect, with both males and females of *C. carnea* complex being attracted. These results are in line with previous research performed in Hungary on green lacewings.On the other hand, methyl salicylate did not attract *Chrysopa formosa* on its own. When tested in combination with nepetalactol, a known attractant for *C. formosa* males, it synergized male attraction; however, in combination with squalene, no synergistic effect was found. The results suggest that the responses of green lacewings to methyl salicylate are context-dependent, and the ecological role of the compound may be more complex than previously expected.

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Keywords: *Chrysopa*, *Chrysoperla*, methyl salicylate, attraction, synergism

## OC096. Uncovering regional variation in sexual communication in the invasive pest species *Spodoptera frugiperda* in Africa

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Successful invasive species likely adapt rapidly to new environments, where they encounter and possibly compete with local closely related species. A particularly successful invasive pest species is the fall armyworm (*Spodoptera frugiperda*), which is native to the Americas and an economically important pest on staple crops. Since its first reported invasion in West-Africa in 2016, it has since been found throughout the continent, as well as in the Middle East, Asia and Australia and Oceania, causing major damage and high economic losses to cultivated staple crops, especially maize. To understand the evolutionary potential of this pest, our research focuses on the question whether, and to what extent, sexual behaviors vary between geographic populations that could interact and possibly hybridize with locally occurring *Spodoptera* species. By comparing the sex pheromone from wild-caught fall armyworm females between Benin and Kenya over two years, we identified some geographic variation in the sex pheromone composition. We also found variation in male responses in field trapping experiments with sex pheromone lures based on the identified female pheromone variation and laboratory windtunnel experiments. Additionally, hybridization experiments have not resulted in viable offspring so far. Given that two strains of this species have been identified, we are currently investigating the role of mitochondrial variation on the sexual communication system in this species. We will discuss our results in light of evolution and pest management of the fall armyworm in Africa.

**Keywords:** fall armyworm, sexual communication, pheromone lures, hybridization, local adaptation

## OC097. Semiochemical pre-treatment reduces the response of *Bactrocera dorsalis* to methyl eugenol under semi-field conditions

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Male flies in the genus *Bactrocera* (Diptera: Tephritidae) are attracted to phenylpropanoid semiochemicals. Due to its attractiveness to *Bactrocera* males, methyl eugenol is a highly effective lure that is used for male annihilation technique (MAT). MAT and sterile insect technique (SIT) are usually implemented sequentially to avoid SIT males being attracted to MAT baits. Prior semiochemical exposure may reduce subsequent lure responses of male *Bactrocera* and allow synchronous application of MAT and SIT. We determined how interactions between weather, physiology and prior semiochemical exposure affect the response of male *Bactrocera dorsalis* to methyl eugenol baited traps. Response of 20 males was determined in a semi-field setting in relation to time of day, temperature, relative humidity, semiochemical pre-treatment (methyl eugenol, eugenol, or none), diet (protein supplemented and protein deprived) and age (4, 10 and 20 days old) (n=5). Semiochemical pre-treatment involved supplementing the diet with either semiochemical at a concentration of 1.25% for 48 hours prior to testing. Exposure of mature males (10 and 20 days) to either semiochemical reduced their response to methyl eugenol by as much as 95.97%. Diet did not affect the response of semiochemical pre-treated flies. The number of flies that responded to methyl eugenol decreased with temperature, with poor response occurring below 23°C. These results highlight the potential of synchronous application of MAT and SIT by using semiochemical pre-treatment. We also show that eugenol can be a viable alternative to methyl eugenol, as it is as efficient, cheaper, and less toxic than methyl eugenol.

**Keywords:** trap response, attractants, methyl eugenol, semiochemical, SIT, IPM

## OC098. Development of Three-Dimensional Models for Numerical Simulations of Insect Exposure to Radio-Frequency Electromagnetic Fields

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Wireless telecommunication networks operate using radio-frequency electromagnetic fields (RF-EMFs). This results in environmental exposure to RF-EMFs amongst others on insects. Potential effects of RF-EMF on insects and other living organisms is of increasing interest. Within the EU-Horizon funded project ETAIN, we quantify the exposure of insects by carrying out numerical simulations, which require anatomically accurate three-dimensional (3D) models. For this purpose, insect samples are collected through different streams: (i) western honey bee workers (*Apis mellifera carnica*) from Belgian hives, (ii) mason bees (*Osmia bicornis*) and blue bottle flies (*Calliphora vomitoria*) from commercial suppliers, and (iii) other pollinator species collected in the wild in Greece and Cyprus. Sixty-one (61) samples representing at least 15 species with varying morphologies (O(mm$^3$)-O(cm$^3$)) were shipped to UGent, BE. Twenty (20) insects were digitized using X-ray micro-computed tomography, which were compiled into homogeneous 3D models, suitable for import into an electromagnetic solver. In the solver, insect models are assigned insect-specific dielectric properties and are exposed to RF-EMFs at frequencies between 0.1-120 GHz. The solver then returns the absorbed power inside of the insects as a function of frequency. These results allow the interpretation of RF-EMF dosage of insects in the wild and/or near antennas, necessary to evaluate potential effects of the exposure on insects. Our future work aims at making these models heterogeneous, measuring insect dielectric properties, and validating the models using measurements.

**Keywords:** insects, radio-frequency, micro-computerized tomography, electromagnetism

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## OC099. Modelling the impacts of light pollution on UK moth populations at two temporal resolutions

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In the UK, there has been a 33% decrease in moth abundance since the 1960s, with 41% of common, widespread species in decline. This has largely been attributed to changes in habitat availability and climate. However, the concurrent increase in light pollution has led to speculation that artificial light may be a contributory factor. We modelled the impact of light pollution on UK moth population trends over two timescales (daily/monthly and annually). The first approach used Unihedron Sky Quality Meters to measure the ambient lighting over two years at a subset of traps from the Rothamsted Insect Survey (RIS) light trap network. These data were analysed with daily moth counts to test whether cyclical patterns in moth catches, driven by lunar phase, were dampened or absent at light polluted sites. For the second approach a novel high-resolution light pollution map of the UK was developed using night-time imagery from the Luoija1-01 satellite, launched in 2018. This map was used in conjunction with pre-existing time-series light pollution data (from the DMSP and VIIRS satellites) to compare trends in long-term moth abundance between lit and unlit sites. These complementary approaches provide new insight into the possible role of light pollution as a driver of declines in British moth populations.

**Keywords:** Lepidoptera, trends, nocturnal behaviour

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## OC100. Impact of ozone on the behavior of pollinators

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Ecological interactions among organisms underpin the stability of ecological networks that are responsible for species biodiversity in our ecosystems. These interactions are currently threatened by environmental risks such as air pollution, especially tropospheric ozone ($O_3$). Indeed, due to its strong oxidizing potential, $O_3$ has major effects on human and plant health. However direct effects of $O_3$ on the behavior of insect have not been considered so far, even though insect behavior is the key to participation in ecological interactions. In this study, we evaluated the potential effects of $O_3$ on the behavior of the fig wasp *Blastophaga psenes,* a specialist pollinator, and of the bumble bee *Bombus terrestris*, a generalist pollinator. Particularly we investigate if $O_3$ exposure at different realistic concentrations can alter their attraction towards the volatile organic compounds (VOCs) emitted by their host plants. Behavioral assays using Y-tube olfactometer showed a modification of their behavioral responses to a VOC stimulus after ozone exposition, depending on $O_3$ concentration. Electrophysiological experiments highlighted that such changes are at least partly due to changes in volatiles perception. Besides we have monitored exposed fig wasps hourly for 5h after exposure and found that ozone exposure induced major changes in their behavior, with individuals displaying moving troubles. The tracking over time clearly showed that exposed individuals might only have a reduced chance of recovery, with a decreasing proportion of active fig wasps despite the cessation of $O_3$ exposure. These findings illustrate that $O_3$ can affect pollinator behavior, which may have detrimental implications for pollination systems.

**Keywords:** air pollution, behavioral response, electrophysiology, bumble bees, fig wasps

## OC101. Comparative assessment of heat tolerance in weevils associated with a fire-prone ecosystem

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Fire is an important cause of disturbance that directly shapes many ecosystems worldwide, the frequency and intensity of which are expected to increase with climate change. While the effect of fire on floristic dynamics has been widely documented, little is known about how phytophagous insects respond when facing these disturbances. We explored the survival strategies of a set of sixteen weevil species with divergent lifestyles and geographic distribution in fire-prone ecosystems of the Cape Floristic Region (South Africa). More specifically, we investigated how the lifestyle of species correlated with heat tolerance. For instance, wingless species were hypothesized to show higher heat tolerance as they have a limited ability to escape fire and usually remain hidden in the soil.

The thermal tolerance of the insects was first measured using a standard heat knockdown set-up. Thermo-limit respirometry was then performed on the most resistant species using a thermal ramping protocol.

Our results show that weevils exhibit high variation in thermal tolerance across taxonomic groups. Species ecology better explains thermal tolerance than flight ability or the fire-proneness of ecosystems. Furthermore, some non-flying weevil species were found to be highly heat tolerant with CTmax values reaching up to 51.9°C.

This study highlights the diversity of strategies developed by arthropods to escape extreme heat in fire-prone ecosystems. Further work is necessary to examine the generality of these patterns across other fire-prone ecosystems to better understand behavioural compensation and evolutionary responses.

**Keywords:** Bogert effect, fynbos, heat knockdown, thermolimit respirometry, Coleoptera

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## OC102. Two common invasive whitefly cryptic species (B and Q) interact differentially with old-world and new-world begomoviruses

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Sweetpotato whitefly (*Bemisia tabaci* Gennadius)- transmitted old-world (monopartite) and new-world (bipartite) in the genus *Begomovirus* are major constraints to vegetable production in the United States. Monopartite tomato yellow leaf curl virus (TYLCV) and bipartite cucurbit leaf crumple virus (CuLCrV) and sida golden mosaic virus (SiGMV) affect crops such as tomato, cucurbits, and snap bean. *Bemisia tabaci* cryptic species B (MEAM 1) is predominant outdoors, and *B. tabaci* Q (MED) cryptic species is limited to greenhouses. However, in recent years, *B. tabaci* Q has been found colonizing outdoor

ornamentals and field crops in Florida and Georgia. This has raised concerns on whether the dispersal of *B. tabaci* Q cryptic species into the landscape could exacerbate the extant virus epidemics situation. Competence in the transmission of CuLCrV, SiGMV, and TYLCV was compared between *B. tabaci* B and Q. *Bemisia tabaci* B efficiently transmitted all three viruses, whereas the Q did not transmit the two bipartite viruses. TYLCV loads in both cryptic species following acquisition was similar, but bipartite virus levels were substantially lower in *B. tabaci* Q than in B. Virus loads in various whitefly tissues revealed a similar trend. A series of molecular and Omics approaches were undertaken to decipher the differences in interactions between monopartite (old-world) and bipartite (new-world) viruses with both cryptic species. An exciting number of transmission-influencing candidates were identified, and some were functionally validated. These findings will be discussed.

Keywords: sweetpotato whitefly, cryptic species, begomoviruses, transmission

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## OC103. Biological oscillator activity in relation to seasonal migrations of two European nymphalid species – *V. atalanta* and *V. cardui*

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The relationship between the biological oscillator located in the brain and seasonal migrations has been characterized in the North American monarch. However, oscillators which generate rhythms in other organs have not been detailed for migrating species. Our objectives were to characterize the brain oscillator activity that controls daily rhythm of locomotion and the peripheral oscillator that controls the rhythm of sperm release (RoSR) in two European migrating butterflies, *Vanessa atalanta* and *Vanessa cardui*. Among *V. atalanta* males migrating to SE Poland, we found that both, the brain and locomotor activity rhythms were similar to those found in overwintering individuals. However, parameters of these rhythms changed in two successive generations of breeding and migrating individuals. Among *V. cardui* males arriving for breeding, the brain and locomotor activity rhythms were significantly different from those of their migrant offspring. In *V. atalanta*, shortly after spring immigration, we did not observe the RoSR present in overwintering males. The RoSR in migrants was on activated after a few weeks in Poland. It also had different parameters than those in overwintering males. The RoSR in migrants was similar to the RoSR found in parent generation of migrants fleeing winter. In *V. cardui* arriving from the south, the RoSR became established after a few weeks (in the second half of May). Whereas in the summer-autumn generation migrating south, sperm release does not occur. Supported by the funding of the University of Warsaw IDUB grant #BOB-IDUB-622-322/2022 (PSP: 501-D114-20-1004310) awarded to P.B.

**Keywords:** seasonal migrations, biological clock, sperm release, molecular oscillators, Nymphalidae

## OC104. Myrmecomorphy in the predatory mirid *Pilophorus gallicus*: living between the devil and the deep blue see

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Myrmecomorphy is a mimetic strategy involving morphological, behavioural and/or chemical similarities to resemble ants in order to gain protection from other predators. Sometimes, myrmecomorphic species are also myrmecophiles living in close association with ants. Myrmecomorphy is very common in arthropods and particularly in mirids (Hemiptera: Miridae). *Pilophorus gallicus* is a myrmecomorphic predatory mirid living among ants colonies in pear orchards, where spiders are also common, in the southern Mediterranean area. A laboratory essay was carried out to test whether myrmecomorphy reduced predation rates in *P. gallicus* when confronted with spiders and ants. The predatory behaviour of two spider species (*Icius hamatus* and *Cheiracanthium mildei*) and the ant *Lasius grandis* was compared when they were offered the myrmecomorphic mirid *P. gallicus* and the non-myrmecomorphic mirid *Nesidiocoris tenuis* as prey. Additionally, the behaviour of ants was observed when confronted with the two spiders species. The two spiders species showed lower predation rates for *P. gallicus* than for *N. tenuis*. Ants showed low and similar predation rates for the two mirid species. *Cheiracanthium mildei* showed an avoiding behaviour when confronted with ants. In contrast, *I. hamatus* showed both avoiding and attacking behaviours. The results of the present research show that Myrmecomorphy in *P. gallicus* can be considered as a type of Batesian mimicry.

Keywords: ants, mirids, spiders, Batesian mimicry, behaviour, trophic interactions

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## OC105. Poison or potion: An infected bee story

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Bees depend entirely on floral resources for their nutrition. However, there is increasing evidence that bees also rely on floral resources, specifically on specialized metabolites (SM), to deal with endoparasites, either enhancing their tolerance (i.e., increasing fitness) or resistance (i.e., reducing parasite load). Thus, plant co-evolutions with not only herbivores but also pollinators have undeniably shaped SM profiles among their tissues. Although this topic has gained attention, research addressing bee-parasite-plant relationships remain scarce. To narrow this gap, we first determined the SM profiles in sunflower (SM: phenolamides) as well as cherry and heather (SM: flavonoids) tissues. Then, using microcolonies fed with control, natural or SM-enriched diets, we assessed how different pollen diets and their SM impacted the economically important bumblebee *Bombus terrestris* and its most prevalent gut parasite, the trypanosomatid *Crithidia bombi*. Finally, we tested whether infected bumblebees were able to self-medicate using choice bioassays. We found that SM were distributed unevenly among plant tissues with species-specific patterns. Besides, bumblebees were impacted differently according to the provided pollen diet but as expected, SM-enriched diets mostly had detrimental consequences (e.g., impeded colony development). We further found that SM could both boost and reduce parasite load. Finally, contrary to our expectations, infected bumblebees did not prefer therapeutic pollen diets when offered a choice. We discuss the limitations of our experiments in light of the criteria underlying self-medication. We argue that highly virulent parasites are more likely to trigger self-medicative behaviors and that designing a SM-free artificial diet as control is considerably needed.

Keywords: pollen, bee, parasite, phytochemical, therapy, self-medication

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## OC106. The sugar diet and associated environmental compounds in the crop of the mosquito *Culex pipiens*

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The common house mosquito *Culex pipiens* is of medical relevance due to its ability to transmit West Nile virus to animals and humans. The ecological relevance of the species for terrestrial and aquatic food webs is well described. However, floral nectar is usually used by adult mosquitoes to cope with the energetic demand linked to various physiological processes like longevity and vectorial capacity. Despite this frequent plant-mosquito interaction, the quality of flora-mosquito interactions is largely unexplored.

The aim of present study is to identify and quantify metabolites in the crop of female *Cx. pipiens* mosquitoes. The crop is part of the digestive system and devoted to the storage of sugar-based meal obtained from various nectars sources. We harvested the crops from field collected and experimentally nectar-fed mosquitoes (nectar offered from field-collected or purchased flowers). The metabolites in the crops have been qualified and quantified by UHPLC-MS offering a low limit of detection (10 ug/L for sucrose, glucose and sucrose) and a high resolution of chemical compounds.

The floral nectar source determines the metabolome in the crop of the mosquito *Cx. pipiens*. After nectar feeding with specific ornamental plants, the sugar profiles in the mosquito crops are comparable to those of field collected mosquitoes. Alarmingly, next to sugar compounds and secondary metabolites (2 amino acids, 12 natural products), nine environmental pollutants have been up taken from nectar sources.

Our findings merit further research to explore mosquito´s nectar preferences and the role of chemical exposure during nectar feeding for insecticide resistance and vector competence.

Keywords: sugar meal, floral nectars, plant-mosquito interaction, crop, metabolome, pesticides

## OC107. Food plants and their localization by the sugar beet weevil *Asproparthenis punctiventris*

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*Asproparthenis punctiventris* Germar (Coleoptera: Curculionidae) is widely distributed in Eurasia and one of the most important pests of sugar beet (*Beta vulgaris* subsp. *vulgaris* Altissima group). Since 2017, the mass occurrence of the weevil in eastern Austria has led to major damage in sugar beet crops. Besides sugar beet, other Amaranthaceae species have also been described as food sources for the weevil. To find out whether and which plants can maintain or even promote the pest population outside sugar beet fields, the leaf consumption of *A. punctiventris* females on sugar beet was compared with different weeds and other plant species. In addition, differences in behaviour between mated females during oviposition and unmated females during maturation feeding were investigated in the laboratory. Irrespective of mating status, females located food plants by the odour bouquets of the leaves. Unmated females consumed the greatest amount of leaf mass from Beta spp. and about a third less from Atriplex spp., indicating that these plants have the highest nutritional value for *A. punctiventris*. Considerably less was fed on *Chenopodium album* and *Amaranthus retroflexus*. Mated females generally consumed more leaf mass than unmated, especially from plants with a lower feeding value. It could be shown for the first time that larvae can feed and develop not only on sugar beet roots, but also on weeds and other crops from the Amaranthaceae plant family. These findings show aspects that can contribute to reducing the overall population of the weevil in a sugar beet growing area.

Keywords: *Bothynoderes punctiventris*, leaf consumption, larval development, olfactometer experiments, female mating status

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## OC108. Are aphids a source of medicine for ants?

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The ability of ants to incorporate biologically active compounds as a form of self-medication in response to the threat of pathogens has so far been shown using strict artificial diets, yet natural sources of medicine remain unknown. Here we explored whether aphids could provide ants with medicinal benefit. $H_2O_2$, a reactive oxygen species which ants can use for medicinal benefits against fungal pathogens in artificial foods, has been shown to be present in high concentrations in *Megoura viciae* aphids feeding on broad bean plants. Using a diet supplemented with different concentrations of aphids, we explored whether *Formica fusca* ants would alter their foraging choices to medicate themselves against a fungal pathogen. We observed that exposed ants switched their preference on foraging on a high concentration aphid food during the acute phase of the disease in a way which

carried a medicinal benefit, but only when part of a varied diet. Our results are the clearest suggestion to date, that aphids might act as a source of natural medicine for ants during the acute phase of infection. While *M. viciae* is not an ant-affiliated species, ants do engage in mutualistic interactions with many species of aphids, that they also predate on if needed. Understanding how pathogens impact the evolution and stability of ant – plant – aphid interactions, as well as how healthy and diverse ecosystems affects the health of animals is an important topic should be studied further using more natural setups.

Keywords: self-medication, *Formica fusca*, co-evolution, adaptive plasticity, mutualism, multitrophic interactions.

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## OC109. Fungal infection alters collective nutritional intake of ant colonies

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Animals are known to alter their feeding behavior when infected to help combat various parasites. However, parasites can manipulate host foraging behavior to increase their own development, survival and transmission. The mechanisms by which nutrition influences host-parasite interactions are still not well understood. Using Nutritional Geometry, we investigated the role of key nutrients: amino acids and carbohydrates in a host-parasite system: the entomopathogenic fungus, *Metarhizium brunneum*, and the ant, *Linepithema humile*. We first established that the fungus grew and reproduced better on diets comprising four times less amino acids than carbohydrates (1:4). Second, we showed that the fungus facing various food pairings, was always able to exploit the two complementary food resources to reach the same performance as on the optimal diet. Third, we demonstrated that a diet comprising 1:4 (AA:C) decreased host lifespan in uninfected but not in infected ants compared to a more carbohydrate-biased diet (1:199). Lastly, when given a binary choice between two diets of different amino acids to carbohydrate composition, the foragers of uninfected colonies chose their optimal carbohydrate-rich (1:199) diet, whilst the foragers of infected colonies selected a diet comprising a 1:4 ratio of amino acids to carbohydrate. This means that pathogen infection induced workers to alter their nutritional intake target towards a more amino acids-rich diet, which would be a costly, lifespan-reducing choice for healthy colonies. Under fungal infection, however, the negative impact of high amino acids diet seems counterbalanced by its mitigating effects against disease.

Keywords: host-parasite interactions, nutritional geometry, social insects, ants, parasites

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## OC110. A beta-glucosidase of an insect herbivore determines both toxicity and deterrence of a dandelion defense metabolite

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Gut enzymes can metabolize plant defense compounds and thereby affect the growth and fitness of insect herbivores. Whether these enzymes also influence feeding preference is largely unknown. We studied the metabolization of taraxinic acid β-D-glucopyranosyl ester (TA-G), a sesquiterpene lactone of the common dandelion (*Taraxacum officinale*) that deters its major root herbivore, the common cockchafer larva (*Melolontha melolontha*). We have demonstrated that TA-G is rapidly deglucosylated and conjugated to glutathione in the insect gut. A broad-spectrum *M. melolontha* β-glucosidase, Mm_bGlc17, is sufficient and necessary for TA-G deglucosylation. Using cross-species RNA interference, we show that Mm_bGlc17 reduces TA-G toxicity. Furthermore, Mm_bGlc17 is required for the preference of *M. melolontha* larvae for TA-G-deficient plants. Thus, herbivore metabolism modulates both the toxicity and deterrence of a plant defense compound. Our work illustrates the multifaceted roles of insect digestive enzymes as mediators of plant-herbivore interactions.

**Keywords:** plant-herbivore interactions, digestive enzymes, glucosidases, metabolism, plant specialized metabolites, insect behaviour

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## OC111. What's on the menu? Investigation of the feeding behaviour of the brown marmorated stink bug *Halyomorpha halys* using a molecular gut analysis

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The brown marmorated stink bug (BMSB) *Halyomorpha halys* is native to Northeast Asia, and during the last decades, it spread into several countries in North America and Europe. It is considered a severe threat to agricultural production due to its highly polyphagous nature, which includes more than 300 different host plants, and due to the damage it has caused in the invaded regions. Currently, the feeding behaviour throughout the season is unknown and because of its wide variety of host plants, it is of high interest. Since host plants are often identified by time-consuming visual inspections that do not define what can be considered a feeding host, a metabarcoding approach was developed to determine what *H. halys* feeds on. For this purpose, individuals were collected in Northern Italy from early summer to

late autumn. The gut of the stink bugs was dissected and used for DNA extraction, followed by a PCR using primers amplifying the ITS2 region of plants. The amplicons were sequenced on the Nanopore Flongle device. This approach allowed the detection of a broad variety of different feeding hosts, including several ornamental plants. This study helps to draw conclusions about the ecology of the BMSB in invaded areas and the potential of the method to assess the feeding behaviour of phytophagous insect pests.

Keywords: *Halyomorpha halys*, Nanopore, DNA metabarcoding, invasive species, molecular gut analysis

## OC112. Life history traits and immune investment in the black soldier fly are influenced by larval density

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Conspecific density can severely affect the fitness of any organism. It is apparent that even in voluntary aggregating insects, high larval densities (i.e., crowding effects) may be detrimental to their life histories and fitness. However, despite fundamental and applied importance, larval crowding effects on life history traits have still received surprisingly little attention in insects. Moreover, while most key life history traits are predicted to lead to decreased fitness in environments when larval density is high, the immune response is sometimes shown to be stronger at high than at low density conditions, potentially leading to higher fitness at high larval densities.

By avoiding the methodological problems of previous studies, we explored the effects of larval density on life history traits (including phenoloxidase activity (PO)) in commercially important black soldier fly (BSF). Results revealed a significant increase in pupal mass, survival, fat content, adult mass, adult longevity and reduced larval and pupal development time at lowest density tested (1 larva/cm$^2$). BSF larvae reared at high larval densities had significantly higher PO activity than those reared at lowest density, a result broadly consistent with our literature review of larval density effects on insect immunity. We also observed higher PO activity in larvae exposed to entomopathogenic fungi, indicating that both larval density and exposure to pathogens lead to a stronger immune response in BSF.

High larval density thus increases immune response but negatively affects other studied life history traits, and thereby may lead to decreased or increased fitness in BSF and other insects.

Keywords: density-dependent prophylaxis, crowding, *Hermetia illucens*, fitness, trade-offs

## OC113. Host size overrides maternal effects on development of a secondary hyperparasitoid wasp

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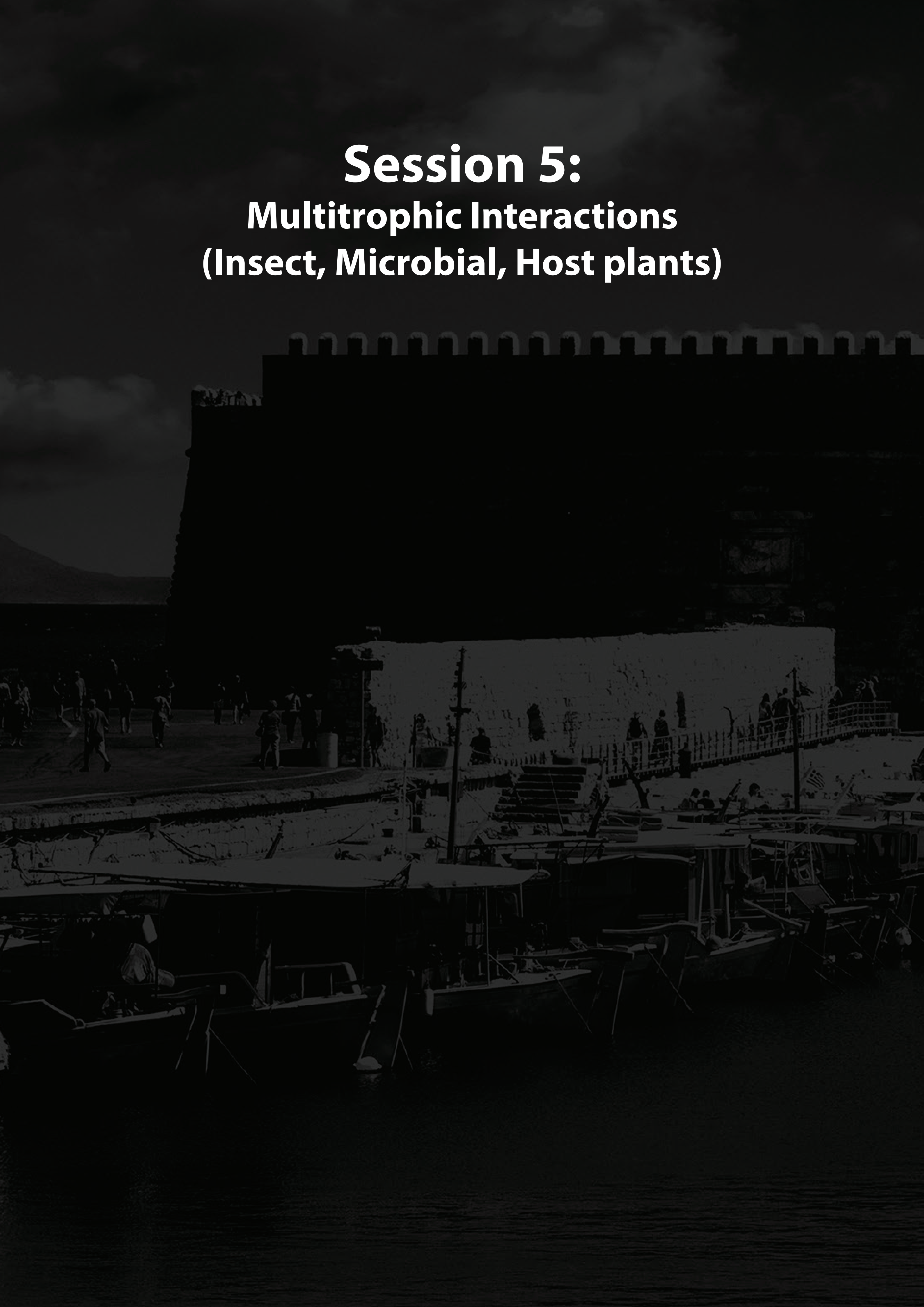
Offspring phenotype in most organisms is significantly mediated by genetically-based traits inherited from both parents. Traits like body size and morphology are heritable in arthropods and are often more strongly correlated with biotic and abiotic parameters like food quality and availability and temperature and are more influenced by short-term ecological factors. We evaluate maternal and host size-related effects on the development of an asexually reproducing (= female only) idiobiont ectoparasitoid, *Gelis agilis* on pre-pupae in cocoons of its host, the primary parasitoid, *Cotesia glomerata*. Female *G. agilis* from two adult size classes, 'small' (mean 0.7 mg) or 'large' (mean 1.2 mg) were allowed to parasitize cocoons of differing size along a continuum from ~1.2 mg to ~4.0 mg and the body size and development time of their offspring were measured. In both body size classes of *G. agilis* mothers, there was a strong correlation between host size and offspring size. However, there was no effect of adult *G. agilis* size on this parameter: for a given host size, the size of *G. agilis* offspring did not differ between small and large mothers. Our results reveal that host quality completely overrides the effects of maternal size on the development of *G. agilis*. Given that parasitoids are under strong selection for optimal exploitation, allocation and utilization of limited resources contained in individual hosts, we argue that host quality is far more of a constraint on parasitoid development and fitness than traits of their parents.

Keywords: hyperparasitoid, maternal effect, host effect

# Session 5:

## Multitrophic Interactions (Insect, Microbial, Host plants)



## OC114. Chemically-mediated insect-plant interaction across space and time

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Plants and insects have coexisted for millions of years, with a range of intricate and dynamic interactions occurring between them. One of the most important ways that plants interact with insects is through chemical compounds, which can attract, deter or even manipulate the behaviour of the insects. In some cases, these interactions can be adversarial, such as when plants produce toxins to defend against herbivore, while in other cases, they can be mutually beneficial, such as when plants send signals for attracting predators or parasitoids. These interactions vary across ecological gradients, including differences in climate, elevation and habitat, as well as differences in plant and insect species diversity. In this talk, I will expand on our current understanding of how plant chemistry changes along large-scale ecological gradients, and in turn, how such chemistry impacts multitrophic interactions between plants, insect herbivores and predators. Understanding the complex relationships between plants and insects across large-scale ecological gradients is essential for predicting the impacts of environmental change on ecosystems and for developing sustainable agricultural and conservation practices.

**Keywords:** plant-herbivore interaction, chemical ecology, tritrophic interactions, phytochemical diversity, ecological gradients of biodiversity

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## OC115. How plant microclimates modulate the vulnerability of insects to climate change

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Plants host a huge diversity of interacting ectotherms, especially at the surface of leaves or flowers. The ecophysiology of tiny arthropods, as well as the rate of biotic interactions, depends on the temperature of these plant surfaces. Most of these organisms are tiny enough to occupy small spaces within plant boundary layers making them largely under the influence of plant functioning. The temperature of leaf or flower surface can deviate from air temperature sometimes quite strongly. In this presentation, I will detail the biophysical mechanisms determining the microclimate of arthropods living at the plant surfaces. A comprehensive understanding of the leaf/flower-air temperature deviations as well as the temperature heterogeneity at the organ surface scale, is important to identify the extent to which ectotherms can exploit these thermal patterns to thermoregulate. Then, arthropod herbivores can fundamentally modify the functioning of the leaf when feeding on it; these changes have cascading effects on the plant microclimate. These cascading effects can help explain the patterns of thermal adaptation among arthropod herbivores and predict the consequences of competitive interactions. Finally, the plant microclimate can be placed in the context of climate change impacts: will the plant surface provide ectotherms with a buffer of extreme heat or by contrast will it magnify the atmospheric changes? This knowledge of the plant microclimate helps us determining the vulnerability of arthropods to warming.

**Keywords:** thermal ecology, microclimate, body temperature, biophysical ecology, thermal stress

## OC116. Biological control of Lepidopteran grapevine pests: plant and insect host mediated bottom-up effects of elevated CO2. Lessons from a VineyardFACE facility

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The increasing atmospheric carbon dioxide (CO2) concentration is expected to influence interactions between plants and associated insects. Especially the indirect, plant and insect host mediated bottomup effects on natural enemies may affect biological pest control. The European Grape Berry Moth *(Eupoecilia ambiguella)* and European Grapevine Moth *(Lobesia botrana)* are important, multivoltine insect pests in vineyards. Biological control strategies for these pests rely on pheromone-based mating disruption, microbial biocontrol agents, and natural enemies occurring in the vineyard. Will climate change affect their efficacy? In the Geisenheim VineyardFACE facility (Free-Air Carbon dioxide Enrichment; ca. +20% CO2) and in the laboratory, we conducted experiments to investigate the direct and indirect effects of elevated CO2 on the tritrophic system grapevine – moth – natural enemy. On the 1st trophic level, elevated CO2, grapevine cultivar and plant phenology affected plant quality (nutrients and defense compounds). On the 2nd trophic level, they changed life history traits, larval behavioral and immunological defenses, and the larval transcriptome. We found no indications of reduced mating disruption efficacy. On the 3rd trophic level, egg parasitism was impacted. Mostly, advancing host plant phenology had a stronger impact than grapevine cultivar or elevated CO2,suggesting that the changes currently occurring during one season are stronger than those due to elevated CO2 in the near future. This generalization, however, may not hold true for all the specific interactions in the vineyard food web and future impairment of biocontrol may still occur, especially considering the interactive nature of co-occurring climate change factors.

**Keywords:** multitrophic interactions, *Vitis vinifera, Trichogramma*, immunity, gene expression

## OC117. The effect of climate change on a *Bemisia tabaci* based pathosystem

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Whiteflies from the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) are a significant threat to agriculture and the economy worldwide. They cause direct harm to plants by feeding on phloem sap and indirectly harm them by transmitting hundreds of economically important viruses, such as the Tomato Leaf Curl New Delhi Virus (ToLCNDV). ToLCNDV, which is primarily spread by whiteflies from the *Bemisia tabaci* complex,

was first discovered in South-East Asia and subsequently spread to the Mediterranean region, including Greece, Italy, Spain, Portugal, Tunisia, Morocco, Algeria, France, and Slovakia. The virus causes severe economic losses in cucurbit crops grown in protected and open fields. Climate change may exacerbate the spread of *B. tabaci* and its associated diseases. Therefore, to understand how climate change affects the epidemiology of ToLCNDV disease in cucurbits, we are investigating how climate impacts the acquisition and inoculation phases of ToLCNDV in *B. tabaci* (MED). We are also studying the gene expression of target genes to determine the effects of climate change and virus presence on the whitefly vector. Two climate chambers with independent air temperature, humidity, CO2 concentration, and light intensity regulation are being used to simulate current and future climatic conditions. This research will provide valuable insight into a previously unexplored area, which will help to address future plant protection challenges.

Keywords: *Bemisia tabaci*, ToLCNDV, cucurbits, climate change

### OC118. Under the radar: The preference for volatiles from plants infested with small and young aphid colonies may help aphid parasitoids to avoid intraguild predation

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One key factor that determines the fitness of endoparasitoids is the survival of their larvae that feed inside the insect host and are at risk to fall victim to intraguid predation if their host is attacked by a predator. Aphid predators are known to accumulate in large aphid colonies and aphid colonies often last only a few day in the field before predators eradicate them. Aphid parasitoids may try to avoid this fate of intraguild predation for their larvae by selecting young and small aphid colonies for oviposition and indeed, we found in an olfactometer experiment that the aphid parasitoid *Diaeretiella rapae* was most strongly attracted to volatiles emitted by plants infested for just one day with 25 aphids. Volatiles from plants that were infested for more than one day or with 100 aphids were less attractive. *D. rapae* was even attracted to volatiles from plants that had been infested with just one adult aphid for one day. The ability of parasitoid females to detect even very small and young aphid colonies may provide their larvae with enough time to finish larval development before predators detect the growing colony. By using the red and green color morph of the pea aphid, we were able to show that survival of individual parasitized aphids (one color morph) and unparasitized aphids (other color morph) and mummification rate in the presence of predators was highest in small aphid colonies, as these received less hoverfly eggs in a cage and semi-field experiment.

Keywords: intraguild predation, HIPVs, plant volatiles, aphids, parasitoids

## OC119. Defense priming in cauliflower (*Brassica oleracea*) by the entomopathogenic fungus *Metarhizium robertsii*

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Plants live in association with a multitude of microorganisms, some of which may improve the plant's defense towards herbivores. In this study, we assessed the effects of the root colonizing entomopathogenic fungus *Metarhizium robertsii* on cauliflower (*Brassica oleracea*) defenses upon an attack by the aboveground herbivore *Plutella xylostella* larvae. We evaluated whether the glucosinolate-myrosinase plant defense system in the leaves was primed by *M. robertsii* in the root system and whether priming reversed after removal of *P. xylostella* larvae. Conidia of *M. robertsii* were inoculated to experimental cauliflower plants as root drenches. Before larval herbivory, leaf glucosinolate content and myrosinase activity did not differ between fungal-inoculated and control plants. Immediately after herbivory, in contrast, both the glucosinolate content and myrosinase activity were significantly higher in inoculated plants, evaluated by reduced leaf material consumption. Twenty-four hours after herbivory, glucosinolates and myrosinase activity were reverted again to before herbivory levels. Total biomass and shoot-root ratio were unaffected by *M. robertsii* root colonization. In conclusion, *M. robertsii* can associate with cauliflower roots and prime aboveground glucosinolate content and myrosinase activity. The increased defense levels were down-regulated 24 hours after herbivory. Root-associated *M. robertsii* may thus affect aboveground herbivores indirectly by host plant priming, in addition to their well-known direct effects as insect pathogens.

**Keywords:** cauliflower, *Plutella xylostella*, glucosinolates, myrosinase, entomopathogenic fungi, *Metarhizium robertsii*

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## OC120. The endophytic role of the entomopathogenic fungus *Beauveria bassiana* in agricultural plant protection against *Spodoptera littoralis* larvae

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Beneficial microorganisms are able to promote plant growth and defence barriers, offering a valuable alternative to synthetic agrochemicals. *Beauveria bassiana* is one of the most important entomopathogenic fungi that can colonize a wide variety of plant species as an endophyte, limiting the growth and survival of plant pests and pathogens. Here we contribute to this research topic by studying the effect of tomato plants colonization by *B. bassiana* on the survival, development, and immunity of *Spodoptera littoralis*

(Lepidoptera, Noctuidae). Endophytic colonization of plants did not affect the survival of larvae feeding on them, which showed a weight increase associated with a higher pupal mortality and a lower adult fecundity. Interestingly, encapsulation and nodulation responses of larvae fed with *B. bassiana* colonized plants were reduced, while, in contrast, phagocytosis slightly increased. These larvae proved to be more sensitive to *B. bassiana* or *Bacillus thuringiensis* infection, given their reduced immune competence. “Omic” studies on tomato plants and S. littoralis larvae aim to unravel the molecular mechanisms underlying these changes. Our study provides key findings toward the understanding of mechanisms underlying this intricate plant-insect-microbe interaction interconnecting the below-ground and above-ground environments. It also sheds light on the evolution of the entomopathogenic lifestyle in soil fungi.

**Keywords**: soil microbiota, insect immunity, plant protection, multitrophic interactions, pest management

## OC121. Symbiotic microbes as driving forces of evolutionary innovation in beetles

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Symbiotic associations with microbes are important driving forces of evolutionary innovation. The hyperdiverse insect order Coleoptera, the beetles, are associated with an astounding diversity of symbionts that provide a wide range of functional benefits to their hosts. Highlighting a few selected beetle-microbe associations, I will report on novel findings of symbiont-provided antibiotic defense, microbe-enabled digestion of plant material, and symbiont-mediated adaptation to the abiotic environment. These examples are intended to show the impact of symbionts on niche expansion and diversification in beetles, but also provide insights into life-style switches from parasitism to mutualism in bacteria as well as the process of extreme genome reduction beyond the well-known intracellular symbioses.

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## OC122. Diversity, transmission and function of the microbiota of a root phytophagous insect

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Gut microbiota of phytophagous insects can contribute to the detoxification of toxic compounds produced by plants and thus to the adaptation of the insect to its host plant. *Brassicaceae* produce isothiocyanates, which can be detoxified by bacteria carrying the *saxA* gene. However, the involvement of insect gut microbiota in the detoxification of isothiocyanates produced by *Brassicaceae* remains understudied. Here, we describe the diversity, composition, acquisition and impact on insect fitness of the cabbage root fly (*Delia radicum*) microbiota. We performed a laboratory experiment where *D. radicum* faced contrasted environments, with a gradient *saxA* abundance. These contrasted environments are represented by rapeseed (*Brassica napus*) genotypes that differ in glucosinolates (directly implicated in the production of isothiocyanates) concentration and on soils that differ in microbiota diversity. We combined the measure of the insect fitness with a metabarcoding approach to exhaustively describe the microbiota communities of the soil, the roots and the insect at different life stages. This DNA-based approach was completed with a molecular approach to characterize *saxA* abundance and diversity in the environment as well as in insect microbiota. We demonstrated horizontal transmissions in bacterial communities: three bacterial genera (*Pseudomonas*, *Serratia* and *Acinectobacter*) known to harbour the *saxA* gene were shared between the insect, the soil and the root microbiota. Insects have a better fitness in environments with high glucosinolates concentration. Our results indicate that *D. radicum* deals with the isothiocyanate toxicity and its microbiota contains bacterial genera able to detoxify toxic compounds released by *B. napus*.

**Keywords:** glucosinolates, isothiocyanates, life history traits, cabbage root fly, microbial communities

## OC123. Effects of caterpillar's diet and gut microbiota on the tomato defense response upon herbivory by two *Spodoptera* species

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The coevolution between plants and insects has selected for specific herbivory resistance traits, driving insect diversification and adaptation to different ecological niches. Plant can perceive molecules present in fluids produced by the attackers, such as oral secretions, and activate downstream signaling cascades that eventually lead to the activation of a defensive response tailored to the attacker's nature. However, additional factors, such as the microbes present in insect's oral secretions or the herbivore's previous diet, can influence the outcome of this interaction in a way that may be species-specific. In this study, we investigated how herbivory-induced defense activation varies under different conditions using a comparative framework represented by two caterpillars belonging to the *Spodoptera* genus (Lepidoptera: Noctuidae): *Spodoptera littoralis* and *Spodoptera exigua*. We first combined transcriptional analysis and proteomics to analyze the response of tomato var. Moneymaker (*Solanum lycopersicum*) to herbivory by *S. exigua* and *S. littoralis* caterpillars reared under different diet conditions. We identified candidate molecules differentially produced by the herbivores that may act as chemical signals and trigger differential defense activation under specific diet conditions. We also investigated whether the oral microbiota of the two caterpillar species may drive caterpillar-specific modulation of plant defense response under different diet conditions.Our results suggest that tomate defense response is modulated after herbivory by closely related *Spodoptera* species under certain diet conditions and in presence of microbes, and identify chemical signals that may be responsible for altering the outcome of these interactions.

Keywords: *Spodoptera*, tomato, herbivory, plant defenses, oral secreted proteins, oral secreted microbes

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## OC124. Parasitic wasps alter the microbiome and body odours of caterpillar hosts, with consequences for higher tropic levels

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Multitrophic interactions are affected by several factors, but little attention has been given to the role of microbes and microbial volatiles (mVOC). Microorganisms living in and on insects may produce mVOCs that characterize their odour. The mVOCs might thereby provide a reliable cue to natural enemies in locating their host or prey. Parasitism by parasitic wasps might alter the microbiome of their hosts, affecting interactions with hyperparasitoids. Odours of parasitised caterpillars aid hyperparasitoids to locate their host, but the origin of these odours and its relationship to the caterpillar microbiome are unknown. In this study, we manipulated and analysed the microbiome of *Pieris brassicae* caterpillars in relation to parasitism by its parasitoid *Cotesia glomerata*, and analysed their body odours. The microbial communities and body odours of unparasitised and parasitised caterpillars were significantly different. Most likely, changes in the external microbiome and body odour after parasitism were driven by the resident internal microbiome of caterpillars, where the bacterium *Wolbachia* sp. was only found in parasitised caterpillars. To test whether *Wolbachia* affected higher tropic levels, unparasitised caterpillars were injected with *Wolbachia* and exposed to the hyperparasitoid *Baryscapus galactopus* in a behavioural assay. Injection of *Wolbachia* increased hyperparasitoid attraction to the caterpillars compared to untreated caterpillars, while no differences were found compared to parasitised caterpillars. Altogether, our findings illustrate the importance of microorganisms as hidden drivers of multitrophic interactions and highlight their role in insect food webs.

**Keywords:** parasitism, microbiome, body odours, multitrophic interactions, *Wolbachia*

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### OC125. CaMV and TuYV modify the post-acquisition behavior of aphids with consequences on virus transmission

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Arthropod-transmitted plant viruses may induce symptoms in the host that can influence vector behavior and thus affect the transmission of the virus itself. Most studies focus on changes in vectors' host selection and feeding behavior, or performances when they are on infected plants. In contrast, knowledge is scarce on post-acquisition effects, i.e. altered vector traits persisting after the vectors leave the infected plant. Here we studied the post-acquisition behavior and physiology of aphid vectors coming from plants infected with two viruses using opposite transmission modes but sharing the same vector, the green peach aphid *Myzus persicae*. Turnip yellows virus (TuYV- genus *Polerovirus*, family *Solemoviridae*) is a phloem-limited virus transmitted in a circulative-persistent manner, whereas cauliflower mosaic virus (CaMV - genus *Caulimovirus*, family *Caulimoviridae*) infects all plant cells and is transmitted in a non-circulative, semi-persistent manner. We show that the mobility of CaMV-viruliferous aphids is increased compared to TuYV-carrying or non-viruliferous aphids. In contrast, both TuYV- and CaMV-viruliferous aphids display a similarly high emigration rate from one healthy plant to another. Further, CaMV-viruliferous aphids have decreased body mass, survival, and energy resources than control or TuYV-carrying aphids. Overall, CaMV modified vector behavior more drastically than TuYV. This might be due to the significantly deteriorated plant quality by CaMV infection, evidenced by metabolic analysis. Interestingly, despite the different effects on aphid physiology and mobility, both viruses promote aphid vector dispersal between hosts equally, which we will discuss in light of virus transmission strategy.

**Keywords:** aphids, behavior, viral manipulation, plant viruses

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## OC126. Is *Tomato yellow leaf curl virus* an attractant or repellant for whitefly predatory beetles?

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The whitefly *Bemisia tabaci* and the *Tomato yellow leaf curl virus* (TYLCV) complex can cause up to 100% yield losses in vegetable crops. The management of whiteflies and TYLCV are primarily dependent on chemical pesticides. However, there are growing concerns about the environmental impacts of chemicals and the rapid evolution of insect-resistance. Biological control is an environmentally compatible and sustainable alternative to chemicals. A predator species such as *Delphatus catalinae* (Coleoptera: Coccinellidae) has a capacity as an effective biological control agent for whiteflies due to its high prey consumption rates and reproductive capacity. Moreover, several studies have provided evidence that insect-virus interactions may confer benefits against host plant defenses. However, could plant viruses also interfere with the interaction between the insect pest and biological control agents? In this study, we showed how TYLCV affect the biocontrol activities of the whitefly predatory beetle *D. catalinae*. Experiments using a Y-tube olfactometer were designed to test preferences of the predatory beetle for TYLCV-whiteflies on tomato (a TYLC host plant) and collard (a non-TYLC host plant) compared to non-infected whiteflies control complexes. In a separate experiment, we compared the performance of the predatory beetle after feeding on TYLCV-infected versus non-infected whiteflies. The overarching goal of this research was to develop a better understanding of how insect-virus-host plant interactions interfere with biological control agents. Furthermore, our study is important for the development of improved IPM (integrated pest management) technologies tailored to reduce reliance on chemical pesticides, and therefore enable a more sustainable vegetable production system.

**Keywords:** biological control, predator, whitefly, begomovirus, tomato, vegetable

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## OC127. Gut content analysis in predatory mites as a tool to reveal trophic interactions in vineyards

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Biological control using predatory mites is an important component of pest management in a wide range of crops, including grapes. However, the trophic relationships established in these environments are still not fully understood. The use of molecular tools can contribute to identify the food sources of predatory mites and disentangle the complex relationships occurring in the field. We hypothesized that using qPCR and TaqMan probes we could quantify the proportion of plant based food sources and animal diet of various predatory mites throughout the season. Hence, our specific objectives were: 1) to design species-specific primers and TaqMan probes to identify the main mite groups in vineyards from La Rioja DOC Region (northern Spain), 2) to detect prey and plant products in predatory mites via PCR and qPCR, and 3) to validate these methods using predatory mites collected from the field. First, we designed specific primers-probe sets to identify the main predatory mite species in the region (*Typhlodromus pyri* and *Kampimodromus aberrans*) as well as to detect their potential prey (e.g. tetranychiids, tydeiids and eriophyid mites) and plant food sources. Then, we developed a predator-prey system (*T. pyri* and *Tetranychus urticae*) to quantify DNA by qPCR and establish post-consumption detection times of plant and animal food sources. Third, we collected predatory mites from the vineyard to unravel their prey items and establish their trophic interactions. Ultimately, detecting food sources within predatory mites could provide a useful tool to better understand the effectiveness of biological control using predatory mites in vineyards.

**Keywords:** gut content analysis, predators, pests, food web, biological control, Acari

## OC128. Aphid herbivory on macrophyte drives real-time evolution of the aquatic community

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Indirect ecological effects are thought to be important for driving evolutionary processes. However, direct evidence remains lacking. Here, by conducting real-time evolution experiments using outdoor macrocosms, we characterized the indirect effects of aphid herbivory on the evolution of aquatic communities. Two-year experiments showed that aphid herbivory reduced the growth of the macrophyte and increased the diversity and abundance of phytoplankton, which in turn increased the abundance of zooplankton that feed on phytoplankton. Whole-genome resequencing and phenotypic assays demonstrated that aphid herbivory altered genotype frequencies and increased parasite resistances in *Daphnia magna*. Transplant experiments further suggested that evolutionary changes in *D. magna* driven by aphid herbivory are adaptive. These findings provide direct evidence that indirect effects in complex multitrophic communities can drive rapid adaptive evolution in nature.

**Keywords:** indirect effects, aphid herbivory, trophic interactions, adaptive evolution, experimental evolution

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## OC129. Endophytic *Beauveria bassiana* modifies flowering phenology, floral volatile profile and pollinator behaviour in melon

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It is notable the potential of entomopathogenic fungi as microbial control agents through their contact mode of action. Recently, symbiotic relationships with plants have been attributed to them, which include evidence of changes in plant volatile emissions. The present research aims at investigating whether systemic *Beauveria bassiana* (Balsamo) Vuillemin colonisation of melon plants reached the floral tissues and affected flowering, pollen production and pollinator behaviour. Melon plants sprayed with *B. bassiana* conidial suspensions were endophytically colonised throughout the entire plant growth cycle and reached the floral tissues. Results showed that melon plants endophytically colonised with *B. bassiana* achieved a significant increase in yield of pollen grains, earlier flowering and advanced flower maturation. Indeed, a foraging preference of honeybees and bumblebees for the control plants in choice assays were detected, as expected if the advanced maturation stage of colonised plants flowers is considered. Noteworthy, changes in the emitted blend of volatiles were observed in the endophytically colonised plants, which emitted more of the pollinator attractants D-limonene and benzeneacetaldehyde than control plants. Future research should consider the mechanisms for endophyte-mediated early flowering and variations in volatile profiles throughout the blooming process, together with their impact on pollination under greenhouse conditions.

Keywords: *Apis mellifera,* biological control, *Bombus terrestris*, *Cucumis melo*, endophyte*,* tritrophic interactions

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## OC130. *Tomato trichomes and its effect* on the development and dispersal of *Tetranychus urticae* and of its promising biological control agent the phytoseiid mite *Typhlodromus (Anthoseius) recki*

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Tomato trichomes are well-known efficient defense mechanisms used by plants against herbivores but at the same time they can also impact natural enemies and the knowledge of the plant/pest/predator interactions are required for defining biological control. Cultivated tomato and its wild relatives present a high diversity of glandular and non-glandular trichomes (types, densities and locations on plants). In this study we evaluate the impact of trichome traits on development, survival, and dispersal of a pest, the two spotted spider mite (TSSM), *Tetranychus urticae*, as well as of its predator, the phytoseiid mite *Typhlodromus* (*Anthoseius*) *recki* under controlled conditions. Eight genotypes of tomatoes and wild relatives (*S. cheesmaniae*, *S. peruvianum*) presenting contrasting trichome traits were considered. Each trichome type was counted in delimited areas of tissues. The numbers of dead mites on glandular trichomes and of alive mites on each plant organ were counted three weeks after infestation (5 females, two introduction leaves, 3-weeks plants). Results suggest that density of glandular trichomes types IV and VI and non-glandular type V affect TSSM mite dispersal and development. We thus also evaluated the development and dispersal of *T. recki* on infested plants (5 predators, two introduction leaves). After three weeks, the predator was found on every organ of the plant whatever the genotype considered and it was able to control TSSM on all genotypes. Altogether, the results are highly encouraging, showing that this predator can cope very well with tomato trichomes and confirms its potential as biological control agent in phenotypically distinct tomatoes.

**Keywords:** *Solanum*, plant defense, Tetranychidae, mite pest, Phytoseiidae, biological control

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## OC131. Induced resistance by arbuscular mycorrhizal fungi in tomato: a new tool for integrated pest management programs

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Beneficial soil microorganisms can boost plant defences increasing their resistance to herbivores. Our research has revealed the contribution of arbuscular mycorrhizal fungi to improve plant direct and indirect defences against chewing herbivores in tomato. Mycorrhizal colonization in tomato reduced the performance of the generalist chewer *Spodoptera exigua* and the specialist leafminer *Tuta absoluta*. The reduction was associated to a primed accumulation of antiherbivore metabolites in challenged leaves including alkaloids and polyamine conjugates. Moreover, the volatile blends in mycorrhizal and non mycorrhizal plants differ, and enhanced attraction of natural enemies of the pests-commonly used in biocontrol programs- have been observed in challenged mycorrhizal plants. Comparisons across different experimental scales from controlled lab set-ups to commercial production conditions evidenced the robustness of the effects, the compatibility with other biocontrol methods, and accordingly, the potential of mycorrhiza induced resistance to be incorporated in current Integrated Pest Management Programs.

**Keywords:** *Tuta absoluta*, tomato, primed defenses, Nesidiocoris tenuis, HIPVs, multitrophic interactions

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## OC132. Beneficial microbes to optimize pest control in sustainable tomato production

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Plants are continuously challenged by biotic stressors such as herbivorous arthropods and pathogenic microbes. Nevertheless, plants also interact with beneficial organisms such as certain soil microbes which are known for their ability to improve plant growth, antagonize pathogens and prime plants against future attacks via plant defense elicitation. In this work, we hypothesized that beneficial soil microbes can protect plants also against herbivores hence, serve as biological control agents in Integrated Pest Management programs. We assessed the plant-mediated effects of a series of beneficial soil fungi and bacteria against key pests of tomato namely, *Tetranychus urticae*, *Trialeurodes vaporariorum*, *Frankliniella occidentalis*, *Tuta absoluta* and *Myzus persicae*, and their natural enemies, and studied the molecular and chemical mechanisms underlying beneficial microbe-tomato interactions which enhance tomato resistance against key pests. Our results identified promising bacterial and fungal strains with efficacy against tomato pests via the plant, as well as specific molecular and chemical components of tomato direct and indirect defense that were differentially affected by tomato inoculation with these microbes.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “1st Call for H.F.R.I. Research Projects to support Faculty Members & Researchers and the Procurement of High-and the procurement of high-cost research equipment grant” (Project Number: 50).

**Keywords:** arthropod pests, beneficial fungi, plant defense, predators, tomato

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## OC133. The multifaceted nature of the modulation of plant responses to insect herbivory by beneficial microbes

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Inoculation of plants with beneficial microbes is increasingly recognized as a powerful tool to enhance plant defense against insect pests. Arbuscular Mycorrhizal (AM) fungi can sensitize the plant’s immune system, resulting in priming of defences against leaf-chewing insects. However, meta-analyses show that effects of AM fungi on plant defense range from increased resistance to increased susceptibility. We present two studies in ribwort plantain (*Plantago lanceolata*) showing that such contrasting effects may reflect ontogenetic changes during plant life, and unexpected ways in which AMF interfere with plant responses to herbivory.First, we show that the direction of effects of AMF on the leaf chewing insect *Mamestra brassicae* shifts during plant ontogeny. In young plants, AMF enhances leaf nutritional quality, increasing the insect’s efficiency of conversion of ingested food (ECI). However, as plants age, effects of induction of leaf defenses by AMF override nutritional effects, reducing caterpillar ECI and growth. Second, we show that leaf transcriptional responses of *P. lanceolata* to insect herbivory by *Spodoptera exigua* can be either reinforced or counteracted by AMF. Surprisingly, when considering transcripts affected by both herbivory and AMF, 40% showed changes in opposite directions in response to AMF and herbivory. These include antagonistic effects of AMF on plant responses to herbivory, e.g. suppression of herbivore-induced plant nitrogen storage. We conclude that plant beneficial microbes have multifaceted effects on plant responses to herbivory, contributing to their variable effects on plant defense, that should be taken into account when developing products intended for microbially-based pest control.

**Keywords:** Plant-microbe-insect interactions, microbe-induced resistance, AMF, *Spodoptera exigua*, *Mamestra brassicae*

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## OC134. Microbe-Induced Resistance against Tuta absoluta: from the lab to the field

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The tomato leaf miner *Tuta absoluta* is an invasive insect pest and a major threat to global tomato production. The integrated management of this pest includes chemical and biological control, mass trapping and mating disruption, as well as agronomic and cultural control. Yet, little is known about the efficacy of microbe-induced plant resistance against *T. absoluta* and its potential to contribute to the integrated management of this devastating pest. In this study we tested diverse plant beneficial bacteria and fungi for their ability to trigger induced resistance in tomato plants against *T. absoluta* and explored possible underlying mechanisms. We performed bioassays to assess microbe-induced resistance under controlled, semi-controlled and agronomic conditions. To explore the possible underlying mechanisms, we conducted an untargeted metabolomics analysis to identify defense-related metabolites with primed accumulation in the plants displaying induced resistance. Functional analysis of these primed metabolites was performed to test their potential anti-herbivory activity on *T. absoluta*. We found that *Trichoderma harzianum* and the arbuscular mycorrhizal fungi *Rhizophagus irregularis* and *Funneliformis mosseae* consistently triggered induced resistance under all the conditions tested, reducing *T. absoluta* performance or incidence. We showed that these beneficial fungi can modulate plant defense responses through metabolic reprograming and primed accumulation of defensive compounds with deleterious effects on *T. absoluta*. Our results under agronomic conditions supported the compatibility of microbe-induced resistance with current crop management practices. Thus, microbe-induced resistance can be incorporated in integrated pest management programs, improving the sustainable control of *T. absoluta*.

Keywords: arbuscular mycorrhizal fungi, field research, metabolomics, microbe-induced resistance, *Trichoderma*, *Tuta absoluta*

# Session 6:

## Insect Biotechnology

## OC135. RNA-based biocontrols: successes, challenges, and learnings in different insect species

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RNA-based biocontrols use the natural antiviral defence mechanism, RNA interference (RNAi), to selectively control insect pests. One challenge in developing these biocontrols against a broad range of pest insects is the variable sensitivity to dietary uptake of the biocontrols (dsRNA) between insect species. For example, certain beetle (Coleoptera) species are highly sensitive to small amounts of ingested dsRNA, while in caterpillars (Lepidoptera) it has proven difficult to achieve a commercially relevant activity level using an insecticidal RNA-based biocontrol. Here, we discuss these differences in sensitivity between insect species and the loss mechanisms that can cause them. We will have a closer look at these differences in species from 3 different orders (Coleoptera, Hemiptera and Lepidoptera) and evaluate them more precisely in a quantitative way. We will also discuss the expansion of our ADME-toolbox, which allows us to further investigate these loss mechanisms. Various techniques, including RNA sequencing and fluorescent confocal microscopy have been developed in-house to investigate the fate of dsRNA in the insect body. While sequencing allows us to understand what is happening to the dsRNA molecules in vivo, our fluorescent/confocal microscopy-based approaches allow us to track fluorescently labeled dsRNA in the insect body and investigate cellular uptake more closely.While our investigations have already revealed some interesting differences between these species, further investigations are needed to fully understand the role of these physiological barriers and how they affect RNA-based biocontrols efficacy. Assessing these factors in different insect species could direct us towards solutions to overcome these barriers.

**Keywords**: RNAi, RNA interference, biologicals, insect pest control, ADME, insect physiology

## OC136. RNAi in Integrated Pest Management

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The biodegradability and target-specificity of double-stranded RNA (dsRNA), puts RNA interference (RNAi) at the forefront of biosafe insecticide technology. RNAi-based applications are being developed to improve plants by modifying endogenous gene expression as well as to target pest and pathogen genes both within plants (i.e. host-induced gene silencing) and as topical applications (e.g. spray-induced gene silencing). Although these RNAi-based plant protection products show much potential, there remain critical knowledge gaps in each of these areas. Especially for the SIGS approach, RNAi efficacy needs to be improved by overcoming the barriers of RNAi. In addition, there is an urgent need to develop appropriate science-based risk assessment procedures for topical RNAi applications. And particular emphasis must be placed on ensuring RNAi's compatibility with integrated pest management (IPM).

**Keywords:** RNAi, IPM, insects, SIGS

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## OC137. The quest for the best dsRNA target sequences for pest control by a genome wide screen

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RNAi is an emerging technology for eco-friendly and species-specific pest control. One of the challenges is the identification of the best RNAi target sequences, i.e. those that lead to the death of the target at minimal concentrations and with minimal side effects on other species. The difficulty in performing large-scale screens in pest species has limited the number of genes that have been tested in the past. One way to gain a comprehensive view is to use genetic model systems, which are more easily subjected to genome wide RNAi screens. However, it has remained an open question, in how far target genes identified in one species can be transferred to other species. In a genome wide RNAi screen, we have identified the most efficient RNAi target genes in the red flour beetle *Tribolium castaneum*. We present the results of that screen and show what we learned about efficienttarget genes in our ongoing follow-up analyses. Further, we discuss the variability that is found when transferring RNAi target genes to other pest species. At the end, we outline a procedure to establish RNAi in pest species and to identify the best target genes for that purpose.

**Keywords:** RNAi, target genes, *Tribolium castaneum*

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## OC138. Advances towards RNA interference-based management of *Brassicogethes aeneus*

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Oilseed rape (*Brassica napus* L.) is a widely grown crop in several continents and one of the most intensively grown crops. Management of major oilseed rape pests has relied on synthetic insecticides, resulting in resistance development in pest populations, loss of insect biodiversity in agricultural landscapes, and pesticide contamination in the environment. Therefore, there is urgent need to develop pest management strategies that are both effective and environmentally sustainable. In this regard, crop protection based on RNA interference (RNAi) represents a potentially favourable approach compared to broad-spectrum pesticides, as the nucleotide sequence-dependent mode of action of double-stranded RNA (dsRNA) makes RNAi uniquely selective compared to conventional pesticides, and may poses no- or negligible risk to non-target organisms.Our studies we focus on research findings regarding the potential efficacy of applying RNA pesticides towards managing a major oilseed rape pest in Europe – the pollen beetle *Brassicogethes aeneus*. Studies have shown that microinjection with dsRNA and dietary exposure (sugar water, oilseed rape anthers and buds) have an effect on pollen beetle survival. Mortality rates depended on the method of dsRNA administration, targeting gene selection, duration of dietary exposure to treatments, dsRNA concentration, and pollen beetle development stage. Altogether, results suggest potential for developing RNAi approaches to control pollen beetle populations. However, further research is necessary in order to better understand- and optimise these approches.

Keywords*:* pollen beetle*, Melighetes aeneus*, pest management, oilseed rape

## OC139. Development of a Novel Biopesticide using an RNA Interference Approach Targeting Potassium and Fibroblast Growth Factor in *Helicoverpa armigera*

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Crop protection is a serious issue that is a concern globally. Insect pests are responsible for 10 – 20% of crop damage worldwide, and levels of crop damage have increased by up to 40% in developing countries in recent years. *Helicoverpa armigera* is a Lepidopteran insect and a global pest, causing huge damage to many economically important crops, including tomatoes, cotton, maize and peas. The annual estimated cost associated with this insect is approximately US$5 billion globally, representing both crop damage and the use of pesticides. In China and India, 50 % of pesticides used are applied to control this pest. RNAi is an innovative approach for pest control and has been successfully used with several lepidopteran species. The aim of this project is to develop a novel and sustainable form of pest control based on RNAi targeting potassium ion channel and fibroblast growth factor genes in *Helicoverpa armigera* and to evaluate the biosafety of the use of dsRNA on non-targeted organisms such as bumblebees. Knockdown of both target genes (potassium ion channel and fibroblast growth factor) was observed by qPCR and the mortality rate was measured after delivery of dsRNA through microinjection, feeding and soaking eggs. Biosafety studies were performed by using adult bumblebees in which no significant changes in gene expression and mortality rate were observed.

Keywords: RNAi, *Helicoverpa armigera*, biosafety

## OC140. RNAi-mediated knockdown of essential central nervous system genes as a potential *Spodoptera littoralis* control strategy

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Globally, more than 700 million people are undernourished and, with an estimated population of 10 billion by 2050, this figure is likely to increase. *Spodoptera littoralis* is a highly polyphagous lepidopteran pest capable of extensive damage to a range of crops including *Solanum lycopersicum*, an important food crop in low-middle income countries. S. littoralis is predominantly controlled by chemical insecticides but, due to environmental concerns, there is increasing demand for more sustainable options. RNA-interference (RNAi), a natural mechanism that regulates the expression of endogenous genes, can be used to knock down the expression of target genes within an organism via the application of dsRNA. Here, the ace-1 (acetylcholinesterase), para (voltage-gated sodium channel) and naChR (nicotinic acetylcholine receptor) genes were selected, all of which are targets for current insecticides and are essential to the functionality of the insect CNS. Initially, PCR amplified each target gene at each life stage and RT-qPCR demonstrated that, for all genes, expression was highest in the pupae stage. In terms of RNAi, although some phenotypic changes were observed, the application of gene specific dsRNA did not lead to significant mortality, regardless of insect life stage or administration method. Consequently, various methods were used to enhance RNAi susceptibility, including the use of nanocarriers and immune system priming. Extensive bioinformatic analyses suggested that the dsRNA-based strategies would be safe to use and would not affect a range of non-target organisms. Furthermore, bioassays confirmed that the dsRNA molecules were safe against an important pollinator species, *Bombus terrestris*.

Keywords: biopesticides, RNAi, nanocarriers, bioinformatics

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## OC141. Optimization of small interfering RNAs derived from double-stranded RNAs for enhanced pest control

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The discovery that exogenous double-stranded RNA (dsRNA) is able to induce the cleavage of complementary mRNA via RNA interference (RNAi) has initiated efforts to develop RNAi-based pest control strategies, especially against insects. Although RNAi-based strategies have various advantages over chemical insecticides such as superior species-specificity, they are currently inferior in terms of their killing potency. Previously, parameters that distinguish efficient siRNAs have been described. In this study, we hypothesized that a rational design of the dsRNA sequences — by considering the downstream generation of small interfering RNAs (siRNAs) — could maximize the lethal effect. In order to test this idea, an application was established that uses several previously described predictors of efficient siRNAs to identify the best siRNAs. It was applied to target the most effective lethal genes previously revealed by our lab in the red flour beetle, *Tribolium castaneum*. The dsRNAs were designed such that the processing of the prospective siRNA sequences was favored considering the hypothetical cleaving properties of insect *Dicer-2* homologs. After injecting the dsRNAs into *T. castaneum* larvae, we investigated the survival rates and the generated siRNA pools via small RNA sequencing for the confirmation of our approach. Overall, the study gave insights into how a rational design of dsRNAs could lead to more competent RNAi-based insect pest control.

Keywords: dsRNA, siRNA, small RNA-seq, RNAi, pest management, biotechnology

## OC143. PIWI proteins play an antiviral role in lepidopteran cell lines

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Insect antiviral immunity primarily relies on RNAi mechanisms. While a key role of small interfering (si)RNAs and AGO proteins has been well established in this regard, the situation for PIWI proteins and PIWI-interacting (pi)RNAs is not as clear. In the present study, we investigate whether PIWI proteins and viral piRNAs are involved in the immunity against single-stranded RNA viruses in lepidopteran cells, where two PIWIs are identified (Siwi and Ago3). Via loss- and gain-of-function studies in *Bombyx mori* BmN4 cells and in *Trichoplusia ni* High Five cells, we demonstrated an antiviral role of Siwi and Ago3. However, small RNA analysis suggests that viral piRNAs can be absent in these lepidopteran cells. Together with the current literature, our results support a functional diversification of PIWI proteins in insects.

**Keywords**: Ago3, antiviral immunity, Argonaute, RNA interference, Siwi, small RNAs

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## OC144. Assessment of insect virus-like particles as nano-vehicles for efficient dsRNA delivery in insect tissues and cells

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RNA interference (RNAi) is considered as an important tool in the arsenal of future pesticides that minimize the negative impact on the environment or human health. To be able to control most insects, however, the efficiency of delivery of RNAi needs to be improved considerably, which has led to research into various "carrier" molecules that encapsulate dsRNA and promote its cellular uptake. One approach is based on the use of viruses as vehicles for RNAi but current legislation in Europe discourages genetic modification of agents that multiply in host cells. An interesting alternative would be the employment of virus-like particles (VLPs) that lack replicative genetic material but still contain the (protein-based) functions to protect cargo (such as dsRNA) and penetrate tissue and cellular barriers. In our research, VLPs of various compositions could be produced after the over-expression of capsid proteins of insect-specific viruses by the baculovirus expression vector system (BEVS). Successful purification was achieved after sucrose density ultracentrifugation of cellular extracts and different protocols were investigated for the loading of dsRNA cargo into the VLPs. Of note was the observation that the BEVS was not very successful for the co-production of dsRNA molecules with capsid proteins that would have allowed loading of dsRNA during VLP assembly. On the other hand, the approach of dis-/re-assembly of VLPs in the presence of large amounts of exogenous dsRNA proved successful and "RNAi-VLPs" are currently being tested in dipteran and lepidopteran cell lines and larvae for delivery of dsRNA and silencing of gene targets.

**Keywords:** RNAi, virus-like particle, pest control, Diptera, Lepidoptera

### OC145. Leveraging the role of *Dnmt1* in early development for potential use in whitefly pest management

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The whitefly *Bemisia tabaci* is a globally important crop pest that is difficult to manage through current commercially available methods. RNA interference (RNAi) is a promising strategy for managing this pest. However, effective target genes remain unclear. The use of reproduction-related target genes is largely unexplored, despite pest management methods that interfere with insect reproduction and induce infertility being generally considered efficient and safe. We suggest *DNA methyltransferase 1* (*Dnmt1*) as a potential target gene due to its effect on inhibiting reproduction in other insect taxa. We investigated the role of *Dnmt1* in *B. tabaci* oogenesis and embryogenesis to identify its usefulness as a target gene. Using RNAi membrane feeding, we downregulated *Dnmt1* in virgin, asexual females and show that reduction of DNMT1 protein decreased egg production and egg viability. Using immunohistochemistry, we show that knockdown-induced oocyte abnormalities potentially resulting in egg inviability. We also show that knockdown of *Dnmt1* negatively affects embryo development. Together, these results suggest that *Dnmt1* is crucial to *B. tabaci* egg oocyte and embryo development, making it a suitable target gene for pest management.

**Keywords:** *DNA methyltransferase*, RNA interference, *Bemisia tabaci* , reproduction, embryogenesis, oogenesis

### OC146. RNA Spray for Plant Protection - Knockdown of p300 reduces lifespan and causes early onset and reduction of offspring in *Myzus persicae*

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Green Peach Aphids (*Myzus persicae*) are the main transmitter of several yellowing viruses in sugar beet, thereby reducing sugar content and causing significant crop loss. They developed resistances against many commercially used chemical compounds due to their fast reproduction cycle and quick adaptation. To prevent crop loss, innovative environmental friendly insect pest control methods should be elaborated. RNA interference (RNAi) has due to its mode of action species specific effects and is a promising alternative to chemicals. In previous studies in *Acyrthosiphon pisum*, the knockdown of p300, a CREB-binding protein resulted in reduced survival and premature offspring. We hypothesized that a p300 dsRNA treatment, could cause similar effects in *Myzus persicae*. We monitored the dsRNA-injected aphids for 10 days on sugar beet seedlings under greenhouse conditions. The p300-injected aphids had an increased mortality of ~64 % compared to non-specific dsRNA-injected and non-injected aphids. During the monitoring, we observed several premature nymphs within the p300-treated group; further research needs to be conducted. Knockdown of specific genes in *Myzus persicae* resulted in reduced survival and potentially other effects exceeding previous results with *Acyrthosiphon pisum*. The discovery of highly specific functioning dsRNA could result in the development of new biological insecticides, reducing environmental and non-target damage – opening a new path to biological safe pest control.

Keywords: RNA interference, *Myzus persicae*, pest control, p300

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## OC147. Effective and Sustainable Control of *Halyomorpha halys* (stink bug) using a Novel Alginate-Encapsulated dsRNA Formulation

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*Halyomorpha halys* (Hemiptera; Pentatomidae) is an invasive pest that causes significant economic damage to crop production in Europe and many American countries. As synthetic chemicals are becoming less available due to being forbidden or no longer effective against resistance development, effective and selective "green" alternatives are needed. One promising solution is RNA interference-based methods, but the stability of dsRNA in the natural environment presents a major challenge due to various external and internal factors. To overcome these challenges, encapsulation technologies are ideal for delivering various formulation components with different features. However, the identification of components that can interfere or block nuclease activity is a major challenge in developing effective formulations. Here we provide evidence that dsDNA can compete with nucleases and improve the stability of dsRNA in the saliva of *H. halys*, making it a promising formulation component for a dsRNA delivery system. Our finding suggests that the use of dsDNA could increase the efficacy of oral RNAi as an insect pest control strategy in the field. More research is needed to determine the optimal ratio of dsDNA to dsRNA and test the effectiveness of this formulation in vivo. Nonetheless, this finding represents a crucial step towards developing a new, low-cost, and eco-friendly approach to insect pest management. Overall, RNA interference-based methods

using encapsulation technologies are a promising alternative to synthetic chemicals for pest control. By identifying and incorporating components that interfere or block nuclease activity, the stability of dsRNA can be improved, making it more effective in controlling insect pests.

**Keywords:** RNAi, *Halyomorpha halys*, dsRNA encapsulation, formulation, nucleases

## OC148. Species-specific dsRNAs designed against agricultural pests show no significant cross-species activity against *Coccinella septempunctata*

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RNA interference (RNAi) mediated crop protection promises targeted insect control due to its sequence-dependent mode-of-action, a strategy that also offers the potential to be implemented in integrated pest management (IPM). RNAi can be used in combination with biological control agents like parasitoids and predators, however, potential non-target effects to beneficial insects are poorly understood and prompt the demand for risk assessment studies. To investigate this, we designed species-specific double stranded RNA (dsRNA) constructs targeting the RNAi target genes *Rop* and *ncm* of four different coleopteran species. These included three pest insects *Tribolium castaneum*, *Leptinotarsa decemlineata* and *Brassicogethes aeneus*, as well as the biological control agent *Coccinella septempunctata.* First, the susceptibility of all four beetle species was verified in a worst-case exposure scenario by selectively killing the target species via injection of species-specific dsRNA. Knockdown via injection resulted in ~80 % mortality in all four species 10 days post-treatment with species-specific dsRNA. In contrast, no significant mortality was observed in the non-target insect *C. septempunctata* when injected with non-specific dsRNAs. Once the risk of exposure was confirmed, *C. septempunctata* larvae were fed with aphids previously injected with target pest specific dsRNA and no lethal or sub-lethal effects were identified. This study can provide preliminary indications about real world exposure scenarios and guidance for safety assessment of future RNA-based pest control strategies.

**Keywords**: RNAi, risk assessment, pest control, non-target effects, lady beetle

## OC149. Recoding immunity in the malaria mosquito midgut

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The mosquito midgut is a fascinating system to study the interactions between the immune system and microbes. The mosquito encounters a variety of microbes throughout its life history, some of which colonize the midgut, forming rich microbial communities that help with diet digestion and defense against pathogens. Our research in the malaria mosquito *Anopheles gambiae* has revealed a gradient of immune resistance and quiescence along the anterior-posterior midgut axis. Antimicrobial peptides and other effectors are highly expressed in the cardia, the anterior-most part of the midgut, but their expression is significantly reduced or totally abolished in the posterior midgut, leading to a highly immunotolerant environment. Therefore, the cardia appears to act as an immune organ that filters diet-borne microbes before they reach and colonize or infect the posterior midgut. This pattern is developmentally regulated, and preliminary work has identified some of the transcription factors involved in establishing this pattern. Capitalizing on this knowledge, we engineered transgenic mosquitoes to express peptides with strong antimalarial activities, either constitutively in the cardia or in the posterior midgut upon mosquito blood feeding. These modifications were shown to alter the capacity of mosquitoes to transmit the human malaria parasite *Plasmodium falciparum*, by either reducing the numbers of malaria parasites that survive to infect the midgut tissue or by interfering with parasite growth and differentiation. Computational models confirmed that these modifications, when combined with gene drive towards mosquito population replacement, hold a great potential to suppress or eliminate malaria transmission in various malaria-transmission settings.

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## OC152. An efficient method for CRISPR/Cas9 mediated gene editing in difficult to transform arthropods

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The control of arthropod pests is crucial to protect crops and to sustain animal and human health. Biotechnological approaches are essential for discovering alternative safe products for the management of these detrimental pests. Gene editing by CRISPR/Cas9 has revolutionized genetic work in many organisms, and methods mostly rely upon delivery of the Cas9 ribonucleoprotein (RNP) complex into the egg/embryo via microinjection. Unfortunately, in many species embryo injection has proven extremely difficult if not impossible. This is not necessarily because of biological embryonic constraints or technical issues with injection, but also because eggs/embryos are extremely difficult to collect. Within the Chelicerata, the second largest group of arthropods, only two species have been genetically transformed so far, with limited success. For one of them, *Tetranychus urticae*, embryo injection always fails despite much dedicated research. An alternative method, is to deliver the RNP complex to the germline by injecting female adults near or in the ovaries. Using this approach, *T. urticae* was recently genetically transformed, but the low efficiency hampers the implementation of CRISPR. We present here a novel Cas9 method with gene editing efficiencies up to 30% for knock-outs, and around 5% for knock-ins. This method was devised using *T. urticae*, but we also reveal the wide application breath of the method to other pests that have been impossible to transform. We therefore anticipate that the novel method can be a game changer for genetic research in previously hard to transform arthropods.

**Keywords:** CRISPR, delivery, gene editing

## OC153. Decifering the role of ROS/CncC signaling pathway in the adaptation of *Spodoptera frugiperda* to plant allelochemicals and insecticides using Sf9 cells

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The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a polyphagous pest feeding on numerous host-plants including important crops such as maize, rice and sorghum. FAW has developed sophisticated adaptive mechanisms to eliminate xenobiotics (plant secondary metabolites and insecticides), among them, an efficient machinery of detoxification enzymes. They are often expressed at low basal level and induced when the insect is exposed to xenobiotics. While the role of these enzymes is well characterized in several pest insects, the transcription factors controlling their expression remain largely unstudied. We explored the role of the transcription factor Cap'n'collar C (CncC) using the FAW cell model, Sf9 cells and with the strategy of either over-expressing it or suppressing its expression by Crispr/Cas9. Our results show that CncC has a central role in tolerance to xenobiotics such as methoprene and indole-3-carbinol. The activation of this signalling pathway is mediated by reactive oxygen species. A transcriptomic approach allowed us to explore the genes expressed under the control of this transcription factor. This knowledge helps to better understand pathways in detoxification gene expression and can be helpful to design next generation insect control measures.

**Keywords:** detoxification, CncC, resistance, methoprene, indole 3-carbinol

## OC154. Development of a next-generation CRISPR-mediated population control strategy for Tephritid pests as a replacement for traditional sterile insect technique

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*Ceratitis capitata*, commonly known as the medfly, is a Tephritid pest known best for its global distribution, vast economic impact, and flexible host selection. Here, we developed a highly efficient CRISPR/Cas9 toolkit

for use in the medfly to establish a fully-fledged precision-guided sterile insect technique (pgSIT). PgSIT, which utilizes CRIPR/Cas9 technology to simultaneously target genes vital for female development and male fertility generating a sterile male-only progeny, was recently developed (Kandul et al., 2019) as a replacement for the traditional, cost-ineffective SIT. To date, pgSIT has already been successful in *Drosophila* and *Aedes aegypti*, and in this project we showcase the feasibility of this approach in the medfly.PgSIT involves crossing separate guide RNA (gRNA) and Cas9-expressing lines to obtain hybrid progeny with working CRISPR machinery. Our toolkit development consisted of gRNA and Cas9 promoter selection for optimal CRISPR/Cas9 activity. Transgenic lines were established with piggyBac constructs harbouring either Cas9 or gRNA under the control of different promoters targeting the model *white eye* gene resulting in over 20 stains with unique integrations. We assessed the efficiency of two new endogenous U6 promoters for gRNA expression and 4 promoters for Cas9 expression through eye phenotype screening, where *D. melanogaster* polyubiquitin promoter resulted in 93% desired phenotype prevalence upon crossing with the best-performing gRNA line. Next, this Cas9 line will be crossed with gRNA-expressing lines targeting a female development gene, *transformer*, and a male fertility gene, *beta2-tubulin*, both of which are well-conserved among Tephritids and can be applied to other species.

**Keywords:** SIT, pgSIT, CRISPR, medfly, Tephritids

## OC155. Investigating Chemosensory Protein Ligands as Novel Synergists for Insecticides

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Insect control largely relies on chemical insecticides; however, insects have an intriguing ability to develop insecticide resistance. The elucidation of the underlying molecular mechanisms and their interactions is critical for in-depth understanding of resistance, to inform management strategies and develop efficient, environmentally friendly new products. Our recent research, using the *Drosophila* genetic toolbox and CRISPR/Cas9 genome modification, has provided significant insights on relevant mechanisms as well as their synergistic interaction. Furthermore, we investigate the potential role of chemosensory protein family (CSPs) genes. It has been demonstrated that a mosquito CSP, SAP2 binds pyrethroids and participates in a hitherto non-characterized mechanism, which likely manifests through synergistic interactions with other resistance pathways. In this research, we expand on these findings to: a) investigate the potential role of specific CSPs, b) test potential synergism of candidate CSPs with other mechanisms and c) suggest lead compounds that act antagonistically to CSP/insecticide binding, thus suppressing associated resistance.We have functionally expressed biologically relevant candidate CSPs and propose a framework to validate their insecticide-binding potential. This will allow the use our established *Drosophila* “test-tube” system to demonstrate that CSPs can confer resistance *in vivo* and examine CSP synergistic action with other mechanisms. Finally, we propose a framework to biophysically characterize selected promising candidates for their interaction with insecticides and use a ligand-based strategy to explore chemical space for identifying new pharmacophore structures that will lay the groundwork for innovative research on the development of lead inhibitory molecules, generating significant application potential for the biotechnology industry.

Keywords: functional expression, chemosensory proteins, ligands, synergists, lead compounds, pharmacophore backbone

# Session 7:

## Symbiosis and Insect Pathology

## OC157. Invasion dynamics of *Wolbachia* in cherry fruit flies

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Microorganisms are ubiquitous in the insect world. Therefore, knowledge on how they invade, spread, and establish within natural insect populations is crucial. *Wolbachia* are common endosymbiotic bacteria that are widely distributed especially in arthropods. Key factor of their successful lifestyle is their ability to manipulate the reproduction of their hosts and promote vertical transmission. To establish within a new host and to invade a new host species, *Wolbachia* must be able to shift the species border and establish a stable association within the new host. Although many studies documented horizontal *Wolbachia* transmissions between species on evolutionary timescales, examples of horizontal transmission in field populations, particularly the early stages of *Wolbachia* spread in new hosts, are still poorly understood.

Here I will present the dynamics of *Wolbachia* on two cherry fruit fly systems. I show the dynamics of a currently spreading *Wolbachia* strain in the European cherry fruit fly *Rhagoletis cerasi* and present a novel example of a recently horizontally acquired *Wolbachia* strain from the European cherry fruit fly to the invasive American cherry fruit fly *Rhagoletis cingulata*. These unique examples of a recent intraspecifically acquired *Wolbachia* provide new insights into the invasion dynamics of *Wolbachia* in natural field populations.

Keywords: *Wolbachia*, Infection dynamics, horizontal transmission, genomics, cytoplasmic incompatibility, *Rhagoletis*

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## OC158. Complex host modifier systems control two symbiont-mediated reproductive phenotypes in a tetranychid mite

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Maternally transmitted symbionts such as *Wolbachia* induce sex allocation distortion (SD) and cytoplasmic incompatibility (CI) in haplodiploid arthropods. SD and CI are typified by great variation in strength across host-symbiont systems and numerous hypotheses have been suggested to explain the underlying mechanisms. Here, we tested whether and how natural genetic variation of the host modulates these *Wolbachia*-mediated reproductive phenotypes. Using a single *Wolbachia* variant, a nuclear genotype reference panel of the spider mite *Tetranychus urticae* was created that consisted of infected and cured near-isogenic lines. To unravel the role of host modulation in CI, we performed a highly replicated age-synchronized full diallel cross. We observed striking variation in CI and uncovered that infected males modulate different features of CI compared to uninfected females. Both male and female modulation interacted with the genotype of the mating partner, suggesting that these modifier systems are underpinned by complex genetic architectures. To unravel the role of host modulation in

SD, we first demonstrated that this *Wolbachia*-mediated reproductive phenotype is driven by increasing egg size, promoting egg fertilization. We gathered evidence that host modulation is an important determinant for SD strength and acts by regulating the egg size effect. Using a parametrized deterministic model, we simulated *Wolbachia* invasion in different host populations. Our simulations uncovered a prominent role of the mite genotype in the spread of *Wolbachia*. Together, these findings underscore the importance of host genetics for symbiont-host interactions and help elucidate the mechanisms that underlie the widespread occurrence of maternally transmitted symbionts in haplodiploids.

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## OC159. Zombie-flies: Is symbiosis at the heart of behavioural manipulation by an insect-destroying fungus?

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Certain insect pathogens can manipulate the behaviour of their hosts to increase their chance of transmission. The specialist fungal pathogen *Entomophthora muscae* turns their housefly hosts into so-called 'zombie-flies' after six days of infection. There are three stereotypical and sequentially observed manipulated behaviours: (1) summiting, whereby the fly climbs to an elevated position, (2) proboscis affixation, where the fly 'glues' itself to the substrate surface, and (3) wing raising, where the wings raise to not obstruct the fungal spores that will be shot out of the abdomen. We are using comparative transcriptomics to assess how this pernicious hijacker provokes these striking moribund displays and how the host responds. Preliminary analyses have uncovered a handful of candidate genes, including two secreted effector proteins. Moreover, we have found an overwhelming amount of RNA reads that correspond to a known mycoviral symbiont. Iflaviruses are known to have a role in insect manipulation, e.g. bodyguard behaviour in ladybirds. This supports the idea of a possible manipulation role of our virus, potentially redefining what we know about host-fungus interactions. I will present our insights into the genetic underpinnings of the co-evolutionary processes of this tri-Kingdom interaction leading to the extended phenotypic response of zombie-flies.

**Keywords:** behavioural manipulation, entomopathogenic fungus, housefly, virus, host-pathogen interactions

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## OC160. Diversity in symbiont-mediated killing and integrity of defensive symbiosis at extreme temperatures underpin protective efficacy in an anti-parasitoid insect-bacterial symbiosis

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Internal symbioses between insect hosts and bacteria are ubiquitous in nature. Among the most famous of these is the relationship between the pea aphid (*Acyrthosiphon pisum*) and the bacteria *Hamiltonella defensa*. *H. defensa* protects its aphid host from the specialized braconid parasitoid *Aphidius ervi* by killing the parasitoid as it attempts to develop inside the aphid. While it is known that the *H. defensa* genotype is diverse and environmentally sensitive and associated with commensurately variable rates of anti-wasp protection, our understanding of the killing phenotype itself is still nebulous. To further elucidate the diversity of defensive phenotype in aphids infected with *H. defensa*, we tested for strain specificity in the symbiont's effect on parasitoid development and mortality. Carrying out mid-parasitism dissections in aphids of identical host background, but different symbiont strain, we reveal a variety of killing strategies, which display strain-level diversity in the symbiont's effect on wasp development and time of mortality. These strain-specific killing strategies also differentially affect particular components of the wasp's parasitism arsenal, namely specialized cells called teratocytes. Additionally, we demonstrate that even within single clades of *H. defensa*, there is strain-level variety in integrity of defense in the face of sub-optimal temperatures. Together, these results further enhance the picture of the *H. defensa* symbiosis as a highly varied, versatile and durable phenomenon, which ultimately has broad agroecological implications.

Keywords: facultative symbiosis, endoparasitoid, parasitism, heat resistance

## OC161. *Wolbachia*-induced cytoplasmic incompatibility to control *Drosophila suzukii*

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The development of environmentally friendly pest management practices is generally based on processes that occur in the wild, but which can be intensified for the benefit of cultivated crops. *Wolbachia*-induced cytoplasmic incompatibility causes reproductive barriers. It is therefore one such natural process that potentially underpins alternative pest control strategies. In this context, we studied *Drosophila suzukii* population dynamics during planned invasions of incompatible *Wolbachia* strains. A theoretical model suggested that pest populations at carrying capacity could be driven to extinction following the introduction of a small number of conspecifics harboring an incompatible *Wolbachia* strain. In contrast, laboratory experiments on *D. suzukii* populations at carrying capacity did not show such a predicted effect. Effects of *Wolbachia* inoculation did occur, but only when populations were small and growing or controlled by extrinsic processes. Our results suggest that control strategies based on *Wolbachia*-induced cytoplasmic incompatibilities are sensitive to negative density dependence. Therefore, they should be applied early in the reproductive season or in combination with other strategies.

**Keywords:** density-dependence, Allee effect, transient populations dynamics, pest management

## OC162. Evolutionary history of *Cacopsylla* species and their little helpers: diversity of endosymbionts and their co-evolution

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*Cacopsylla* species are phloem-sucking insects (Hemiptera; Psylloidea), which feed on a wide range of plants. Some species are considered important agricultural pests since they act as vectors of the plant pathogen phytoplasma. Like other sap-feeding insects, *Cacopsylla* maintain obligate, heritable relationships with symbiotic bacteria. These symbionts help the insects in overcoming nutritional deficiencies caused by a diet low in amino acids and vitamins. The primary endosymbiont '*Candidatus* Carsonella ruddii' together with a co-primary symbiont play an essential role in the supplying of these nutrients. Here we report the presence of an undescribed second endosymbiont affiliated with the *Enterobacteriaceae* family (proposed name '*Candidatus* Psyllophila symbiotica') in the apple psyllids, *C. melanoneura* and *C. picta*. Even though both symbionts have small genomes (170-230 kb) characterized by a low GC content, the second co-primary symbiont *Psyllophila* complements the genes missing in *Carsonella*. Such genome reduction indicates an ancient association with the psyllid host. Additionally, we assembled and analyzed the genomes of bacterial symbionts in other five European *Cacopsylla* species. *Carsonella* was found in all the analyzed psyllids, whereas *Psyllophila* was present in only four species. Interestingly, a *Sodalis*-like bacterium with a much bigger genome (1 Mb) replaced *Psyllophila* in the pear psyllid *C. pyrisuga*. Its relatively big genome size suggests that the symbiont replacement happened only recently. We present a comparative genome analysis of the different psyllid symbionts as well as a genome-based phylogeny of the symbionts and their hosts and discuss their co-evolution history.

Keywords: *Cacopsylla* species, nutritional dual-symbiosis, *Carsonella*, comparative genomics, phylogenomics, mitogenomes

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## OC163. Effects of high temperatures exposure on *Halyomorpha halys* (Hemiptera: Pentatomidae) and its bacterial endosymbiont *Pantoea carbaekii*

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*Halyomorpha halys* (Hemiptera: Pentatomidae) is an invasive insect able to cause remarkable economic losses in different crop productions. This pest became a severe problem due to its rapid invasion, the number of host plants, and the lack of effective control methods. *Halyomorpha halys* coevolved with a Gammaproteobacterial endosymbiont located in its mid-gut as for other Pentatomid species. This endosymbiotic bacterium Candidatus *Pantoea carbekii* is vertically transmitted to the offspring and plays a vital role in metabolism and nutrient assimilation throughout *H. halys* development. Here, in the context of climate change, we investigated the effect of high temperatures on insect-endosymbiont relationships. We simulated long- and short-term exposure to constant and variable high temperatures in the laboratory. The impact of high temperatures was evaluated on the mortality and development time of the different life stages of *H. halys* and the endosymbiont presence. Results showed how the increasing temperatures lead to a high mortality rate of the *H. halys* adult with a delay in the developmental time of the juveniles. Long-term exposure to high temperatures negatively affected the bacterial endosymbiont. The results are discussed regarding climate change's impact on *H. halys* populations.

Keywords: *Halyomorpha halys, Pantoea carbekii*, high temperatures, endosymbionts, climate change

## OC164. Contribution of microbiota in mosquito nutritional ecology

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Although the role of the microbiota in insect biology does not require further proof, much remains to be deciphered in mosquitoes. Along their life cycle, mosquitoes interact with different ecological niches colonized by various microorganisms and diet sources that likely modulate their performance. Larvae acquire most of their microbial communities from the water they colonize while adults feed on plant nectar, mainly composed of fructose. We used surface-sterilized eggs re-associated or not to conventional bacterial microbiota upon a range of diet concentrations and addressed the impact of microbial inoculum and diet concentration variation on several mosquito performance traits. We also used $^{13}$C-metabolomic and stable isotope probing approaches coupled to high-throughput sequencing to reveal fructose-related mosquito metabolic pathways and the dynamics of the active gut microbiota following fructose ingestion in adults. We showed that mosquito juvenile survival depends on the interaction between bacterial inoculum load and diet concentration in the breeding water. Exposure to bacteria in rearing water shorten larval development time although it impacted larvae survival in an inoculum and diet concentration-dependent manner. Our results also revealed competitive and synergistic interactions of diverse fungal taxa within the active mycrobiota following fructose ingestion. Significant differences in metabolic pathways between males and females were highlighted suggesting different modes of central carbon metabolism regulation. These findings argue the importance of deciphering mosquito-microbe trophic interactions and open promising perspectives for vector management of this vector in the field.

Keywords: mosquito, microbiota, nutrition, trophic interactions

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## OC165. Probiotic bacteria can improve insect health of mass-cultured housefly larvae reared for animal feed

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The use of insects for animal feed production has become a fast growing industry and is considered a sustainable alternative to the traditional feed production. As a fast-developing sector, insect feed comes with certain challenges concerning suitable rearing substrates for insect growth and best safety practices to avoid disease outbreaks. To tackle these challenges, beneficial microbes with probiotic properties can help boost insect performance and safeguard the insect culture against microbial pathogens. We used the common housefly *Musca domestica*, because of its good capacity to convert organic matter, and cultures of the common gut commensal *Lactinibacillus plantarum* (strain HBUAS57019) to test three main hypotheses about the effect of probiotics: a) provision of probiotics in the rearing substrate has a direct effect on housefly development; b) probiotics can directly fight-off opportunistic microbial pathogens from the insect culture; and c) probiotics can regulate genes involved in nutrient uptake and immunity of the host. Housefly performance was assessed by measuring the larval biomass of third-instar larvae, pupation rates and adult emergence rates, while bacterial assays were used to measure the antimicrobial effects of *L. plantarum* against commonly found opportunistic

pathogens. qPCR was used to measure the expression of genes involved in immunity and nutrition in the housefly larvae. We found that the substrate supplementation led to higher survivability and larval biomass, and that common opportunistic pathogens were inhibited. To unveil the mechanisms through which probiotics can boost insect performance, RNA sequencing will unveil gene expression changes in treated housefly larvae.

**Keywords:** insect-feed, housefly, probiotics, larval biomass

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## OC166. Effects of *Asaia* bacteria on mosquito fitness for improvement of SIT programs

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Arboviruses are responsible for several diseases in both humans and animals. The well-known yellow fever mosquito, *Aedes aegypti*, is the primary vector of yellow fever, dengue, chikungunya and Zika virus. Currently, disease-control strategies strongly rely on mosquito population management through insecticides and larvicides. However, insecticide resistance is leading to a less effective vector control outcome. The sterile insect technique (SIT) is a method that has been developed for insect population management. To successfully apply SIT, healthy and competitive mosquito colonies are required, to better allow sterilised males to successfully compete with their wild counterparts. It has been shown that mosquito microbiota plays an essential role in larval development and fitness. Bacteria of the genus *Asaia* were proposed for paratransgenic techniques and as a probiotic, but little is known about the effects of *Asaia* spp. on adult fitness of *Ae. aegypti* and other mosquito species of public health interest. Here, we inoculated first instar larvae of *Ae. aegypti*, *Ae. albopictus*, *Culex pipiens* biotype *molestus* with different species of *Asaia* for different exposure times. We analysed several fitness parameters, as well as the microbiome composition. Larval developmental time, and adult size, were positively affected by *Asaia*, and interestingly the effects were species-species specific. In addition, larval microbiota underwent changes in composition. Ongoing efforts include exploring potential microbiome manipulations on male attractiveness and competitiveness in laboratory and semi-field contexts and vector competence. Our findings underline the importance of understanding host-microbe interactions. Improving mosquito fitness will ultimately be beneficial for large-scale sterile male production.

**Keywords:** mosquito, sit, bacteria, fitness, microbiome

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## OC168. Assessing the risks of co-infection by a fungal and a bacterial insect pathogen in *Tenebrio molitor*

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The yellow mealworm, *Tenebrio molitor* is an important species mass-reared for human consumption and feed for livestock and aquaculture. Insects kept at high densities are at an increased risk of infections by various insect pathogens, potentially leading to economic losses. Different pathogens may occur alone or in combination in mass-production systems, leading to multiple possible infection outcomes. The interaction between pathogens may be additive (as predicted from their combination effect), synergistic (more severe effects than predicted), or antagonistic (less severe effects than predicted). Understanding the impact of pathogen co-infections is essential for the risk assessment and subsequent prevention of disease outbreaks in mass-production systems. Here, we assess interactions between two pathogens of *T. molitor* larvae, the fungus *Metarhizium brunneum* and the bacterium *Bacillus thuringiensis*. We use the ecotoxicological MIXTox model, originally developed to investigate interactions between chemicals, to assess the intricate interactions between the pathogens. The MIXTox model proves to be a powerful tool to investigate co-infections in the field of insect pathology. In mixtures in which the effects are mainly caused by *M. brunneum*, interactions are antagonistic leading to higher survival and larval biomass than predicted. In mixtures in which the effects are mainly caused by *B. thuringiensis*, however, interactions become synergistic. Interactions between pathogens are complex and these results help to estimate the risks associated with co-infections in mass-rearing systems.

**Keywords:** co-infections, yellow mealworms, mixtures, risk assessment

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## OC169. Both transcriptomic response and gut microbiota composition of *Nosema ceranae*-infected honeybees differ between laboratory and semi-field conditions

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The intestinal parasite *Nosema ceranae* causes honeybee nosemosis. Unfortunately, fumagillin, the only effective treatment, is currently banned in many countries. Because of the significant economic losses in the beekeeping industry, feed additives are being tested as alternatives, and probiotics are seen as a promising solution.To determine whether a putative probiotic affects both gut microbiota and transcriptomic responses of the infected honeybees, we simultaneously monitored honeybees from the same hives under laboratory and semi-field conditions.We performed whole shotgun metagenomics sequencing of the microbiota sixteen days after infection to analyse the impact of the infection and probiotic supplement on bacterial communities and to identify markers related to the infection and/or housing conditions. We also used RNA sequencing to look for significant changes in gut gene expression.Surprisingly, at least 5 bacterial species were overrepresented in cages compared to the hives regardless of the treatments applied. Among them *Apilactobacillus kunkeei* and *Fructobacillus fructosus*, two beneficial lactic acid bacteria are only present in honeybees reared in pain cages with a considerable relative abundance of about 5%. According to RNAseq analysis, infected honeybees in pain cages have 155 down-regulated and 120 up-regulated genes, whereas bees in hives have 182 down-regulated and only 86 up-regulated. The addition of probiotics had no effect on gene expression in either the cage or the hive.Our findings highlight the importance of using a representative model to investigate both microbiota and transcriptomic responses of bees to various products intended for use in natural settings.

Keywords: honeybees, *N. ceranae*, gut microbiota, transcriptomic, probiotic

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## OC170. Studying insect egg microbiomes as a first step towards the identification of microbial elicitors to enhance plant defenses against insect eggs

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Plants activate direct and indirect defenses in response to insect egg deposition as an important first line of defense. Although several abiotic elicitors present on the egg surface have been identified, recent studies suggest a potential role of egg-surface microorganisms, but compelling evidence is still lacking. Nevertheless, so far only very little is known about insect egg microbial communities. In this study, we used high-throughput amplicon sequencing and qPCR to get a better understanding of the microbiome of insect eggs and how they are established using the Southern green stinkbug *Nezara viridula* as study object. First, in order to determine the bacterial community composition, egg masses from two natural populations were examined. Subsequently, microbial community establishment was assessed using eggs from laboratory strains. Eggs from the ovaries were completely devoid of bacteria, indicating that egg-associated bacteria were deposited on the eggs during or after oviposition. Further, bacterial diversity was very low in and on the eggs, while the community composition and density remained

stable over time, suggesting limited bacterial growth. A *Pantoea*-like symbiont previously found in the midgut of *N. viridula* was found in every sample and generally occurred at high relative and absolute densities. It is reasonable to assume that plants are able to perceive and react to such symbionts consistently associated with eggs as signals to trigger plant defenses. This would make them promising candidates for the development of microbial elicitors to aid biological pest management. Further research is needed to determine whether such scenario is at play.

Keywords: microbial community, *Nezara viridula*, *Pantoea*, plant defense, symbiont

## OC171. Comparative Genomic Analysis of Fruit Fly associated bacteria: New Insights into bacteria-host interactions

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The increased use of-omics approaches has greatly advanced microbiome characterization in insects but still the functional role of the insect associated bacterial is eluding. We focused on the characterization of the bacteriome in fruit flies. *Enterobacter* species are commonly found in the gut microbiota of fruit flies, and they are known to play an important role in the physiology of their host. Some *Enterobacter* spp. have been shown to benefit fruit flies by promoting their growth, and overall health. The knowledge regarding the genomic features of *Enterobacter* spp. and the mechanisms of interaction with their host remains limited. Genomic sequencing revealed that each species harbors two *Enterobacter* spp., one unique to each insect host while the other was shared between them, except for *A. fraterculus* which is found to harbor a single *Enterobacter* sp. The unique isolates were identified as *Enterobacter mori* (n=2) in *Z. cucurbitae*, *E. asburia* (n=1) in *C. capitata*, and *E. roggenkampii (n=1)* in *B. zonata*. While the shared isolates were identified as *E. hormaechei* (n=8). The *Enterobacter* spp. draft genomes ranging in size from 4.5-to-5.1Mb and a CG content of around 55%. The genomic analysis revealed the presence of at least one cluster of the 13 core genes encoding for T6SS. In addition to transporting proteins into the extracellular environment, T6SS had the ability to deliver a diverse array of effector proteins directly into target cells, including other bacteria, and eukaryotic cells. The T6SS in *Enterobacter* spp. is supposed to promote the health and growth of their host by eliminating competing pathogenic bacteria. This is achieved by giving a competitive advantage to *Enterobacter*, which can then provide essential nutrients to the host and help it resist pathogen infections.

Keywords: bacteriome, genomics, symbionts, secretion systems, T6SS

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## OC172. Silencing of leafhopper vector genes to disrupt phytoplasma transmission

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Phytoplasmas are phloem-limited plant pathogenic bacteria causing diseases in many plant species. They are transmitted by Hemipteran insect species through a persistent-propagative modality. Phytoplasmas are wall-less and their membrane proteins are involved in pathogen internalization into host cells. We focused on the immunodominant membrane protein (Imp) of Flavescence dorée phytoplasma (FDp), a grapevine quarantine pest and a major threat to European viticulture. *Scaphoideus titanus* is the main natural vector of FDp to grapevine, whereas *Euscelidius variegatus* is commonly used as laboratory vector. Previous works indicated that recombinant Imp external domains of two FDp strains selectively interact with proteins from vector species rather than those from non-vectors. Here, similar patterns of interacting insect gut proteins were obtained from both vector species, following pull-down based on Nickel beads saturated with His-tagged FDp Imps. The insect interacting proteins were identified by mass spectrometry on protein dataset from *S. titanus* and *E.*

*variegatus* transcriptomes. Three *S. titanus* and five *E. variegatus* proteins interacting with Imp were further characterized by measuring expression of their corresponding transcripts in different insect tissues and in healthy vs infected insects. Two selected genes, namely natterin and legumain, were silenced to evaluate the effects on phytoplasma acquisition. As control of phytoplasma diseases is based on insecticides against vectors, this work is an initial step to elucidate key stages of FDp transmission specificity and vector infection mechanisms, which might be targeted to disrupt the epidemiological cycle, providing tools to integrate chemical treatments for more sustainable pest control.

**Keywords:** *Euscelidius variegatus*, *Scaphoideus titanus*, grapevine Flavescence dorée, RNA interference, vector-borne plant pathogens

## OC173. Food envy: Interactions between entomopathogenic nematodes, their mutualists and insecticidal pseudomonads

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The use of biological control agents (BCA) for controlling insect pests is an environment-friendly alternative to synthetic pesticides. Entomopathogenic nematodes, associated with mutualistic *Xenorhabdus* or *Photorhabdus* bacteria have emerged as effective BCA to fight below ground insect pests. In nematode infected cadavers, bacteria of the genus *Pseudomonas* are recurrently found, which is interesting, since several fluorescent pseudomonads themselves exhibit insecticidal activity. Furthermore, the combination of insecticidal pseudomonads and entomopathogenic nematodes might increase the efficacy and consistency of biocontrol, however direct negative interactions between the different BCA need to be excluded. Therefore, we investigated the direct interactions between nematodes, their mutualists and insecticidal pseudomonads. We assessed the growth/colonisation dynamics of the different organisms during *in vitro* competitions as well as during insect infections. Co-culturing strains of the two bacterial groups in liquid media or co-injecting them into the hemocoel of *Galleria mellonella* larvae showed that *Pseudomonas* spp. could grow in the presence of *Xeno-/Photorhabdus* spp., whereas the latter seemed to be inhibited *in vitro* but less so *in vivo*. Force-feeding *Pseudomonas* spp. to *G. mellonella* larvae and afterwards exposing them to entomopathogenic nematodes increased the pseudomonads' chance to colonize the host, whereas the nematode reproduction was not affected. This indicates that insecticidal pseudomonads can co-exist with entomopathogenic nematodes and their mutualists inside insects and that they might even profit from nematode infections. Our results highlight that interactions between BCA might differ between *in vitro* and *in vivo* systems and that applying combinations of BCA is promising for pest control.

**Keywords:** biological control, insecticidal pseudomonads, entomopathogenic nematodes

## OC174. Legume flowers as bridge for interspecific virus transmission among sympatric honey bees (*Apis mellifera*) and long-horned bees (*Eucera spp.*)

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Among various causes for bee decline, viral diseases are considered a major concern. Since there are no known direct or vector-borne interactions between bees of different taxa, shared floral resources are considered the main pathway for virus transmission among bee species. While flower mediated inter-specific virus transmission between bee species was experimentally demonstrated, little is known about the extent of virus spread at the community level. Here we investigate the extent of overlap in the viromes of sympatric honey bees (*Apis mellifera*) and wild bees sharing floral resources along the activity season. A field survey was conducted in a Mediterranean agroecological landscape in central Israel, in which we monitored bee-flower visits, and collected wild bee and honey bees, repeatedly along the activity season. Using deep-sequencing, we mapped the virome of co-existing honey bees and the dominant wild bee genera mining bees (*Andrena*), long horned bees (*Eucera*) and masked bees (*Hylaeus*), using pools of 191-645 individuals/genus. We found that honey bees and *Eucera* bees, both with long proboscis, shared the Lake Sinai Virus (LSV) and LSV-3, while no overlap was found between the other bee genera viromes. Interestingly, we found that those two genera had the greatest overlap in foraging patterns, especially on legume flowers. The tubular structure of leguminous flowers requires intimate interactions with the visiting insect, and might protect viral particles from environmental factors such solar radiation and precipitation that can decrease their infectivity.

**Keywords:** pollinator health, wild bee conservation, bee pathogen

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## OC175. Filamentous viruses constitute a novel family among *Naldaviricetes* preferentially associated with parasitoid wasps

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Nuclear arthropod-specific large dsDNA viruses (NALDVs) correspond to the *Naldaviricetes* class, which also includes *Baculoviridae*, *Nudiviridae*, *Hytrosaviridae*, and *Nimaviridae* families. The systematic classification of "Filamentous viruses" within one of these families remains yet unclear. Up-to-date genomic data, infection process and particle shape description link them to *Hytrosaviridae*, however phylogenetically distant. In this study, we provide the first comparative genomic analyses of these filamentous viruses. We explored the genomes of seven filamentous viruses, six of which were newly

obtained, in addition to the previously sequenced Leptopilina boulardi filamentous virus (LbFV). In fact, these data led us to infer the core genome, gene order and phylogenetic relationships showing that "Filamentous viruses" constitute a new family. Moreover, these filamentous viruses share all genomic features with *Naldaviricetes* and their core genome comprises five specific genes that clearly distinguish them from other viral families. Finally, we propose a taxonomical revision of the *Naldaviricetes* class in which filamentous viruses constitute a fifth family within the order *Lefavirales*. This study lays the foundation for the description of a new viral family, opening an area for research on wasp-virus interaction and showing a more global picture of the importance of virus discovery.

**Keywords:** dsDNA virus, filamentous virus, *Naldaviricetes*, *Lefavirales,* EVE, parasitoid wasp

## OC176. Symbiotic endobacteria modulate virus effects on plant-aphid interactions

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Aphid-transmitted plant viruses frequently alter both plant and aphid phenotypes in ways that influence interactions between them and thereby enhance transmission. Meanwhile, there is increasing evidence that aphid-plant interactions can also be modulated by the presence of non-pathogenic endosymbionts. Yet, few studies to date have explored how endosymbionts influence pathogen effects on aphid behavior or aphid-plant interactions. We explored how different aphid endosymbionts influenced the effects of pea enation mosaic virus (PEMV) on interactions between pea aphids (*Acyrthosiphon pisum*) and fava bean plants. Metabolic analyses showed that virus infection compromises plant defenses against aphids. Furthermore, we observed striking interactions between virus infection and endosymbionts, with virus effects frequently apparent only for aphids harboring specific endosymbiont strains. Two endosymbiont strains in particular, *Regiella insecticola* Ri and *Hamiltonella defens*a, exhibited strong interactions with the virus across a range of assays, and their presence was often associated with effects that would be expected to favor virus transmission. In performance assays, for example, aphids harboring these strains exhibited significantly higher population growth on virus-infected plants, despite being the worst performing lines on uninfected plants. Similar patterns emerged in behavioral assays, where aphids harboring these strains frequently exhibited virus effects on dispersal and host-plant preference that would appear conducive to virus transmission, while such effects were absent for aphids harboring other endosymbionts. Taken together, our results demonstrate that the presence of different facultative aphid endosymbionts can modulate virus effects on aphid-plant interactions, with potential implications for disease transmission.

**Keywords:** gut microbiome, tritrophic interactions, symbiosis, pathogens

# OC177. Assessment of nematode and microbiome diversity present in the most destructive forest pest in Europe

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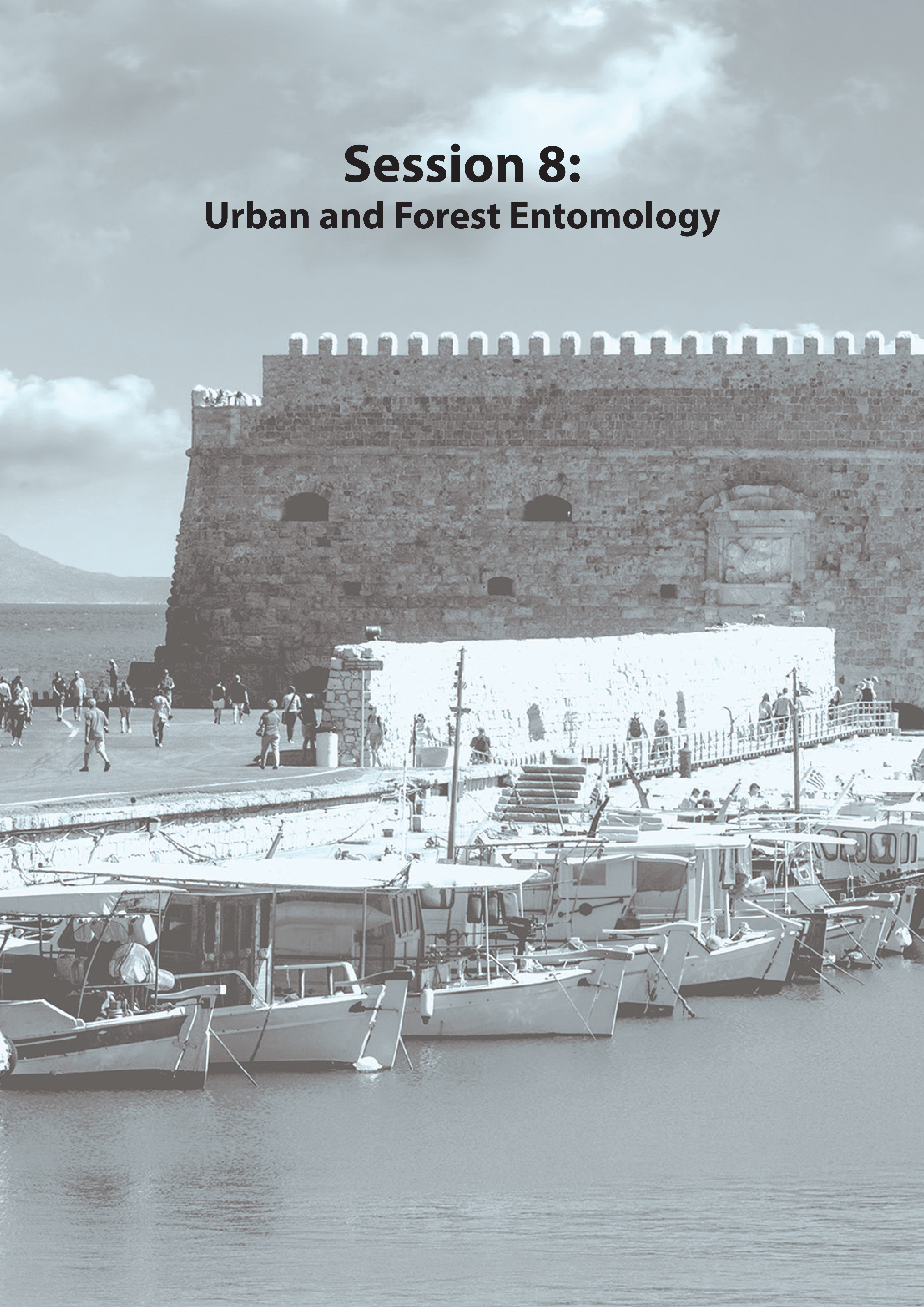
Controlling spruce bark beetle (*Ips typographus*) populations has become one of the most pressing issues as beetle outbreaks intensified in southern and central Europe. A straightforward solution for this problem has not been achieved yet, but it has been suggested that certain nematodes species can serve as biological control. Despite growing numbers of studies on *Ips typographus* numerous interactions with symbiotic and parasitic species, there is a lack of large-scale studies investigating nematode variation across species range in relation to intraspecific microbiota variation. Here we present a study on nematode as well as microbiome diversity in 24 populations of spruce bark beetle across Europe, with special emphasis on northern and southern margins of its host species in Europe. Our initial diversity screening used *Ips typographus* whole genome resequencing data and metagenomics approach and it was later complemented with Nanopore and Illumina amplicon sequencing. We found out that different populations highly differed in nematode as well as microbiome diversity. Besides, we noticed that the most abundant nematode species in northern populations was *Contortylenchus sp.*, a nematode recommended in previous studies as a biological control for *Ips typographus*. We report intraspecific nematode and microbiota variation and compare efficiency of different sequencing approaches in species identification. Our results provide insights on the symbionts and parasites variation in spruce bark beetle, that can potentially improve forest management and conservation.

Keywords: spruce bark beetle, nematodes, parasitic interactions, forest management

# Session 8:

## Urban and Forest Entomology



## OC178. Invasive insects in urban landscapes – pests, friends and allies

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For a long time, European woody plants were spared from invasive insects, contrary to what was observed in other regions of the world. However, in recent decades, many exotic pests of trees and shrubs have appeared, due to the exponential increase in intercontinental commercial traffic and to less restrictive legislation in Europe than elsewhere. Most of these invasive pests occur on ornamental and orchard woody plants rather than on native forest species, which probably reflects the importance of the trade of ornamental and orchard plants in biological invasions. Some pests, such as the horse-chestnut leafminer and the box tree moth, have changed urban landscapes, others such as the spotted wing drosophila have changed fruit production management practices. These invasive insects have become pests mostly because they were introduced without their natural enemies that control them in their area of origin. Some of these invasive insects have been adopted by local natural enemies, with or without effect on their control, whereas others have remained more or less free from natural enemies. In such cases, classical biological control through the introduction of natural enemies from the area of origin of the pest may be a solution. The chestnut gall wasp has been successfully controlled in Europe by the introduction of a parasitoid. Other invasive insects have seen their original natural enemies mysteriously appearing in the region of introduction. The presentation will discuss future biological control options for the main invasive pests of woody plants in Europe.

**Keywords:** biological control, invasive insects, natural enemies, urban pests

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## OC179. Is ash sawfly a problem in Ireland?

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Ash sawfly (*Tomstethus nigritus* F.) is typically a sporadic pest of European ash (*Fraxinus excelsior* L.) It is a defoliator of ash in the larval stage, with the potential to cause significant damage. It is found across Europe, including Great Britain (GB), and in some areas of Europe it has completely defoliated established stands of ash. However, in GB the damage has been limited to urban environments. Ash sawfly was first recorded on the island of Ireland in Belfast, Northern Ireland, in 2016. In the Belfast area, there has been consistent defoliation of ash trees in parkland, hedgerows and riparian habitats, with the sawfly gradually extending its range, with in some cases evidence of inadvertent carriage on motor vehicles. In 2021, ash sawfly was found in counties Kildare and Dublin in the Republic of Ireland. The consistent defoliation of ash trees each year is not typical of ash sawfly outbreaks elsewhere, which normally subside after a few years. This project examines the impact of annual ash sawfly defoliation on ash tree growth and considers whether the outbreak of ash sawfly in Ireland is facilitated by lack of parasitoid natural enemies and/or interaction with ash dieback (*Hymenoscyphus fraxineus*).

**Keywords:** ash sawfly, *Tomostethus nigritus*, tree pest

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### OC180. Community composition of psylliphagous ladybirds in a tropical island environment in La Réunion island, France

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Predatory ladybirds have long been used in biological control. These species often share the same prey and are therefore organised in communities. In Reunion Island, located in the south-west of the Indian Ocean, three quarters of the 26 species present have been introduced for biological control. Since 2006, Réunion has been invaded by a psyllid originating from Australia: *Acizzia uncatoides*, which is spreading and impacting an endemic tree: *Acacia heterophylla*. In order to evaluate the relevance of a biological control programme against this psyllid, we studied the psylliphagous ladybird communities in Réunion. A regular sampling of 11 sites was carried out between 2020 and 2021. At each site, 10 trees hosting psyllid colonies were sampled with Garden Vaccum for one minute and then visually examined to estimate the abundance and richness of psyllids and ladybirds. Fifteen species of ladybirds were identified at the adult stage and five at the larval stage. The larvae of three species feed on psyllids. The first and the most abundant, *Exochomus laeviusculus*, is a species with a generalist diet, is positively influenced by the psyllids' abundance and negatively by the landscape's diversity. The second, *Olla v-nigrum*, is a psylliphagous species voluntarily introduced in Reunion in 1990. Its abundance is positively influenced by meteorological variables such as the average monthly temperature. The last, *C.septempunctata*, is a species known for its aphidiphagous diet. Introduced since 2020 in Réunion, its presence could be due to the scarcity of its prey which would incite them to consume the psyllids.

**Keywords:** community ecology, biological control, ladybirds, psyllids

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### OC181. Exotic aphid species of Mediterranean forests of Türkiye

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Exotic species can directly or indirectly disrupt the ecological balance and threaten biological diversity in new habitats. This study was carried out to determine aphid species which are important exotic invasive insect groups in forest ecosystems, and their densities in different forest habitats (*Pinus nigra*, *P. brutia*, *Juniperus* spp., *Quercus* spp., *Robinia pseudoacacia*, *Abies cilicica*) in the Mediterranean Region. For this purpose, a total of 34 areas were sampled in 2018-2020. Using systematic and random sampling, 9,252 specimens in 68 species from the families Aphididae and Phylloxeridae (14 species at genus level only) were identified. It has been determined that 12 (*Appendiseta robiniae, Cinara curvipes, C. juniperensis, C. obscura, C. orientalis, C. wahluca, C. watanabei, Eulachnus pumiliae, E. thunbergii, Hoplochaitophorus dicksoni, Mindarus kinseyi* and *Pseudessigella brachychaeta*) of these species are alien species. *A. robiniae* (538 specimens, in *R. pseudoacacia*) and *C. orientalis* (466 specimens, *Pinus nigra* and *P. brutia*) were the highest number of species and attracted attention due to their population density. Although these species reach high populations from time to time, it can be said that the main reason why they do not cause significant damage to the trees is the rich diversity of flora and fauna in these forests. The diversity of natural enemies and competition are thought to be in balance. However, it should not be ignored that the deterioration in the natural balance due to climate change and the introduction of new alien species into the ecosystem may increase the population growth of these species.

**Keywords:** population density, alien, invasive species, forest tree

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## OC182. Human impact on mosquito breeding habitats in Urban Green Space and consequences for mosquito proliferation in urban areas

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The urban mosaic created suitable mosquito habitats that have favored the establishment of anthropophilic vector mosquitoes such as the invasive mosquito species *Aedes albopictus*. Meanwhile, environmental pollutants emitted through large-scale human activities also pose *increasing* threats to human health in the Antropocene. Several studies showed that anthropization affects mosquito physiology and shapes their microbiota, mainly acquired through larval aquatic habitats. Moreover, physicochemical and microbiological factors influence breeding site selection of gravid mosquito females. Following these observations, the aim of this study was to understand how human activities impact *Ae. albopictus* proliferation in community gardens in Lyon Metropolis (France). To that end, we compared physicochemical parameters and microbial composition between water habitats colonized or not by the mosquito. Metabarcoding analyses revealed different patterns of microbial community structure depending on water colonization status and pollution context. Liquid chromatography coupled with time-of-flight and ion trap mass spectrometry allowed the detection of enriched pollutants in colonized waters. Finally, behavioral assays allowed to identify some cultivable microorganisms with attractive potential to gravid females. These results argue the importance of studying the interplay between biotic and abiotic factors in order to better understand the urban ecology of *Ae. albopictus* and thus be able to propose more efficient tools for the control of this mosquito species. This study is also part of an integrated project that aims to evaluate human behavior and already showed that attitudes, more than knowledge, influence the practices of community gardeners in relation to mosquitoes.

**Keywords:** *Aedes albopictus*, human activities, microbiota, pollutants, citizen science, urban areas

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## OC183. Mosquitoes of Turkey

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Mosquitoes are hematophagous arthropods that pose a huge threat to public and veterinary health. They are responsible for the transmission of large group pathogens that includes viruses, bacteria, protozoa and nematodes and in this way spread of important diseases. These insects belong to the Culicidae family which has 112 genera and 3,547 known species. Certain species from the genera *Anopheles, Aedes and Culex* are of huge public health interest in view of their biting nuisance and the numerous protozoan and viral diseases that they are capable of transmitting. They are capable of serving as vectors of important disease agents that can and caused explosive epidemics affecting the whole world. Turkiye is situated between Europe and Asia. It is bordered by three seas and divided into seven regions and these regions have a distinct climate change. Turkiye is vulnerable to climate change and has experienced upward trends in annual temperatures and rising sea levels, and greater fluctuations in precipitation rates. It is a potential hotspot for important vector species because the climate in various regions is conducive for several insect and serves as a conduit for refugees and immigrants fleeing areas troubled with armed conflicts and natural disasters which have increased substantially in recent years. So, all these situations cause arthropod vectors for transmission from vertebrate host to another vertebrate hosts. In Turkiye, there are 64 identified species based on morphological and molecular studies. This presentation aims to explain the status of the mosquito species and their future dissemination in Turkey.

**Keywords:** Turkey, mosquito, vector, diseas

## OC184. Control of *Xylotrechus chinensis* (Chevrolat) (Coleoptera: Ceramycidae) using four conventional insecticides

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*Xyloterchus chinensis* (Chevrolat) (Coleoptera: Cerambycidae) is an important wood-boring insect pest that is endemic to several Asian countries, mainly infesting mulberries but also grape vine, *Malus* spp, and *Pyrus* spp. Few years ago it invaded Europe and became a serious pest of mulberries. Since 2019, *X. chinensis* is a major pest of mulberry trees in the Municipality of Athens (Greece). Injecting insecticides into the trunk of trees is a promising method for the control of xylophagous insects. In the present study, we evaluated the efficacy of the conventional insecticides fipronil, imidacloprid, spirotetramat, and abamectin through the trunk injection method against *X. chinensis* in a two-year trial. Spirotetramat was the least effective insecticide against *X. chinensis* reducing the percentage of exit holes by 37.4%, while imidacloprid and fipronil were highly effective causing a reduction of exit holes by 76.1 and 71.8%, respectively. Abamectin was the most effective insecticide, decreasing the proportion of exit holes by 85.6% in the second year of application. The highest mean number of exit holes was recorded in treated trees with spirotetramat (24.7 and 17.9 exit holes/tree in 2021 and 2022, respectively), followed by fipronil (12.1 and 8.8 exit holes/tree in 2021 and 2022, respectively), imidacloprid (9.5 and 5.0 exit holes/tree in 2021 and 2022, respectively), and abamectin (4.7 and 3.3 exit holes/tree in 2021 and 2022, respectively). Our results indicate that the injection of abamectin into mulberry trees is an effective method against *X. chinensis*.

Keywords: *Xylotrechus chinensis*, mulberries, trunk injection, insecticidal formulations

## OC185. Syngenta TreeCare application: an effective treatment against *Xylotrechus chinensis* in mulberry trees

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*Xylotrechus chinensis* (Coleoptera: Cerambycidae) is a wood boring insect, commonly known as the tiger longhorn beetle, which damages *Morus* spp. (Rosales: Moraceae) and potentially Malus spp. (Rosales: Rosaceae) and *Pyrus* spp. (Rosales: Rosaceae). In Greece, soon after its invasion in 2017, it became a severe pest of mulberry trees, which is the only host plant of this pest, till now. Larvae mines the bark and leads to the disruption of the transportation of water and nutrients in the tree, which leads to the weakening of the tree and gradually to its death. In Greece, mulberry cultivation is an important industry, mainly for sericulture, and the introduction of *X. chinensis* poses a significant threat to the sector. In the present work, we evaluate Syngenta TreeCare, the commercial name for the exclusive tree micro injection system that enables the precise and targeted application of Revive II, direct into targeted trees and palms. A systemic insecticide with imidacloprid as active ingredient applied with tree injection and soil drench was used as a positive control, while mulberry trees without any kind of insecticide application were used as untreated control. For all the treatments and the untreated control, mulberry trees were classified in three different classes, according to the infestation level: a) heavily infested, b) medium infested, c) trees without infestation symptoms (dead branches or adult exit holes). Significant differences observed between the treatments, which will be thoroughly discussed during the presentation.

Keywords: tiger longhorn beetle, *Xylotrechus chinensis*, Syngenta TreeCare, Revive II, mulberry

## OC186. New and emerging bark and ambrosia beetles in Europe

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Bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) are amongst the most important wood-boring insects worldwide. They are a large and diverse group commonly recognized as one of the most important tree mortality agents. Two groups of factors, namely international trade and climate change, strongly affect presence and harmfulness of new and emerging bark and ambrosia beetles recently infesting European forests. Largely assisted by global trade, alien species are being introduced into Europe at unprecedented rates. Many alien species recently arrived to or spread into new European territories. However, biological invasions may be caused also by internal trade, which promotes the spread of native species within European countries enlarging their distribution range. Finally, the introduction of individuals of species native to Europe but belonging to non-European populations may cause cryptic invasions related to the arrival of both new haplotypes and different strains of the associated fungi, which could be more aggressive. Activity and development of native bark and ambrosia beetles are directly or indirectly (i.e., via associated symbionts) regulated by climatic variables such as temperature and rainfall. The strong and quick climate warming affecting southern Europe in the last decades increased the populations of many native species, which in some case became new emerging pests. Wide outbreaks of *Ips typographus* and *I. acuminatus* affect respectively spruce and pine forests in central Europe and Alps, whereas Mediterranean pine forests are infested by *Orthotomicus erosus* and *Tomicus destruens*. A new international forest management strategy is needed to contrast these phenomena.

Keywords: biological invasions, climate change, new pests

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## OC187. Demographic history of the spruce bark beetle

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Demographic history of species and populations inferred using whole genome data can provide quantitative information about the parameters of biological relevance i.e. size changes, time of divergence and rates of gene flow. This information is essential for understanding of the processes that shape species evolution. However, to obtain reliable estimates one need to use data which is not affected by selection. Here we use genome wide data from over 300 bark beetle (*Ips typhogaphus*) individuals, from 23 populations sampled across its European range to reconstruct species demographic history. We assessed if the demographic model of spruce bark beetle is similar to its host plant Norway spruce. We used Pairwise Sequentially Markovian Coalescent (PSMC) model and coalescent-based simulation approach based on site frequency spectrum (SFS). We accounted for multiple large inversions identified in the bark beetle genome and performed demographic inferences for datasets with and without inversions. Bark beetle genetic structuring and demographic history seems to be incongruent with its host plant Norway spruce. Our analyses show that populations of spruce bark beetle diverged to northern (non-outbreaking) and southern (outbreaking) groups within the Last Glacial Period. Inferred demographic parameters suggest that populations of spruce bark beetle went through a serious change in the effective population size over time, reaching highest effective population size just before the Last Glacial Period and that southern populations until recently had higher population size than northern but now, their size has evened out.

**Keywords:** demographic history, bark beetle, *Ips typhographus*, inversions, outbreak

### OC188. Comparing methods of mapping the infestation by insects of a Mediterranean forest in Karpathos, Greece, using Sentinel 2 data

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Detection of forests affected by pathogen insects constitutes a procedure of high importance considering that insects are one of the major threats for forest ecosystems in Europe. Development of a mapping methodology capable of detecting the spread of such disasters is crucial in order to protect the Mediterranean forests and minimize the disastrous effects. Our research took place in a Mediterranean forest of Calabrian pine in Karpathos, Greece and we took advantage of the remote sensing data in high spatial and spectral resolution that Sentinel 2 satellite provided us. Two methods of detecting the infestation were applied; the first one using the spectral indices NDVI and SIWSI and the second one trying to interpret the difference between digital signatures of healthy and infested vegetation. The results for the detection of the affected forest over time by the NDVI spectral index method showed satisfactory accuracy, as an overall accuracy of 91% and a Kappa coefficient of 0.75 was achieved. In short, the research demonstrated a reliable way of detecting and measuring the area of the infested forest by which similar phenomena can be monitored in Mediterranean forests with similar characteristics to those of the island of Karpathos.

**Keywords:** Mediterranean forest, remote sensing, Sentinel 2, spectral indices, digital signatures

## OC189. Bark beetles of Greece – refining species checklist and assessing complementary management approaches

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The intense population outbreak of pine bark beetles affecting the suburban forest of Thessaloniki since 2019 highlighted the lack of up-to-date knowledge on the native Scolytinae and thus reignited the research on them. To this direction, the native species checklist is now under revision, using both morphological identification keys and even DNA barcoding to resolve the identity of species that have been recently reassessed. Moreover, as the only plausible and efficient management approach against bark beetles remains the logging and removal or debarking of infested trees, a complementary approach that includes the use of pheromone traps is being evaluated. To do that, a network of Theysohn® traps (77 in total) has been installed in the spring of 2022 in the pine (*Pinus brutia*) forest of Samothraki (Greece), and baited with kairomone lures (KaiPin®) which are being replaced every 40 days to retain their attracting capacity. After the first year, more than 25.000 bark beetle individuals belonging to six different Scolytinae genera have been trapped. As this project will carry on for a second year (2023), it will ultimately assess the potential of mass-trapping as a complementary and sustainable management tool that, in concert with sanitation loggings, can effectively protect Mediterranean pine forests from bark beetles.

**Keywords:** bark beetles, checklist, mass trapping, pheromone traps, biological control

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## OC190. National Biodiversity Future Center (NBFC): assessment of impact of alien ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) on Coleoptera native community in Italian Protected Areas

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The recent increase in the introduction of non-native species worldwide is currently considered an overriding concern. This phenomenon seriously impacts local ecosystems, until causing a strong reduction of biodiversity.Among the plethora of species, ambrosia beetles (AB) are deemed among the most successful groups of invasive organisms. They are known to easily reach new territories, usually through wood products trade, and to attack a wide range of tree species of agriculture and forest interest, affecting their distribution and abundance.This three-year PhD study, as part of National Biodiversity Future Center Project, aims to explore the effects of non-native AB on structure and functioning of ecosystems dominated by *Fagus sylvatica* L. in some important Italian Protected Areas (PAs) (*i.e.*, Abruzzo Lazio and Molise National Park). This study also aims to investigate the effectiveness of zonation strategies at different levels of protection in Italian PAs in preserving these areas from invasive ABs and thus whether specific diversity and community structure can help preserve these valuable ecosystems. For three seasons, from March to October, we will deploy different types of traps on the plants at 2 m height in divergently managed beech forests, to estimate the populations of AB in terms of species and abundance. Traps will be inspected fortnightly for counting and identifying the specimens and for replacing the ethanol lure.
This project will assess the infestation trend of alien ABs species over time in protected beech forests and their impact on biodiversity.

**Keyword:** ambrosia beetles, invasive alien species, beetle native community, beech forests

## OC191. Methods of early detection of insect outbreak in a Mediterranean forest in Karpathos, Greece, using SENTINEL 2

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Considering the extensive forest mortality that insect outbreaks cause globally, early detection of forest infestation is of high significance in order to prevent the uncontrolled spread of the forest destruction they cause. In our research, we applied two methods of early detecting the infestation in a Mediterranean forest in Karpathos, Greece. For the first method we developed our own spectral index combining bands 7 (NIR) and 11 (SWIR) of Sentinel 2 Multi-Spectral Imager (MSI) while for the second one we looked for the differences between the digital signatures of healthy and infested green vegetation. The results for the early detection of the infestation with both methods presented similar accuracy and they were considered satisfactory as the actual purpose of our research was to early detect the beginning of an outbreak and not to predict the exact size of the forthcoming destruction.In short, as it arises from our research, early detection is not impossible, since the methodologies developed show that they can assist in detecting the beginning of the insect outbreak, giving authorities the chance to monitor the phenomenon and encounter on time the catastrophic consequences of insect infestations in Mediterranean forests like those of Karpathos.

**Keywords:** Mediterranean forest, early detection, Sentinel 2, spectral index, digital signatures, insect outbreak

## OC192. The effect of irrigation on the mesofauna of Mediterranean vineyards

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The identification of factors driving vulnerability and resilience of agroecosystems to climate change has become a major challenge. In the European Mediterranean, mean annual temperatures and the length of drought periods are increasing threatening agriculture and related biodiversity. In southern France, the proportion of irrigated vineyards has increased in recent years to cope for higher evapotranspiration and lower rainfall. While irrigation is clearly beneficial to viticulture under water stress, the effects on biodiversity have rarely been studied.The objective of our study was to evaluate the effects of irrigation on soil characteristics and mesofauna in Mediterranean vineyard systems. We hypothesize that irrigation extends the activity period of the mesofauna to the summer whereas summer drought largely reduces mesofauna activity in unirrigated vineyards. We selected 22 vineyards in the Luberon region (Southern France), of which 11 were irrigated and 11 not irrigated. In each vineyard, we collected four samples for soil and mesofauna analysis in April and four samples for mesofauna in August. We didn't find any effect of irrigation on mesofauna in April while August data are still under analysis. The results contribute to a better understanding of the potential effect of global change-related management changes on ecosystem services such as organic matter decomposition and nutrient cycling in Mediterranean vineyards.

**Keywords**: irrigation, biodiversity, mesofauna, vineyard, climate change, southern France

## OC193. Old friends – new challenges, forest insects in Central Europe

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In the last two decades, the species composition (diversity) of the insects found in European forests has changed significantly, as well as the possible damage caused by them. This can be traced back- as in any complex system- to several reasons. Climate change has significantly altered the abiotic environment, which in most cases has become more beneficial for insects. At the same time, the biotic environment has also changed significantly. Many new insect species appeared in the last years, partly by intentional or unintentional introduction from other continents, partly by the area expansion as a result of climate change. New, invasive species have often displaced native species and significantly changed the species composition of some ecological groups (e.g., saproxylic), but also interrupted previous feeding relationships. The role of invasive insects as vectors of pathogenic fungi is also not negligible. However, the changes taking place in forest management should not be ignored either! Multi-functional forestry, the need for continuous forest cover, or the increased amount of dead wood influence significantly the insect species composition and the number of insects found in forests. The presentation illustrates the changes that have taken place through various examples and points out the importance of monitoring, knowledge transfer and research.

**Keywords:** forest insects, climate change, native insects, invasive insects

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## OC194. pclim.net, a database and web application to explore larval phenology of the pine processionary moth *Thaumetopoea pityocampa* across its range since 2015

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PCLIM is an international network of scientists interested in the responses of the pine processionary moth (PPM) *Thaumetopoea pityocampa* to climate change, a winter-active insect whose northward expansion is acknowledged as a bioindicator of winter warming. This collaboration resulted in 2015 in the publication of a book involving more than 100 authors from countries throughout the range of the species. From there, as studies had emphasized the phenological variation among and within populations depending on local conditions, the network started monitoring larval phenology of the PPM. PCLIMdb is the database of these observations, consisting in an annual monitoring of caterpillar colonies to score the most advanced larval instars occurring in mid-winter, when temperatures are the lowest and moulting is the most unlikely (thereby making the survey less sensitive to small variations in sampling dates). These annual snapshots are being completed since 2015 and now comprise more than

5500 observations, which allow benchmarking phenological differences among regions and generations over a geographic extent unique for an insect.
An *R Shiny* web application has been developped at https://pclim.net to dynamically map the observations, filter according to multiple variables, and export corresponding data. SQLite tools are provided to interact with the database directly and run custom queries to compute summmary statistics, view data points (raw or resulting from custom queries) in tables and maps, plot charts or export text data for further analyses. The database is temporarily restricted to authenticated PCLIM members, but will ultimately be made publicly available with a DOI.

Keywords: climate change, database, insect, phenology, pine processionary moth, web application

## OC195. In the context of climate change, the pine processionary moth is no longer facing suitable conditions at the southern edge of its range in Tunisia

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Climate change readily affects insect distributions, survival and persistence due to the strong dependence of ectotherms' development on temperature. Yet, empirical evidence of species shifting their range in response to climate change remains scarce. The pine processionary moth (PPM) *Thaumetopoea pityocampa*, however, is acknowledged as one of the few species for which a causal relationship between air warming and range expansion has been firmly demonstrated. We used this model species : (1) to explore the reproductive success change over three decades based on field observations in various climate clusters in Tunisia, and (2) to study the change in its southern range combining field observations and field experiments on a latitudinal gradient.We found that the fecundity and hatching rates decreased over the last three decades, while average temperature and climate variability increased. Egg hatching failure was mainly attributed to egg abortion and egg sterility. In addition, we revealed the retraction of the PPM from southern Tunisia due to increasing mortality rates of early life stages in populations situated along a thermal gradient, which could be ascribed to a significant local warming. We thus demonstrate that climate in a large part of Tunisia could become too warm for the PPM, and that at the scale of its spatial distribution, PPM is actually shifting rather than expanding northwards.

Keywords: *Thaumetopoea pityocampa*, climate change, egg hatch, range retraction, Tunisia

## OC196. Effects of temperature-related mortality factors and urban heat islands in the phenology of the pine processionary moth, *Thaumetopoea pityocampa*

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Physiological and ecological processes of insects are strongly influenced by temperature. Therefore, climate change is a prominent driver of their distribution and phenology. The pine processionary moth (PPM), *Thaumetopoea pityocampa,* is a forest and urban pest which primarily infests pine trees. Additionally, PPM larvae can release urticating setae responsible for health issues. PPM distribution is moving northwards as a direct consequence of winter warming, and PPM phenology also appears to be disturbed by climate change. A mechanistic model previously developed, using daily air temperature to simulate PPM development from the egg to the last larval stage, provided the first evidence of the effects of climate change on PPM phenology. However, PPM larvae spend a part of their time inside a silk nest whereby microclimatic conditions may affect developmental rate. Furthermore, PPM eggs are laid in summer and larvae generally complete their development in the following spring, which may induce exposure to both hot and cold stressors and influence mortality. In addition, microclimates found in urban areas may have contrasting effects on the PPM phenology. To better understand the PPM phenology, we refined the developmental model to consider: 1) a combination of observed air and predicted nest temperatures, as well as the larval behaviour (entering or leaving the nest), and 2) temperature-related mortality factors. We also compared phenologies on a gradient of urbanization from forests to city in degraded ocean climate versus Mediterranean climate. We highlight how temperature-related mortality factors and urban heat islands can shape PPM phenology on the field.

**Keywords**: pine processionary moth, phenology model, development rate, climate change, urban heat island

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## OC197. Back to the future: Assessing the pine processionary moth range shifts according to past, current and future climate conditions

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The pine processionary moth (PPM), *Thaumetopoea pityocampa*, is an important forest pest of Mediterranean origin whose geographical range is currently changing in relation to climate warming. The larvae build silk tents and feed on pine foliage during winter. Their survival is known to be limited by winter minimum air temperature that drives their feeding activity. Increased winter temperatures have recently led to higher larval survival and PPM range expansion both towards higher latitudes and altitudes. On the other hand, the PPM range is shrinking at its southern edge in North Africa because early larval stages cannot survive the high summer temperatures that are now reached in some southern localities due to climate change. The present study aimed at assessing the PPM geographical distribution using species distribution modeling tools and climate data. We calibrated our model using a dataset of historical occurrence for the PPM. The resulting model performed well and was further used to assess the PPM potential range according to current climate and different scenarios of climate change based on 6 General Circulation Models (GCM), 4 shared socioeconomic pathways of gas emission and 4 time periods (2030, 2050, 2070, 2090). The model was also projected according to paleoclimate data depicting the climate conditions in the mid-Holocene (about 6000 years ago) and at the last glacial maximum (about 22,000 years ago). The results highlight the localization of past glacial refugia and the displacement of climatically suitable areas towards the north in the future.

**Keywords:** pine processionary moth, climate change, geographical distribution, species distribution modeling, range shift

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### OC198. Does phenology drive population genetic divergence in the pine processionary moth in Bulgaria?

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Phenology, the timing of the different steps in a species' life cycle, depends both on genetic characteristics and local environmental conditions. In some cases, the timing of sexual reproduction can differ between localities, which tends to reduce gene flow and increase genetic differentiation between populations.The pine processionary moth *Thaumetopoea pityocampa* is a forest pest occurring in Mediterranean regions. Its gregarious larvae can spray airborne urticating hairs responsible for health hazards. They typically develop in winter in conspicuous silken tents. At the end of larval development, the caterpillars leave their tent in a head-to-tail procession in search for an underground pupation site. Phenology of the species varies strongly among Bulgarian localities: larval development usually ends in late winter or early spring (typical winter form found throughout the species' range) but as soon as fall in some populations (known as summer form). We tested whether phenology was driving genetic differentiation by characterizing phenology (summer form, winter form, intermediate) in 22 sites across the country and genotyping 289 individuals using 23 microsatellite markers and 1 mitochondrial gene. We found a fairly reduced genetic diversity in Bulgaria compared to western Europe, which is probably due to eastward post-glacial expansion from a distant refugium. We showed that natural barriers and geographical distances affected genetic structure, rather than phenological differences. Our results suggest that the strong phenological differentiation does not translate into allochronic differentiation in this particular case, which contrasts with results obtained in Portugal in previous studies focusing on another case of phenological divergence.

**Keywords:** phenology, gene flow, genetic diversity, differentiation, pine processionary moth, Bulgaria

## OC199. Upper and smoother! A 50 year-long survey revealed altitudinal changes in forest outbreaks of the larch budmoth in response to climate warming in the French Alps

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Both dendrochronological data and forester's observations showed that populations of larch budmoth, *Zeiraphera griseana* (Lepidoptera: Tortricidae), presented for centuries cyclical, huge outbreaks reddening every 8-10 years the subalpine larch forests over large geographical ranges of the Alps at optimal elevations of 1,850-2,000m. However, a 50-year long survey in the southern French Alps revealed that since the early 1980s the outbreak frequency became irregular, with a significantly decreased amplitude in this altitudinal range. Using the same collection methods as those used by earlier scientists to survey the 1972- 1982 outbreak development in the Briançonnais region, we carried out in the same stands an annual survey of the larval population densities during the period 2011- 2022. Although still exhibiting a cycle at 1,850-2,000m, population densities were at any phase of this cycle significantly lower than those observed 40 years ago. By contrast, densities increased at higher elevations (2,000-2,200m) but never reached the previous maxima observed in the subalpine range. Re-analyzing former data about moth phenology, we suggested that recent winter and spring warming may have resulted in advancing the hatching of moth eggs with regard to the timing of larch bud burst in the subalpine range, leading the most precocious larvae to starve there. Due to the shortening of the season, a better synchrony between eggs and larch phenology is likely to exist at present above 2,000m, and may explain the increase in density at upper elevations

**Keywords:** climate change, budmoth, insect response, Alps, larch, phenology

# Session 9:
## Medical and Veterinary Entomology

# OC200. CLIMOS PROJECT - In-depth study for knowledge and comprehension of climate and environmental drivers of sand fly borne diseases

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Spread of sand fly vector populations and sand fly-borne diseases (SFBDs), caused by mitigation and climate change, induce over the last two decades several research consortia (EDEN, EDENext and Vectornet) to improve their knowledge, surveillance and control in Europe and neighboring countries. In this context CLIMOS project (Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation with Cost-benefit and Climate-policy Measures) started in 2022, involving universities, institutes, research centers and health ministries from 16 European countries. The purpose is to conduct innovative and applied research seeking to be better prepared for current and future impacts of climate and environmental changes on human and animal health. In particular, the program aims to: i) develop a general public health risk assessment method for SFBDs through integration of climate, environmental and one health disciplines and data sciences; ii) utilize big data

from earth-observing satellites and ground-level surveillance records, to map the locations of disease-carrying insects and provide health, climate and environmental services to keep communities safe; iii) integrate economic and social sciences, to enable socio-economic assessments of impacts of the incidence and spread of SFBDs on individuals and societies. Study is co-funded by European Commission grant 101057690 and UKRI grants 10038150 and 10039289, and is catalogued by the CLIMOS Scientific Committee as CLIMOS number XXXX (http:// www.climos-project.eu). The contents of this publication are the sole responsibility of the authors and the funders had no role in study design, data collection and analysis, decision to publish, or abstract preparation.

### OC201. Mosquito and avian-based West Nile virus (WNV) surveillance systems as an integral component of integrated vector control (IVM) programs

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West Nile virus (WNV) is a mosquito-borne virus circulating in a sylvatic/rural cycle between birds and mosquitoes of the genus *Culex*, and under certain environmental conditions it spills over to human settlements where it infects humans and equines causing large outbreaks. The unpredictable occurrence of WNV outbreaks dictates the establishment and maintenance of surveillance systems for quantifying WNV activity levels, assessing public health risk and guiding vector control interventions. Mosquito and avian-based surveillance is considered an important tool for gauging WNV circulation and is commonly used in contemporary Integrated Vector Management (IVM) programs. Here we present information retrieved from a multi-year WNV surveillance system conducted in Greece which included monitoring of avian hosts/sentinel birds and mosquito vectors. Information relating to a) virus circulation in mosquitoes and wild/sentinel bird species, b) impact of environmental parameters on virus circulation, and c) vector control response will be presented. The role of WNV surveillance systems, such as this one, in mitigating the risk of disease transmission and enhancing our understanding on the ecology of WNV will be discussed.

**Keywords:** West Nile virus, surveillance, integrated vector management

### OC202. Risk assessment of (re)-emerging arbovirosis in Southern Europe

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In a geological era dominated by human activity, we are experiencing an alarming deterioration of natural environments responsible for environmental disequilibrium and biodiversity loss of unprecedented proportions. The worsening of the ecological imbalance is mainly caused by urban expansion leading to habitat fragmentation resulting in a non-random process of biodiversity loss. This decrease in species richness harmonizes with an increased abundance of invasive mosquito species able to thrive in urban environments thanks to an overabundance of resources. Thus, our interest is to evaluate the risk of (re)-emergence of arboviruses considering anthropogenic and ecological factors (biotic and abiotic) as the driving forces able to modulate the spatiotemporal distribution of vectors, hosts, and pathogens. Based on a coordinated approach of mosquito surveillance, pathogen screening, and environmental monitoring we found *Culex pipiens* mosquitoes infected with USUTU virus and *Setaria* sp. in central Italy. Studying the Usutu reservoirs' biodiversity, we observed a correlation between host biodiversity and USUTU frequency across months. In-deep studies of land cover and degree of urbanization revealed these two variables able to model the distribution of *Cx. pipiens* and *Aedes albopictus*. These analyses repeated over time will allow a detailed map of the risks associated with mosquitoes and the chance to optimize control strategies.

**Keywords:** mosquito, vector borne diseases, arbovirosis

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## OC203. A social-ecological systems approach to tick and tick-borne disease risk: exploring local actor engagement in collective action tick bite prevention strategies in the Occitanie region in southern France

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Tick and tick-borne diseases (T&TBDs) are a One Health problem affecting both veterinary and public health worldwide. Due to anthropogenic and bio-physical factors (e.g. societal, cultural, economic, political and climatic variables) various tick species' geographic distribution, and thus T&TBD risk, is increasing globally. Vectors of the highest pathogen diversity, ticks are now considered the primary zoonotic disease vector affecting public health in Europe. Today, acaricides are the primary control method, having seemingly profound negative impacts on environmental health, as well as potentially increasing acaricide resistance in tick populations. Therefore, T&TBD risk is simultaneously health-related (human, veterinary and environmental), ecological (ecosystem functions and biodiversity) and economic, depending on local social and ecological parameters that influence this risk. We argue that One Health-inspired tick bite prevention strategies that integrate local societal and biophysical parameters, as well as multi-sector actor collaboration are the most effective T&TBD risk defense. This approach requires local actor T&TBD risk knowledge combined with science-driven data within multi-actor T&TBD risk network(s). For these reasons, we propose a transdisciplinary method that mobilizes participatory approaches involving local actors to create a shared vision of T&TBD risk. Local actor typologies were first identified using participant observations and secondary data (e.g. the literature), followed by semi-structured interviews with 23 local actors to determine needs, priorities and perceptions regarding local T&TBD risk. Finally, participatory workshops were organized with local actors to discuss social-ecological T&TBD risk indicators and potential collective action-oriented tick bite prevention strategies based on a shared vision of local T&TBD risk.

**Keywords:** tick-borne disease, One Health, social-ecological systems, local actors, participatory approach

## OC204. Sandflies vectors of *Leishmania infantum* in Greek islands

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*Leishmania infantum*, the causative agent of human and canine leishmaniosis, is one of the most important zoonotic parasites, with a continuous spread from the Mediterranean areas to continental Europe. It is transmitted by female phlebotomine sand flies during successive blood meals. In an effort to evaluate the seroprevalence of leishmaniosis in populations of dogs leaving on the Aegean islands of Crete and Leros, we also collected sandflies from these locations in order to gain information of the species present and their possible role as reservoirs of the parasite. Sand fly collection was performed using the Centre for Disease Control (CDC) miniature light traps and BG Sentinel traps, at regular intervals for two sandfly activity seasons (April to October). A total of 1,299 sand flies belonging to nine species were captured from the 2 islands. The predominant species, based on collected female sand flies, were *Phlebotomus papatasi* (30.2%), *P. similis* (26.3%), *P neglectus* (18.7%), *P. tobbi* (9.9%) and *Sergentomyia minuta* (7.7%), followed by *P. simici, P. perfiliewi*, *Sergentomyia dentata* and *P. mascittii*, several of those being competent vectors of *L. infantum.* Moreover, all female captured sand flies were tested for the presence of *L. infantum*, with a real-time qPCR assay. *L. infantum* infection in dogs was confirmed in both islands (seroprevalence up to 30%), however, none of the sandflies was found to harbour parasites' DNA based on the molecular analyses. The results of our survey confirm that the environment in both islands is favourable for completion of the *L. infantum* life-cycle.

**Keywords:** *Leishmania*, sandflies, *Phlebotomus*, zoonoses

## OC205. Vector borne diseases: an expanding health threat for animals and humans

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Vector-borne diseases (VBDs) are caused by a range of pathogens transmitted to animals and humans by blood-feeding arthropods, e.g., ticks, fleas, mosquitoes and sand flies. On top of threatening human health, they have a major impact on the health and welfare of companion animals and livestock affecting their productivity, resulting in great economic losses. VBDs represent a growing global threat, due to their constant spread from traditional geographical and temporal restraints to new areas, exposing new populations to previously unknown infectious agents and posing unprecedented challenges to practitioners. The constantly changing epidemiology of VBDs is being influenced by

different factors, such as the impact of climatic change on vector distribution and pathogens development rates, the habitat change introduced by humans, e.g., wetland creation, the increased movement of goods, humans, livestock and companion animals worldwide and, for many of those, an increasing insecticide resistance. So far is the spread of VBZ, endemic in southern Europe to northern non-endemic regions of Europe, is well documented. However, their relative significance cannot be quantified, since for most of them there are no surveillance programs or accurate assessment of prevalence in place, combined with gapsin reporting and accurate diagnosis. Combating VBDs is likely to face an increasingly serious combinationof challenges in the coming years. It is therefore important to adapt a collaborative, multinational, multidisciplinary and holistic approach based on the One Health principal. During this presentation lessons learned in Southern Europe will be presented, giving examples on leishmaniosis, tick-borne infections, dirofilariosis and bluetongue.

## OC206. Molecular detection of *Leishmania* DNA and blood meal analysis in wild caught *Phlebotomus* (Larroussius) species

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*Phlebotomus (P.) perniciosus* and *Canis familiaris* are respectively the only confirmed vector and reservoir for the transmission of *Leishmania* (*L.*) *infantum* MON-1 in Tunisia. However, the vector and reservoir hosts of MON-24 and MON-80, are still unknown. The aim of this study was to analyze the *L. infantum* life cycle. Sand flies were collected in seven locations from the center of Tunisia affected by human visceral leishmaniasis. *Phlebotomus* (Larroussius) spp. were screened for *Leishmania* infection using 18SrDNA-PCR and qPCR. *Leishmania* was typed by nested PCR-RFLP targeting the ribosomal Internal-Transcribed-Spacer 1. Blood meal origin was identified using PCR-sequencing targeting the vertebrate cytochrome b gene. A total of 3,831 sand flies were collected belonging to two genus *Phlebotomus* (five species) and *Sergentomyia* (four species). From the collected 1,029 Larroussius subgenus female sand flies, 8.26% was positive to *Leishmania*. Three *Leishmania* spp. were identified by ITS1 nested PCR: *L. infantum* 28%, *L. killicki* 13%, and *L. major* 22%. The average parasite load was 1,174 parasites/reaction and 90 parasites/reaction in unfed and fed flies, respectively. Among the 177 analyzed blood-fed females, 169 samples were positive. Sequencing results showed seven blood sources: cattle, human, sheep, chicken, goat, donkey, and turkey. In addition, mixed blood meals were detected in twelve cases. *Leishmania* DNA was found in 21 engorged females, with a wide range of blood meal sources: cattle, chicken, goat, chicken/cattle, chicken/sheep, chicken/turkey and human/cattle. Our results support the role of *P. longicuspis*, *P. perfiliewi*, and *P. perniciosus* and the identified vertebrate in *L. infantum* transmission.

Keywords: *Phlebotomus perniciosus*, *Phlebotomus perfiliewi*, *Phlebotomus longicuspis*, *Leishmania*, blood meal, Tunisia

## OC207. The difference between able to act and willing to act: The case for keeping the public in public health decisions

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People are warming the planet through their actions resulting in global climate change and an increase in extreme weather events. These changes alter the composition and stability of ecosystems making them more susceptible to introduced pests and pathogens. Simultaneously, the movement of people world-wide continues to increase and public demand for cheaper goods and food has resulted in unprecedented international commerce. Global connectivity increases the frequency, quantity, and diversity of pests and pathogens transported and introduced pests at best are nuisances to the public and at worst transmit pathogens possibly resulting in disease. Therefore, if people are creating, contributing, or suffering from the problem, they must also be a part of the solution. Mathematical/epidemiological models inform management decisions where and when to trap and treat. New technology improves surveillance efficiency and treatment capabilities to target the deployment of novel treatments and active ingredients. However, during an outbreak the public and more importantly public perception determines management decisions. After the crisis, an educated and supportive public maintains program support and sustainability. The goal of community-based science is to recruit and educate the public and most importantly the community leaders/influencers that will advocate and support surveillance and treatment measures while combating misinformation campaigns. People unknowingly create the health hazard, but they must knowingly be part of the solution, which is a substantial challenge. Therefore, scientists and government officials must continuously work to keep the public involved with scientific advances, public health decisions, and community plans as we adapt to the changing world.

**Keywords:** community science, surveillance, public health

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## OC208. Mass spectrometry toolbox: lessons learnt from a decade of applications on phlebotomine sand flies

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Phlebotomine sand flies (Diptera: Psychodidae) are important vectors of pathogens that include *Leishmania* protozoans, bacteria and viruses. Despite many regions suffering from outbreaks of human leishmaniases, often exacerbated by natural disasters and political unrest, and newly discovered phleboviruses with unknown medical significance, sand fly-borne diseases remain neglected. Conclusive identification of vectors and their trophic affinities towards reservoir hosts help to understand local transmission cycles and to assess potential emergence of sand flies in new regions. MALDI-TOF protein profiling of sand flies from various endemic regions of the Old World including the Mediterranean, East Africa or south-east Asia with standardized trapping, storage and sample

preparation enabled conclusive, time-, labour- and cost-effective species identification of field-collected specimens including those yielded by large-scale multinational entomological surveys. Species-specific protein profiles proved as useful in integrative taxonomy of sand flies, supporting formal description of new species, suggesting existence of yet undescribed cryptic species or challenging validity of established taxons while allowing parallel application of other complementary approaches (DNA barcoding, morphological analysis) and screening for sand fly-borne pathogens. MALDI-TOF peptide mass mapping of host-specific haemoglobin peptides in engorged sand fly females successfully identified the host blood, including mixed bloodmeals, up to 48 hours post feeding, a period longer than those covered by other conventional methods. Prospects and challenges of mass spectrometry deployment in vector biology include rigorous application of standard protocols, dissemination of necessary machinery and expertise, modifications that allow parallel pathogen detection and creation of publicly available open-access database of reference protein profiles.

Keywords: sand fly, MALDI-TOF, species identification, bloodmeals

## OC209. A new FTA card-based trapping system for mosquito-borne diseases surveillance

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Entomological surveillance is a key component in prevention of arboviral diseases, providing timely indicators before outbreaks occur in host populations. However, traditional methods may not be appropriate in some contexts or less feasible due to logistical purposes. Recently, honey baited FTA cards have been proposed to detect pathogens from mosquito saliva released during a sugar meal. In this study, a BG-sentinel® trap was modified to include an artificial feeding system consisting of a collection chamber equipped with a honey-soaked FTA card. This surveillance system was first implemented in a temperate area (Veneto, Italy) and compared to the CDC trap already used in West Nile and Usutu viruses surveillance. To define its applicability in a different eco-epidemiological context, this approach was also evaluated in a tropical area (Djibouti). The results obtained are indicative of good performance in targeting several vector species, including *Cx. pipiens*, *Cx. quinquefasciatus*, *Ae. aegypti* and *Ae. albopictus*. The effectiveness of FTA cards in detecting West Nile, Usutu and Dengue viruses confirms the reliability of this novel approach. Additionally, the use of FTA cards ensures nucleic acids preservation without the need for a cold chain, and reduces the efforts required for viral detection. All these features are particularly useful in a multi-target surveillance, remote sampling areas and contexts characterised by high mosquito densities. The integration of this FTA card-based method could enhance the power of current strategies, offering an alternative and/or complementary tool for arbovirus surveillance programs.

**Keywords:** FTA card, arbovirus, mosquitoes, surveillance

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## OC210. Defining wing-beat patterns of mosquitoes for automated detection

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Deepening our understanding of mosquito wing-beat acoustics is essential for the development of novel, automated acoustic mosquito surveillance devices. To support the translation of acoustic mosquito investigations into an automated surveillance device, a novel semi-anechoic measurement arena has been designed and constructed to generate precision acoustic data sets for both free-flight and tethered mosquitoes. The low-noise nature of the constructed arena permits novel measurement metrics to be explored in both the time and frequency domains using calibrated condenser pressure microphones. Using these metrics, an investigation using *Aedes aegypti*, *Anopheles coluzzii* and *Culex quinquefasciatus* was undertaken, to describe their flight acoustics under a number of treatments including the effects of emergence age and blood feeds. This talk will outline the key design features of the novel arena constructed, and explore the rationale of how the methods were designed which were a combination of mechanical design, acoustic analysis and the flight behaviour of investigated genera. The main acoustic findings will be discussed, as well as an outline given for how this could pave the way for future automated surveillance tool development.

**Keywords:** mosquito, acoustics, vector surveillance, design

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## OC211. Is *Aedes albopictus* truly exophagic in Italy? Citizen science data can provide the answer

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In its native range in Asia, the tiger mosquito, *Aedes albopictus*, bites mostly outdoors. This is a major behavioural difference compared to *Aedes aegypti* and one of the reasons for lower synanthropy and lower efficiency of *Ae. albopictus* as vector of human arboviruses. However, whether this exophagic behaviour is maintained also by *Ae. albopictus* invasive populations after adaptation to temperate climates has never been specifically investigated.We exploited 4,914 photographic records provided by citizens during 2-years of implementation of the Mosquito Alert app in Italy to investigate

indoor/outdoor contacts between *Ae. albopictus* and humans relative to *Culex pipiens. Aedes albopictus* represented 90% and 52% of the total number of mosquitoes photographed outdoors (N=1,087) and indoors (N=1,842), respectively. Despite the total number of mosquitoes recorded indoors vs outdoors is likely biased by the higher difficulty of taking a picture outdoors, the observation of such a high proportion of *Ae. albopictus* among mosquitoes recorded indoors represents an objective evidence of a strong endophagic activity of this species in Italy. This may reflect a change in the species behaviour with high epidemiological relevance, as it implies a higher contact with humans than that estimated by data obtained by conventional outdoor collection methods. Moreover, it may imply a change in practices to prevent arbovirus transmission, i.e. no longer personal protection only, but also protection of the indoor environment. Finally, the results show the exciting capacity of citizen science in providing original data in revealing biological aspects difficult to be investigated by conventional monitoring approaches.

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## OC212. Pilot test of a novel, multiplex point-of-need tool to detect pathogen DNA/RNA in vectors for integrated vector borne disease surveillance

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The potential of using molecular xenomonitoring for the surveillance of multiple vector-borne diseases (VBDs) has not been fully explored, even though some VBDs often co-exist in time and space. Most surveillance programmes use a vertical approach, and only focus on their target vector of interest. Yet by-products of collections (non-target vectors) offer opportunities for complementary screening to maximise resources in an enhanced integrated surveillance system. Any haematophagous insect can be perceived as a ‘biological syringe’ and, if they recently blood-fed on an infected human, can be screened for pathogens that they may or may not transmit. We have developed a multiplex point-of-need (PoN) molecular tool that can be used in the field to screen all blood-fed vectors and by-products for a range of pathogen DNA/RNA. The tool uses an isothermal recombinase polymerase amplification technique and ‘home-brew’ ingredients (to reduce costs) and is integrated in end-point lateral flow strips. It does not require a PCR machine, operates at 37-42°C and provides results in less than 20 mins. Using mobile data collection technology (with online and offline capabilities), results can be relayed to the stakeholder timely, efficiently and are less error-prone. Following laboratory validation, the PoN tool has been translated to the field in a pilot study conducted in Bangladesh in sites endemic for visceral leishmaniasis, lymphatic filariasis, malaria and dengue. In our talk, we will present data comparing the effectiveness of the PoN tool with traditional qPCR for pathogen DNA/RNA detection, provide costs involved, and compare vector sampling methods.

**Keywords**: xenomonitoring, vector surveillance, point-of-need, multiplex, tool

# OC213. A change in IRS insecticide and mode of application in the Visceral Leishmaniasis elimination programme in India - the impact on sand fly abundance and disease incidence

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In 2005 India initiated a programme to eliminate visceral leishmaniasis (VL) utilising mainly case detection and treatment and indoor residual spraying (IRS) with DDT. Operational research we carried out in 2013 demonstrated that DDT was not effective and assisted the National Centre for Vector Borne Disease Control to make a policy change to using alpha-cypermethrin with compression pumps in 2015. However, it is not well understood if and how IRS contributes to VL control, despite taking up >3/4 of the budget.

To determine the impact of this policy change, in 2016, we established 8 sentinel sites, 64 villages as a longitudinal study measuring the key indicators, vector abundance of the sand fly vector *Phlebotomus argentipes*, xenomonitiong *L. donovani* the infectious agent, insecticide resistance, resistance mechanisms, IRS coverage, quality of IRS and case incidence.

The data demonstrates a clear decline in *P. argentipes* abundance and infectivity showing an overall reduction in transmission over time. As of March 2023, elimination targets have been met in all but one block (sub-district). Using statistical and mathematical modelling, we were able to show that sandfly abundance changed because of IRS, and that accounts for VL case numbers over time.

**Keywords:** indoor residual spraying, visceral leishmaniasis, elimination

## OC214. The Sterile Insect Technique and its derivatives against *Aedes* invasive species in Europe

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Mosquito-borne diseases represent a major threat to human kind. Dengue is a particular treat and WHO indicates the urgent need for alternative mosquito control methods, including the Sterile Insect Technique (SIT), which has progressed rapidly in recent years. The SIT package against mosquitoes is currently under development and huge progress was made in all components including mass-rearing of larvae and adults, sex-sorting of pupae, handling, transport, irradiation and aerial release of the sterile males. Testing is ongoing in the field following a phased conditional approach (PCA) proposed for the validation and potential large-scale deployment of the SIT against mosquitoes, as a component of an area-wide-integrated vector management strategy. In such a process, support or advancement to the next phase is conditional on the completion of all (or most) activities in the previous phase, and the scope, expense, and commitment increase along the process. In Europe, several countries initiated field trials against *Aedes albopictus*. Albania, Croatia, France (mainland), Montenegro, Portugal and are in phase 1 (baseline data collection) and implemented mark-release-recapture experiments using irradiated males. Croatia, Germany, Greece, and Serbia have conducted small-scale field trials (phase 2). Italy, Spain and Reunion island are currently upscaling these field trials (phase 3). Finally, Cyprus has initiated an eradication trial against *Ae. aegypti*. In our presentation, we will review the progress of these countries along the PCA, showing that SIT was able to suppress target mosquito populations in various settings and present the perspectives in Europe, including the potential of combination of SIT with other methods to improve the control of mosquito-borne diseases (boosted SIT in particular).

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## OC215. Sugarcane molasses as an alternative adult diet for laboratory rearing of *Aedes albopictus*

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High vectorial capacity of *Aedes albopictus* adds pressure on public health. To manage mosquito and diseases spread, a list of control methods and approaches have been developed and implemented. Among them the Sterile Insect Technique (SIT), a biologically sound approach, aiming to suppress wild mosquito populations, was tested and adopted. To support SIT applications, laboratory mosquito rearing is practiced, to guarantee mosquitoes' quality, survival and performance in the wild. Sugar solution constitutes the main adult diet despite issues encountered (e.g. mold). We explored sugarcane molasses as an alternative and low cost adult diet, in two laboratory trials and compared it with a sugar solution (10%). Longevity, blood feeding rate and fecundity was compared in laboratory adults emerged from three different larval diets (catfood, fishfood, mealworm flour). Eggs layed from wild adults were counted and the size of eggs and pupae was recorded. Our results revealed that laboratory-reared females fed on sugar solution lived longer regardless of the larval diet. Male lifespan didn't differ between adult diets, in two out of three larval diets. Blood feeding was affected by larval but not adult diet. Female fecundity wasn't related to either adult or larvae diet. Wild females fed in molasses oviposited larger eggs compared to sugar solution. Female pupae, resulting from females fed in sugar solution, were bigger compared to molasses. Male pupae had similar size in the two adult diets. Our findings provide the first step towards experimentation in alternative adult diets that can decrease rearing efforts without suppressing mosquitoes' quality.

**Keywords:** mosquitoes, adult diet, quality traits, performance

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## OC216. The integrated management of the Asian tiger mosquito in Region of Attica (Greece)

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*Aedes albopictus* is causing a high concern in public health due to its severe nuisance and its vectorial capacity for a plethora of different pathogens. The management plan is a complex system that includes coordinated actions. In this context a pilot integrated management of *Aedes albopictus*, was implemented in the Region of Attica (Greece) including the synergistic action of the sterile Insect Technique (SIT) with the simultaneous implementation of citizens' educational visits ("door-to-door"). During the implementation of D-t-D method, a questionnaire distributed, based on KAP methodology (Knowledge-Attitudes-Practices). In parallel, within the framework of an area-wide integrated pest management (AW-IPM), a total of 55 BG-Sentinel 2 (BGS2) traps and 110 oviposition traps (ovitraps), are inspected weekly throughout the year.

The results from D-t-D revealed that a visit-inspection of the potential breeding sites in their yards can influence the residents' behavior and raise their awareness towards the reduction of mosquito population. Moreover, results from the releases of sterilized males revealed a significant reduction of egg hatchability and egg density. The outcomes from the pilot trials (both D-t-D and SIT) and the systematic entomological surveillance will be further evaluated to develop an updated AW-IPM against *Aedes albopictus* aiming to reduce the use of biocides in urban areas, as foreseen by EU Regulation 528/2012. Future steps involve boosted SIT, aiming to increase our SIT knowhow and to invest on the production of sterilized mosquito males in Greece in all stages (mass rearing, sex sorting, sterilization, releasing) for the first time.

**Keywords:** invasive mosquito species, SIT, surveillance

## OC217. Long non-coding RNAs regulate *Aedes aegypti* vector competence for Zika virus and reproduction

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Long non-coding RNAs (lncRNAs) are a type of RNA molecules produced by all eukaryotic organisms, insects included, that play critical regulatory roles in various cellular and metabolic processes. Although studies on insects have focused mainly on model-organisms such as *D. melanogaster*, recent findings have highlighted their potential suitability for control approaches against insect pests, due to their striking features such as species and tissue specificity. To elucidate their role in mosquito biology we employed a computational and experimental pipeline to study their impact in vector competence of *Ae. aegypti,* the major disease vector of several arboviruses. We focused our research on the interplay between *Ae. aegypti* and Zika virus and characterized mosquito lncRNAs that are stimulated upon infection. Expression data revealed that at least six lncRNAs (designated *Zinc1, Zinc2, Zinc3, Zinc9, Zinc10* and *Zinc22*) were significantly upregulated in response to Zika infection. Moreover, dsRNA-mediated gene silencing of *Zinc1, Zinc2*, and *Zinc22* rendered mosquitoes significantly less permissive to ZIKV infection, while silencing of *Zinc22* also reduced fecundity, indicating a potential role for *Zinc22* in trade-offs between vector competence and reproduction. We also found that silencing of *Zinc9* significantly increased fecundity but had no effect on ZIKV infection, suggesting that *Zinc9* may be a negative regulator of oviposition. Our work demonstrates that some lncRNAs play host factor roles by facilitating viral infection in mosquitoes. We also show that lncRNAs can influence both mosquito reproduction and permissiveness to virus infection, two biological systems with important roles in mosquito vectorial capacity.

Keywords: *Aedes aegypti*, Zika, lncRNAs, vector control, RNAi

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## OC218. Mosquito odour-baited mass trapping reduces malaria transmission intensity

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The substantial decline of the global malaria mortality and morbidity during the past two decades is threatened, predominantly due to the physiological and behavioural resistance of the mosquito vectors. This resistance has resulted in increased outdoor mosquito feeding activity, undermining the conventional indoor vector control strategies, thus hindering the global malaria prevention and control efforts. Thus, there is a need for novel strategies targeting outdoor vectors. In this study, we show that following the implementation of odour-based mass trapping, a significant reduction in vector density, human-biting rate and entomological inoculation rate, as well as malaria prevalence, was recorded in an intervention village, when compared with a control village. These findings provide convincing evidence that odour-baited technologies are suited to be important additional remedies for controlling residual malaria by targeting vectors outdoors. This study clearly demonstrates that odour-baited technologies can be adopted alongside other vector control methods, enhancing our integrated vector management arsenal.

Keywords: *Anopheles,* chemical ecology, vector, control

## OC219. Genome sequence and metagenomic analyses of *Aedes korei*cus and *Aedes japonicus*: implications in vector control

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The Asian tiger mosquito, *Aedes albopictus,* reached Europe in early 90s, rapidly expanding in the entire continent to become the main vector for human arboviruses. Also, two additional invasive Aedes mosquito species, *Aedes japonicus* and *Aedes koreicus*, are circulating in several European countries posing potential health risks to humans and animals. Vector control is the main option to prevent mosquito-borne diseases, and an accurate sequence of the genome of these mosquitoes is essential to better understand their biology and to develop effective control strategies.We used short and long-read sequencing data to perfom de novo assembly. Thus, we obtained Akor1 and Ajap1, the assemblies of *Aedes koreicus* and *Aedes japonicus* genomes. Their quality was ascertained using various metrics. Masking of repetitive elements, gene prediction and functional annotation were performed. Sequence analysis revealed a very high presence of repetitive DNA and, among others, thermal adaptation genes and insecticide-resistance genes. The assembly of the Akor1 and Ajap1 genomes constitutes the first updated collective knowledge of the genomes of these mosquito species, providing the possibility to understand key mechanisms of their biology such as the ability to adapt to harsh climates and to develop insecticide-resistance mechanisms. Moreover, the microbiota of different populations of *Aedes koreicus* and *Aedes japonicus* was analysed by 16S RNA sequencing. These studies highlighted some population-specific symbionts and bacteria plausibly involved in the mechanisms of thermal-adaptation and insecticide-resistance. Collectively, these results may help developing control methods to counteract the dispersal of these vectors.

Keywords: *Aedes koreicus*, *Aedes japonicus*, genome sequencing, metagenomics

## OC220. A robotic system for sex separation of the tiger mosquito, *Aedes albopictus,* based on machine vision at the pupal stage

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The tiger mosquito, *Aedes albopictus,* is vector of various diseases, affecting a large percentage of the world population, as evident by the re-emergence of arboviruses. Mosquito control is difficult, expensive, and still heavily relying on chemical insecticides, with the well-documented adverse effects to the environment. Among environment-friendly vector control methods, the sterile insect technique (SIT), is receiving renewed interest, following advances in mosquito rearing, packaging, transfer, and release. A still-standing bottleneck is the prerequisite of zero females in the male release batch and, despite recent advances, this task is still either manual, or prone to error, or requires either expensive or difficult to obtain equipment. Separating male from female pupae is usually done by exploiting the pupal size sexual dimorphism, a time-consuming process that requires the human involvement. Here we present a robotized system to automate the sex separation process and autonomously separate males from females. The design concept of the robotic system, which can be moved in x, y, z-axes, includes a camera and a suction device to detect and transfer the male pupae from one tray to another. The conceptual design of the robot and its mechanical/electronic components are presented, while the developed image processing software for recognizing the male from the female pupae is demonstrated. The results of the software part of the system show that it is possible to differentiate the pupae by using the size difference and a deployment of the proposed robotized system will be a very promising low-cost sex separation solution.

**Keywords:** tiger mosquito, robot, automation, sex separation, machine vision

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## OC221. There is nothing simple about vector control

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Humanity took two million years to create a population of one billion, 120 years for 2 billion and the next only took 35 years. Unsurprisingly, this population explosion has had an enormous impact on the environment. Habitat loss, pesticides and climate change are threatening insect populations worldwide. In 2019, Biological Conservation reported that 40% of all insects species are declining globally and that a third of them are endangered. Ironically, while the very species we want to cherish are disappearing, many of species that we purposely try to eliminate thrive. Vectors and vermin, such as mosquitoes and rodents, can simply settle in the environments that humans create. With many people in one place, pathogens can easily and permanently circulate, and quickly jump between livestock, wildlife and humans. Marieta Braks will address current trends in vectors and vector borne diseases worldwide and in specific Europe and discuss strategies to prepare against future vector borne disease outbreaks. She will use insights from tropical countries to show that there is nothing simple about control of vector borne diseases.

**Keywords:** vectors, vector borne diseases, trends, solutions

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## OC222. *In vitro* evaluation of the antibacterial and anticancer activity of the peptide fraction extracted from the hemolymph of *Hermetia illucens*

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Antibiotic resistance is globally one of the most crucial health and social emergencies along with resistance to cancer therapies. In the search for safe and effective new drugs, more attention has recently been paid to a class of biologically active compounds: antimicrobial peptides (AMPs), small bioactive proteins, naturally produced by all living organisms as components of their innate immune system, with antimicrobial, antiviral and antifungal activity. The interactions between microorganism membranes and AMPs depend on electrostatic forces between positively charged AMPs and the membrane of negatively charged micro-organisms. Similarly, to bacteria, cancer cells have a net negative charge due to their high expression of anionic molecules on the outer sheet of the membrane and this allows electrostatic interactions between AMPs and the surface of many cancer cells. AMPs are produced by all organisms, but insects are among the richest and most innovative sources. The aim of our research is to identify pharmacologically active principles of natural origin from the insect *Hermetia illucens* (Diptera, Stratiomyidae), to be used for the development of alternative antimicrobial and

anticancer drugs or in support of conventional therapies already in use. Peptide component was isolated from the hemolymph of immunized (by Gram-positive and Gram-negative bacteria) and not immunized larvae and tested to evaluate its antimicrobial and anticancer activities. Antimicrobial properties were analysed by agar diffusion assay and microdilution assay against both Gram-positive and Gram-negative bacteria, while anticancer effect was evaluated by MTT assay in different cancer cell lines.

**Keywords:** black soldier fly, antimicrobial peptides, antimicrobial resistance, anticancer resistance

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## OC223. Influence of climate chance and infection on thermal preferences of mosquitoes

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Mosquitoes (Culicidae) are vectors that spread numerous pathogens of human or veterinary significance, such as dengue virus, malaria parasites and canine heartworm (*Dirofilaria immitis*). The impact of temperature on vector-borne disease dynamics and distributions has been studied intensively, especially to model the effect of climate change. However, these studies used macroclimatic data and did not consider whether vectors have thermal preferences and select specific microhabitats. With this project, the preferred temperatures of mosquitoes are investigated under laboratory conditions in a thermal gradient setup with video tracking. Earlier such experiments had revealed that laboratory reared *Aedes aegypti* and wild caught *Ae. japonicus* behaviourally thermoregulate, preferring cooler resting temperatures than their physiological optimum proposed in the literature. The present study revealed that, *Ae. aegypti* infected with *D. immitis* showed a cold preference at day nine after infection, which presumably is a pathogen-induced stress response against the migration of the larvae 3 of the pathogen into the proboscis. A second set of experiments with the same gradient (19 °C- 35 °C) was done with *Ae. aegypti* lines selected for 20 generations at 24°C or 30 °C and acclimated each at these temperatures. Cold selected and warm acclimated mosquitoes showed a higher abundance in the warmest temperature zone. Since mosquitoes select microhabitats by thermal preference, and this preference is adaptable, it is imperative to include thermal preference with its adaptability in future models.

**Keywords:** Culicidae, microclimate, temperature preference, thermal adaptation

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## OC224. Mayaro virus, an emerging New World alphavirus knocking at our door

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The emergence of arboviral outbreaks represents one of the most challenging issues for human and animal health. Climate change and anthropogenic activities, including the rapid movement of people and goods around the globe, are fostering the emergence of exotic pathogens and vectors in naïve regions. Over the past decade, multiple imported cases of Mayaro virus have been reported in Europe, with growing concerns about the possibility of autochthonous outbreaks, similar to what happened with Chikungunya virus. Mayaro virus is a New World arthritogenic alphavirus, which shows high vector plasticity in experimental laboratory settings, proving capable of infecting not only *Aedes aegypti* and *Aedes albopictus* but also several species of *Anopheles* mosquitoes.
Here, we explored vector competence of European population of *Aedes albopictus* and *Anopheles atroparvus* for Mayaro virus. After exposing mosquitoes to an infectious blood meal containing Mayaro virus (strain BeAn 343102), we analyzed viral titers in body, legs and saliva using a focus forming assay at day 7 and 14 post feeding.
Results demonstrate for the first time that European mosquito populations are able to sustain the replication cycle of the virus and efficiently transmit it through saliva starting from day 7 post feeding. Finally, our results highlight that surveillance plans and entomological control measures should be adapted to include also *Anopheles* species, which may contribute to the transmission cycle of Mayaro virus and potentially other exotic arthropod-borne viruses.

**Keywords**: Mayaro virus, vector competence, transmission, *Aedes*, *Anopheles*

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## OC225. Investigation of the presence of *Dirofilaria* spp infection in *Culex pipiens* mosquitoes and dogs in the Attica Region

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Dirofilariosis is an important parasitic disease that affects the health of dogs and cats globally. In Greece, it is known that the prevalence is higher in the northern part of the country. Epidemiological models, analyzing the risk of the disease, suggest that the temperature in many parts of Greece is suitable for its transmission. Our aim was to investigate the presence of the parasite in Attica region (eastern part of Central Greece) and particularly its circulation in dogs and mosquito vectors. For the above-mentioned purposes we examined 2,090 blood samples from dogs, with ELISA-antibody test and 913 blood-fed *Culex pipiens* mosquitoes, sampled from 55 different areas in the Attica region using BG sentinel traps (equipped with BG-Lure and constant flow rate of $CO_2$-gas cylinders). Based on the blood samples, we identified positive and/or suspected results in 9 different areas in Attica. Additionally, during the study period, there was a report of a clinical case (a dog that died due to dirofilariosis). Regarding the 224 pooled mosquito samples, 23 of them, from 12 areas of the Attica region, were PCR positive for *Dirofilaria* spp. Four (4) areas were identified in the Attica Region that combined both positives and/or suspected *Dirofilaria* serological results from dog blood samples and positive blood fed mosquitoes. These preliminary results suggest that the parasite circulates in the mosquito population in the Attica region and may result in sporadic cases of the disease.

**Keywords:** dirofilariosis, parasitic disease, PCR, blood fed

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## OC226. Entomological surveillance and detection of West Nile Virus in *Culex pipiens* mosquitoes in the Region of Attica, Greece, 2021-2023

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Entomological surveillance is important for the prevention of vector-borne diseases, contributing to their early detection, understanding of their epidemiology and prompt implementation of targeted control measures. Systematic monitoring of mosquito populations and West Nile Virus (WNV) detection were conducted in the Region of Attica, Greece, during the years 2021-2023. A total of 55 BG-Sentinel traps (equipped with $CO_2$ and BG-Lure) were placed in 55 different areas, monitored weekly throughout the years of study. For the selection of the trap locations, the geostatic method of stratified random sampling was used, based on land use criteria. Regarding WNV detection, BGS2 traps (equipped with $CO_2$ and BG-Lure) were placed in 24 of the above locations in Attica region. The sampling was performed for each location, every 15 days for 24 hours. *Culex pipiens* female mosquitoes were collected, grouped in pools (in respect to trap and collection date), in order to evaluate the presence of WNV, applying a highly- sensitive real-time PCR protocol. Eighteen (18) mosquito species have been recorded and identified morphologically. The largest populations were recorded for *Cx. pipiens* and *Ae. albopictus* species. The study is ongoing and up to now more than 610 pools were examined for the presence of WNV, revealing its circulation in more than fourteen (14) areas. Noteworthy findings include constant recording of *Culex pipiens* populations throughout the whole year. Furthermore, preliminary indications about WNV presence in mosquitoes during the winter period in three (3) areas were noted, though replication and mosquito gonotrophic activity remains low.

**Keywords**: entomological surveillance, *Culex pipiens*, West Nile Virus, real-time PCR

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## OC227. Are entomological surveillance data reliable endpoints for risk assessment of *Aedes albopictus* abundance and risk of arbovirus transmission?

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The increasing threat of recurrent autochthonous transmission of arboviruses such as dengue and chikungunya in Europe highlights the need to quantify the risk of disease transmission. This raises the question about the possibility of achieving reliable estimates from invasive *Aedes* vector surveillance data obtained by the currently available tools, particularly for *Aedes albopictus*. Ovitraps are the preferred monitoring tools for their low cost and efforts, but their accuracy and reliability in accurately determining the biting population is debated. Participants in the EC-funded AIM-COST Action (project CA17108) joined forces to assess this issue. Seventeen teams from 13 European countries deployed 20 ovitraps/area (~10 ha), checked weekly during the local *Ae. albopictus* breeding season. All groups used the same ovitrap and oviposition substrate. Ovitrap management was carried out in association with 12 weekly human landing collections (HLC, 5 minutes each). We found high variability in both ovitrap collections - averaging 86 eggs/week but reaching up to 1,299 eggs in a single trap, and HLC - averaging from 2.6 up to 110 mosquitoes/week in a single 5' session. Preliminary results show 0.625 correlation between average values of HLC and ovitraps per sampling date, but this correlation was highly variable among the sampling area. Results from more detailed analyses will be presented and discussed regarding the accuracy of local scale predictions of mosquito abundance, the uncertainty in the data, and the significance of entomological surveillance data as endpoints for epidemiological risk assessment.

## OC228. Two invasions at once: Preliminary results from the delimitation and control strategy of *Aedes aegypti* and *Ae. albopictus* populations in Cyprus

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The recent detection of the invasive species *Aedes aegypti* and *Aedes albopictus* in Cyprus is expected to expand the vector-borne disease risks of the island. In an attempt to mitigate these risks, an intensive effort to delimitate the spread of the species in Larnaka for *Ae. aegypti* and Limassol for *Ae. albopictus* was done indicating isolated and distinctive populations at densities that still permit to target elimination of these species. The main results from the delimitation strategy will be presented along with the control efforts including door-to-door control, chemical control and SIT pilot trials as components of an area-wide integrated mosquito management plan. This work can serve as a case-study on the way by which other geographical areas can react in case of invasions from medical importance. The main challenges for further scaling up the applied methods will also be discussed.

**Keywords:** invasive species, *Aedes aegypti*, *Aedes albopictus*

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## OC229. Characterizing the effect of coinfection ratios on bluetongue virus reassortment in *culicoides sonorensis*

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Bluetongue virus (BTV), epizootic hemorrhagic disease virus, and African horse sickness virus are segmented, double-stranded RNA orbiviruses listed by the World Organisation for Animal Health and transmitted by *Culicoides* biting midges. Segmented viruses are capable of reassortment which can facilitate large genotypic changes that may enable a virus to exploit new ecological niches. Our study evaluated *in vivo* reassortment of progeny virus in *Culicoides sonorensis* midges coinfected with different ratios of BTV-10 and BTV-17. Midges were fed blood containing BTV-10, BTV-17, or a combination of BTV-10: BTV-17 at 90:10, 75:25, 50:50, 25:75, or 10:90 ratios. Midges were collected every other day and tested for infection using pan BTV and COX (housekeeping gene) qRT-PCR. A curve was fit to the ΔCt values (pan BTV Ct - COX Ct) for each ratio group and linear portions evaluated by pairwise comparisons. On day 10, midges were processed for BTV plaque isolation. Genotypes of plaques were determined by next generation sequencing. Comparison of linear portions of ΔCt curves demonstrated no differences between ratio treatment groups. Plaque genotyping indicated that most plaques fully aligned with one of the parental strains. However, there was reassortment evident

in a pool of midges coinfected with BTV-10: BTV-17 ratio of 75:25. Thus, reassortment within the midge may be an infrequent event, but reassortants may have an advantage over the parental strains and overtake parental strain populations. BTV reassortment patterns and resulting biological consequences will add an important dimension to the understanding of orbivirus emergence and evolution.

**Keywords:** *Culicoides*, veterinary entomology, orbiviruses, reassortment

### OC230. Vertical Transmission Rates of Dengue, Chikungunya, and Zika Virus in *Aedes albopictus* and *Aedes aegypti* Mosquitoes

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Vertical transmission in mosquitoes refers to the transfer of a pathogen from a female mosquito to her offspring and can lead to the maintenance and amplification of pathogen transmission within mosquito populations. To better understand this process, we quantified the vertical transmission rates of the dengue, chikungunya, and Zika virus in *Aedes aegypti* and *Ae. albopictus* by means of a systematic review. From the literature, we extracted the minimum infection rates of the offspring (including eggs, larvae, pupae, and emerged adults) of both Aedes species that have been reported for vector populations worldwide and measured under laboratory or field conditions. Moreover, we explore how climatic factors influence the efficiency of vertical transmission. Thus, this study provides new insights into the quantification of vertical transmission rates and highlights the importance of continued research in this area. By providing essential data on the minimum infection rates of the offspring, our study offers valuable inputs for mathematical models that can help to predict the dynamics of pathogen transmission within mosquito populations and help to reveal changing patterns on vector-borne disease transmission risk.

**Keywords:** transovarial transmission, hereditary transmission, public health, epidemics

### OC231. *Phlebotomus perfiliewi* (Parrot, 1930) as a permissive Italian species: putative role of a wild population in the transmission of an Asian strain of *Leishmania tropica*

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*Phlebotomus perfiliewi* (Parrot, 1930; Diptera: Psychodidae) is a sand fly species involved as proven vector of *Leishmania infantum* (Trypanosomatida: Trypanosomatidae) in Italy and across Mediterranean basin. In this scenario, intense human migration and environmental and climatic changes induce a documented increase in Europe of *L. tropica*, agent of anthroponotic disease, and spreading of phlebotomine leading to their potential interaction. According to this context, aim of this study is to demonstrate putative competence of wild *P. perfiliewi* in the transmission of Asian *L. tropica* strain. Five experimental infections were conducted using a total of 3879 wild unfed *Ph. perfiliewi* females collected in Magliano in Toscana (Grosseto municipality, Italy) and artificially infected with stationary-phase *L. tropica* promastigotes MHOM/IT/2016/ISS3183 from Afghanistan. Individual sand flies dissection analysis, conducted to determine a transmissible-infection status from D0 to D11, reveal an overall prevalence of 30,6% (N=113; mean=17.4, min=1, max=19, SD=29.4) with a peak of positivity around one week after feeding (between D6 and D8) and the presence of metacyclic promastigotes in stomodeal valve (D6-D11). Finally, molecular analysis was performed to confirm positive specimens and *Leishmania* species. These preliminary results support the potential vector role of wild *Ph. perfiliewi* in the transmission of *L. tropica* in non-endemic areas where this sand fly species is widely distributed. Further studies are necessary to demonstrate this exogenous host-parasite infection cycle and confirm *Ph. perfiliewi* as a permissive italian vector. Study is co-funded by European Commission grant 101057690 and UKRI grants 10038150 and 10039289.

## OC232. Effect of mating and blood meals on the demographic traits of *Aedes albopictus* adults

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The Asian tiger mosquito, *Aedes albopictus* is an important species of major significance for the public health, because its vectors severe infectious diseases, such as zika virus, chikungunya and dengue fever worldwide. Its anthropophilic, diurnal feeding habits cause extreme nuisance, that impede human activities and touristic development as well. Gaining insights on the relationship between blood feeding, longevity and reproduction of female mosquitoes contribute to the development of population growth models and efficient control interventions. We studied under controlled laboratory conditions (25 °C, 65 ± 5% RH and photoperiod 14:10 L:D) the relationship between longevity and mating in both males and females, and of blood feeding with longevity and egg production in female *Ae. albopictus*. Mating reduced the longevity of males from 73.7 to 68.7 days and of females from 79.3 to 77.6 for virgin and mated individuals respectively. One blood meal dramatically reduced female longevity from 77.6 to 70.5 days. A second blood meal reduced further female longevity to 50.9 days. The 2nd blood meal almost doubled egg laying at a cost of female longevity. The importance of our findings, for developing demographic population models for the Asian tiger mosquito is discussed.

**Keywords:** mosquito, longevity, egg production, blood meals, Culicidae, public health, life history traits

## OC233. Monitoring and management of insecticide resistance in malaria and arbovirus mosquito vectors through access to advanced diagnostic tools (INFRAVEC2- ISIDORe)

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The intense use of insecticides in vector and agricultural pest control programs has resulted in a dramatic selection of resistance in mosquito populations to such an extent that their control has become exceedingly challenging. Monitoring vector populations for insecticide resistance along with other important entomological traits (e.g., species ID, presence of pathogens) should be an integral component of effective vector control programs.

We investigate the mechanisms of insecticide resistance, aiming to understand the role of specific genes, mutations and other mechanisms in the resistance phenotype and we apply this knowledge in practical monitoring studies that could support the more efficient management of vector populations.

IMBB-FORTH as a partner of the INFRAVEC2 EU funded project (https://infravec2.eu/) and of its successor epidemic disease consortium ISIDORe (https://isidore-project.eu/) provides access to infrastructures for insecticide resistance research at no cost to the end-user, such as: (a) the genotyping of mosquito vectors of malaria and arbovirus, for species, insecticide resistance (IR) traits (metabolic resistance and target site mutations) and detection of pathogens (plasmodium, West Nile virus and other arboviruses) and (b) the analysis of the mosquito cuticular thickness and composition, as a marker of cuticular resistance.

This work was supported by the European Union's Horizon 2020 INFRAVEC2 and ISIDORe projects under grant agreements No 731060 and No 101046133, respectively. It is also funded by the Prefecture of Crete under the program: "Integrated Mosquito Control in the Prefecture of Crete".

**Keywords:** molecular diagnostics, vector monitoring, malaria, arbovirus

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## OC234. Development and commercialization of Aprehend, a fungal biopesticide for the control and prevention of bed bugs

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Aprehend is a commercial biopesticide for the control and prevention of bed bug infestations. In contrast to most biopesticide research programs, the development of Aprehend did not follow the commonly adopted path of isolate screening, laboratory bioassays, small-scale field evaluations, formulation development, mass production and large-scale field trials.

An early objective in our development process was to fully understand the issues faced by pest management professionals (PMPs) when dealing with bed bugs. Talking to potential customers enabled us to evaluate the need for a biopesticide product and determine the attributes that such a product would need to address to be competitive on the market. We learned that the key issues that PMPs were experiencing were their inability to detect and access hidden bed bug harborages, limited residual efficacy of available insecticides after application, and concern around insecticide resistance. A fungal biopesticide is well placed to address these issues. Infection by fungal pathogens is not impacted by insecticide resistance, and fungal conidia acquired by a bed bug could be carried back to the colony and infect others. Further, by formulating fungal spores in oil, it is possible to ensure long-term residual efficacy, particularly in a dry environment protected from UV, rain, and temperature fluctuations. We focused on developing a formulation with a long-term residual, using an existing commercial fungal isolate to reduce regulatory hurdles.

In this presentation we discuss how customer discovery and use of a business model canvas can guide the development and commercialization of successful biopesticide products.

**Keywords:** *Beauveria bassiana*, entomopathogen, *Cimex lectularius*, business model, registration

# Session 10:
## Invasion biology and climate change

## OC235. EFSA activities on risk assessment and preparedness for invasive alien insects in Europe

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The European Food Safety Authority (EFSA) is the Agency of the European Union responsible for risk assessment in food safety, animal health and welfare and plant health. EFSA conducts risk assessment for individual plant pests with a two-phase fit for purpose approach, a simpler and narrative pest categorisation first, followed by a quantitative probabilistic pest risk assessment for more complex questions, where quantification of the risk allows the comparison of outputs under different scenarios. EFSA conducts also commodity risk assessment by analysing for each imported plant commodity and its production system in a Third Country the likelihood of pest freedom for exotic and quarantine plant pests. Such activities are carried out by extensive and systematic literature search and data collection. EFSA is also funding research projects to reduce key uncertainties and knowledge gaps by generation and collection of observational and experimental evidence. Trends and examples from risk assessment of invasive alien insect plant pest are presented and discussed.

**Keywords:** pest categorisation, pest risk assessment, quantitative, probability, commodity risk assessment

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## OC236. Efficacy of entomopathogenic fungi and nematodes against *Popillia japonica* adults and larvae in potted plants

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Potted plants provide a suitable environment and are readily used by *Popillia japonica* adults as egg-laying sites where larvae can easily develop by feeding on the present roots. The risk of spread of this pest in relation to the trade of potted plants is therefore very high and for this reason, strict restrictions are imposed on the trade of products from infested areas. Within the H2020 IPM-Popillia project, we investigated the possibility of controlling *Popillia japonica* in a nursery environment with biological control agents. For this purpose, two experiments were set up in a nursery in Biella (Piedmont, Italy). In the first experiment the treatment of the pots before the adults lay their eggs was tested. In the second, the pots were treated with larvae already present in the potting medium. In both experiments, six treatments were tested: a commercial strain of the entomopathogenic fungus *Metarhizium brunneum* formulated on barley grains, an autochthonous strain of the entomopathogenic fungus *M. robertsii* formulated on barley grains, the same autochthonous strain formulated in sodium alginate pellets (SAPs), a commercial strain of the entomopathogenic nematode *Heterorhabditis bacteriophora*, an autochthonous strain of *H. bacteriophora*, and one untreated control. In both cases, the excellent efficacy of the two strains of entomopathogenic nematodes was verified, while none of the strains or fungal formulations showed a significant effect. In any case, even with the use of nematodes, it was not possible to completely prevent the presence of larvae inside the pots.

**Keywords:** *Popillia japonica*, biological control, entomopathogenic fungi, entomopathogenic nematodes, nurseries, potted plants

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### OC237. Bagrada bug in Europe vs America: insights on biological traits, genetics, invasion pattern and management

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Biological invasions are a worldwide threat as they negatively impact people's life, agricultural and natural ecosystems. In Europe, 53% of the alien insects are phytophagous. One of them is Bagrada bug, *Bagrada hilaris* (Hemiptera: Pentatomidae). This stinkbug belongs to the European non-native pentatomid bugs. Bagrada bug is worldwide known as a pest on various Brassicaceae crops. Interestingly, in Europe and Morocco, bagrada is mainly reported as a pest on caper (*Capparis spinosa*). Unlike other alien stinkbugs, bagrada is not yet present in continental Europe although recorded for more than 40 years on the Mediterranean islands of Pantelleria and Malta. This contrasts with its rapid invasion in the Americas with a first record in California in 2008, then a quick spread eastward from state to state, and to other American countries, e.g. Mexico (2014), Chile (2016), and Argentina (2021). Our recent phylogeographical study has pointed an African origin of the Mediterranean Bagradas and an Asian origin of the American ones. It is known that bagrada is well adapted to high temperatures (35-40°C) and may be favored by climate change to invade new areas. A climate modeling study has predicted southern Europe (Spain, Portugal) as a possible threat by bagrada in the future. Here we present an historical review of the invasion of bagrada, together with recent biological data that help understand its success as an invader. We will also present two possible management methods, e.g. importation biocontrol and sterile insect technique, as they respond to a demand for less pesticide use.

**Keywords:** biological invasions, phylogeography, biological control, stinkbug, Pentatomidae,

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### OC238. Hymenoptera parasitoids (Eulophidae and Braconidae) of the Lime Leaf Miner *Phyllonorycter issikii* (Lepidoptera, Gracillariidae): in invasive areas of Europe and Asia

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The study was carried out in 2001-2023 in Central Europe (Slovakia, Bratislava) and European parts of Russia (Voronezh, Moscow, Nizhniy Novgorod, Kazan, Ulyanovsk, Izhevsk), in the foothills of the Ural Mountains (Perm, Ufa, Yekaterinburg) and West Siberia (Kurgan, Tyumen, Tobolsk, Omsk, Novosibirsk). Authors among 61 species known by the beginning of 2023 found 31 species parasitoids of Hymenoptera. Complexes of parasitoids *Ph. Issikii* in the invaded areas is characterized by the following characters: **1**. The complex is represented mainly by polyphagous species that switched to feeding on *Ph. issikii* from other species of *Phyllonorycte*r. **2.** Long-term studies have shown that the complex of parasitoids does not change over the years and the number of species in it does not increase, moreover, there is no change in dominant species. **3**. The number of ectoparasitoid species prevailed over that of endoparasitoids in the parasitoid complexes of *Ph. issikii*, and the number of reared specimens of ectoparasitoids was 4- 7 times greater than that of endoparasitoids. **4.** Mortality from parasitoids of caterpillars and pupae of *Ph. issikii* was from 0.9% to 23.7%. **5**. Parasitoid infestation of the second generation of moths was higher (17.6%) than that of the first (1%). Among parasitoid species, the most noticeable effect on the survival of *Ph. issikii* was provided by *Ph. issikii Sympiesis gordius*, *S. sericeicornis*, *Chrysocharis laomedon*, *Minotetrastichus frontalis* (Eulophidae) and *Pholetesor circumscriptus* (Braconidae).

**Keywords**: Hymenoptera, ecto and endoparasitoid, invasive species

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## OC239. Quantitative risk assessment of the lesser cornstalk borer, *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae), for the European Union

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The lesser cornstalk borer (LCB; *Elasmopalpus lignosellus*)(Lepidoptera: Pyralidae) is a polyphagous, multivoltine insect, native to the Americas. It feeds mostly on seedlings of cereal and legume crops. Over recent years, LCB has been intercepted in Europe several times and the European Commission (EC) requested EFSA to conduct a pest risk assessment. The EFSA Plant Health Panel evaluated the pathways for introduction (entry and establishment), spread and impact for the EU. Judgements made in each assessment step were based on a combination of literature review, meta-analysis, information gathered during interviews with experts and expert knowledge elicitation involving panel members and EFSA staff. Taking into account pest interceptions, the biology of the pest, the import of host plants in a form that could carry the pest, and the volume of trade entry pathways were narrowed down to finally focus on asparagus entering the EU from Peru. Under current climatic conditions around 16% of asparagus spears enter regions of the EU suitable for establishment; this rises to 24% under climate change. However, due to estimated small likelihoods of adults emerging and escaping from discarded waste, finding a mate and the subsequent progeny surviving to initiate a founder population, the median number of populations expected to establish was estimated to be only 0.0003 per year (90% Confidence Range 0.00001 – 0.005). The EC will use the results of the quantitative assessment to inform their phytosanitary risk management decision making and the regulatory status of LCB for the EU.

**Keywords:** *Elasmopalpus lignosellus*, Pyralidae, asparagus, quantitative risk assessment

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## OC240. Genomic architecture informs the spread of *Spodoptera frugiperda* and *Lobesia botrana*

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Invasive species have direct and indirect global ecological and socio-economical impact. Understanding factors contributing to pest spread is necessary to protect the environment, food security, and to improve biosecurity preparedness. Collaborations between biosecurity agencies and scientists are pivotal to solving invasive species challenges and minimise introductions of: (i) new exotic pest species, (ii) undesirable genetic materials into recently established pest populations; and (iii) to disentangle between natural (e.g., migratory ability) versus human-assisted (e.g., via contaminated commodities) spread patterns through analysis of genomic resources. Misidentification of unrealised independent introduction events from presumed natural spread of pest populations could result in the potential introductions of novel genetic traits (e.g., insecticide resistance genes) to complicate management effort and failure to address existing but unrecognised biosecurity weaknesses could increase the risk of introductions of new invasive pest species. Using *Lobesia botrana* and *Spodoptera frugiperda* as examples, we applied population genomics to infer their global spread patterns and potential population origins. *L. botrana* invaded the Americas since the mid-2000s while *S. frugiperda* was thought to rapidly spread across large parts of Africa, Asia, and Oceania since 2016 from an initial west Africa introduction. Analyses of whole genome sequencing data identified signatures of multiple independent introductions, with: (i) previously unrecognised continental-scale movements for *L. botrana*, and (ii) identifying Asia/Southeast Asia as biosecurity hotspots to underpin *S. frugiperda*'s perceived rapid spread across its new invasive ranges. We highlight population genomic lessons learned to empower biosecurity preparedness of emerging transboundary plant pests within a global context.

**Keywords:** European grape vine moth, fall armyworm, Invasion biology, single nucleotide polymorphism (SNP) loci

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## OC241. Niche modelling and landscape genetics of the yellow-legged hornet (*Vespa velutina*): an integrative approach for evaluating central–peripheral population patterns in Europe

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Genetic diversity is an important biological trait for a successful invasion. During the expansion across a new territory, the invasive species may face unprecedented ecological conditions that will determine their demography and the genetic diversity of their populations. The invasion of Europe by the yellow-legged hornet *(Vespa velutina*) began from a single introduction event recorded in France in 2004, and since then the species has successfully spread through many territories including France, Italy, Spain, or Portugal. Integrative approaches are a powerful strategy to detect and understand pattens of genetic variation in central and peripheral populations. Here, we analysed the relationship between genetic diversity parameters based on 15 nuclear DNA microsatellite loci and geographic and environmental drivers such as the distance to the introduction focus, environmental suitability, and distance to native and invasive niche centroid. Our results revealed a central-peripheral pattern with lower allelic richness as we move away from the introduction focus to low environmental suitability areas and near to the native niche centroid. Conversely, no association was detected between observed or expected heterozygosity values and any geographical or environmental driver. These patterns may reduce the fitness of marginal populations to invade new territories and reduce potential distribution for future global change scenarios. This study is of particular concern for conservationists and stakeholders because it may lead directly to the decrease of biological fitness of this invasive species of European concern.

**Keywords**: environmental suitability, genetic structure, invasive species, microsatellite, single introduction

## OC242. Invasapp: early detection tools for invasive insect species on Mediterranean islands

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Global change is one of the main threats to today's society, with greater implications for island territories such as the Balearic Islands. The introduction of invasive species is currently one of the main causes of biodiversity loss, as well as causing great economic costs for society and posing a threat to human health.

The aim of this work is to present the first early detection network of invasive insect species (EU prioritises pests) in the Mediterranean archipelago (Balearic Islands), through two key points: (1) a trapping network in areas of entry and distribution of plant material; and (2) the design of a mobile application that will allow any citizen to report detections. The final objective is to design a specific management plan adapted to the territory for the Early Detection and Rapid Response (EDRR) for the control and eradication of these species, highlighting the fact that in the Balearic Islands we have a differential factor that plays in our favour in this regard: insularity. The first result was the detection of the European hornet (*Vespa crabro*), which is not present in the Balearic Islands. The Ministry considered treating it as an invasive species, applying the precautionary principle and establishing a nest detection protocol.

Keywords: invasive species, prioritises pests, trapping network, Invasapp, Early Detection and Rapid Response.

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## OC243. Spread of the lime butterfly *Papilio demoleus* in Europe: Projection of environmental suitability using citizen science data

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The lime butterfly, *Papilio demoleus* Linnaeus, 1758 is one of the most widespread members of the swallowtail family (Papilionidae) globally and is known for its rapid range expansion and potential threat to agriculture as a citrus pest. This Indo-Australian species was thought to be confined to warm tropical climates, but recent records indicate that the species is more adaptive and able to survive colder winters than was previously considered. This study explores the potential of *P. demoleus* establishing in Europe using a presence-background species distribution modelling (SDM) framework that allows the input of prior biological knowledge of species' tolerances. Climatic and environmental predictors selected for the models were: Mean temperature of the coldest quarter, annual precipitation, precipitation seasonality, Human Influence Index and Enhanced Vegetation Index. In addition, we used

mean temperature of the warmest quarter to define the unsuitable background for modelling. Species occurrence data were compiled by citizen science platforms (iNaturalist, GBIF) and from the authors' own observations (8955 records in total). Our results suggest that many parts of the Mediterranean region are already suitable for establishment of *P. demoleus* in the current climate, and that increasing areas will become suitable in the future. Specifically, *P. demoleus* is projected to expand its western limits to Cyprus (probably already established), Greece, Croatia, Italy, France, Spain, and Portugal. Next steps include the addition of citrus growing regions as a predictor variable in order to further elucidate the potential future distribution and impact of this invasive butterfly species in Europe.

Keywords: biological invasions, Lepidoptera, Species Distribution Model (SDM), citizen science

## OC244. Phenotypic plasticity in initial introduction phase and its discrepancy effect for artificially intelligent identification of economic Thysanoptera

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Recently, artificial neural networks (ANN) seem to have been one of the most promising computing tools for semiautomed identification of economic pests. Analogous to the structure of the human brain the advantages of ANN include an ability to learn from examples and to generalize observed patterns. However, their efficiency is significantly influenced by some degree of phenotypic plasticity. Undisputedly, it is a unique adaptation mechanism complex, which guarantees high fitness of a species along ecological gradients as a predisposition of alternative ontogenies for potentially more successful infiltration into new (secondary) ecological niches as well as within the expansion of the distribution area. For economic alien thrips (Thysanoptera), for example, the initial phase of their introduction includes an increase in morphological (morphometric) intraspecific variation with discrepancy effects on automated online remote identification system modules in artificially intelligent detection of pests. Our previous analyses have emphasized a challenge of specific phenotypic "explosion" curve in forensic reconstruction of the banded greenhouse thrips *Hercinothrips femoralis* Reuter, 1891 (Thysanoptera: Thripidae: Panchaetothripinae) introduction timing and generation assessment with an outstanding postulate for applied phytosanitary care. The experimental population statistical evaluation (Shapiro-Wilk test of normality, ANOVA analysis of variance in R software) of measured morphometric characters has proved significant difference (<0.05) between generations, with total body length, pronotum width, forewing length and ovipositor length intraspecific variation explosion for generation 1 (G1). The principal component analysis (PCA) emphasized more than 75% confidence ellipses to rule out the potential outliers.

Research was supported by VEGA grant No. 1/0286/20.

Keywords: ANN identification, invasion, thrips, intraspecific variation

## OC245. Guardians of plant health – the European Union Reference Laboratory for Insects and Mites

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International trade and travels creates unprecedented opportunities for species to disperse beyond their native ranges. Biological invasions pose a risk for plant health, thus jeopardizing food security, economical flows and ecological balances. EU Plant Health legislation therefore listed the most dangerous pests. Nowadays, 245 insects and mites are included in annexes IIA-IIB of (EU) 2021/2284 regulation amended by (EU) 2022/1941. These are defined Quarantine Organisms (QOs): their introduction, holding, transport and multiplication within Europe is prohibited; 16 of them considered especially dangerous are therefore designated as priority QOs. Regulation enforcement requires fast and accurate identification by officially designated National Reference Laboratories (EU-NRLs). Each Member State has today its own or delegated NRL, i.e. 26 NRLs in charge of the same assignment. In order to support and harmonize their diagnostic efforts the European Commission (EC) nominated the European Union Reference Laboratories (EURLs) in 2019. The EURL for Insects and Mites is a consortium between ANSES (France) and AGES (Austria).

To ensure high-quality and harmonised performances of EU-NRLs, the EURL performs validation studies, trainings and proficiency tests. A comprehensive website offers information and guidance on available identification methods and tests. Up-to-date communication is also provided through newsletters and a web-based workspace for casual exchanges. EURL maintains a reference collection to provide material to EU-NRLs upon request. Furthermore, EURL is assisting in the diagnosis of outbreaks in Member States by carrying out confirmatory diagnosis. Finally, scientific and technical assistance is provided to EC and international partner organisations such as EFSA, EPPO or IPPC.

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## OC246. Predicting the impact of constant and variable temperatures on Neotropical stink bugs using Dynamic Energy budgets

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Understanding the relationship of insects with their environment is fundamental because of their ecological role and influence on agriculture and human health. Global warming and other climate change drivers have a significant impact on the life cycle of insects which in turn affect their population dynamics and geographic distribution. Mathematical models are useful tools in understanding the impact of climate change drivers on the status of insects. Dynamic energy budget (DEB) theory provides a modeling framework to study the dynamics of biological systems from molecules to ecosystems via a mass and energy balance of the individual. We have developed DEB models for two species of the Pentatomidae family. Data on instar duration, adult longevity, nymphal length and wet mass, fecundity and survival were used to parametrize the models and consequently used them to simulate individual and population traits under different environmental conditions. Overall, the models adequately capture the duration of nymphal instars and adult longevity, growth, survival, and fecundity. Temperature has a higher impact on stage durations and survival while food availability (quantity and/or quality) on the size and lifetime fecundity. Among the five instars, instars one and two are the most sensitive in temperature changes. Adult longevity shows a higher inter-individual variability than the nymphal stage durations. The models can be used to simulate growth and survival under different scenarios which could be used to analyze potential distribution of a species, possible de-synchronization with their food sources and to assist the optimization of integrated pest management.

**Keywords :** Dynamic Energy Budget theory, modeling, pest insects, Pentatomidae, insect energetics

## OC247. Influence of temperature on biological control of the codling moth *Cydia pomonella* (L.) by two natural enemies

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Climate change is expected to have a significant influence on insect biology. One concern about agroecosystems is that increased temperature may differentially influence physiology and behaviours of pests and their natural enemies, resulting in altered biological control.
This study aims to determine the potential of two natural enemies to control a major pest of apple orchards worldwide, Cydia pomonella, in the context of global warming. It seeks to determine whether the European earwig *Forficula auricularia* and the parasitoid *Mastrus ridens*, have different thermal optima for development, and whether the two natural enemy species will still be able to provide effective control of *C. pomonella* under future climatic conditions.
Our results indicated that *M. ridens* was able to produce viable offspring at 28°C and temperature had no influence on its parasitism potential. *F. auricularia* was however unable to develop above 25°C and slightly lost its regulatory potential at 30°C. According to the biology of these species, their effective season in orchards and to the results of our study, these two natural enemies present a good potential of regulation of *C. pomonella,* at different periods, in a context of global warming.

**Keywords:** IPM strategies, life history traits, behaviours, thermal optima, phenological mismatch

## OC248. An ecological index for arthropod habitats using climate model data applied to the Circum-Sicilian islands

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Arthropod diversity plays a vital role in ecosystems, as they are excellent indicators of ecosystem integrity. Recently, anthropogenic processes, such as climate change, have contributed to the degradation of ecosystems, especially in vulnerable regions such as the Mediterranean. These processes have been studied with climate models, and with the introduction of Convection Permitting simulations, it is also possible to focus on the climate impacts of small islands such as the Circum-Sicilian islands in the central Mediterranean. This is part of the multidisciplinary project PALEOSIM (PALEOclimate modelling of Small Islands in the Mediterranean and possible impacts on arthropod habitats) which aims to shed light on the processes (natural and anthropogenic) that contributed to the ecological changes of many of these small islands, and provide new insight into potential future habitat changes. As the project is still in its early stages, this presentation is a proof-of-concept that showcases the use of environmental parameters from climate models (e.g., air temperature, rainfall) to establish an ecological index based on the Mahalanobis distance for a selection of arthropod species. The analysis makes use of large climate model ensembles to test this index, which is a spatial distribution ranging from 0 to 1, where 1 describes an ideal habitat, and 0 is an environment beyond the habitat limits determined by arthropod observations. The results may highlight potential habitats for species with known ecology, hot-spots for invasive species, as well as changes in spatial distribution due to climate change.

**Keywords:** habitats, ecological index, climate models, PALEOSIM, climate impacts

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## OC249. Assessing the impact of climate change on fungal pathogens and insect pests in wheat: A joint species distribution model approach

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Species in ecosystems do not exist in isolation; they co-occur and interact among others via competition, predation, and facilitation. In the face of ongoing climate change, these ecological interactions are also expected to change, including economically important interactions, e.g., between a crop and its multiple fungal pathogens and insect pests. Acknowledging and accounting for multi-species interactions remains a challenge for species distribution models. The challenge can be addressed through the use of Joint Species Distribution Models (JSDMs), a relatively new statistical approach for analyzing multiple coexisting species in an assemblage or community. We used JSDM to examine the global distributions and co-occurrence patterns of fungal pathogens and insect pests in wheat to better understand how these threats affect the world's most important staple crop: wheat. Furthermore, we incorporated climate change scenarios into our analyses to make predictions about the future. Consequently, our analyses can identify potential shifts in the hotspots of wheat pathogens and pests. By analyzing together crop, pathogen, and pest distributions, our work can reveal co-occurrence patterns in agro-ecosystems that have important ramifications for optimal control strategies.

**Keywords:** climate change, Bayesian joint species distribution models (JSDMs), HMSC, species associations

## OC250. Is integrated pest management suitable to control a regulated quarantine pest? Prospects and limitations of IPM against *Popillia japonica* in Europe

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The scarabaeid beetle *Popillia japonica* has been detected in northern Italy in 2014, and is since then, slowly but steadily, expanding its range. Reaching favorable habitats, *P. japonica* can build up horrifying population densities, as shown by monitoring traps being blocked within minutes by the shear mass of attracted beetles, and hundreds of individuals aggregating on a single vine. Any eradication attempts of the Japanese beetle population invasive to mainland Europe are probably doomed to failure. Reason is, among others, the wide host range of *P. japonica*, including common crop species as well as prevalent plants of Europe's natural vegetation. Consequently, *P. japonica* adults will never run short of nutritional supply in newly invaded areas. Furthermore, Japanese beetles are abundant in places where eradication measures are technically not feasible or socially not acceptable, e.g. in nature conservation areas or in residential areas. The same is true for *P. japonica* oviposition sites and larval populations, which are mainly found in different types of grassland. Against that background it becomes clear that a reasonable response to the invasion of *P. japonica* in mainland Europe must focus on a sustainable reduction of its populations in the infested zone, and a slow-down of its spread. Furthermore, any control strategy must include measures which are applicable against larval populations in grasslands as well as against adults in different kinds of crop and non-crop areas. The presentation will introduce the Horizon2020 project "IPM-Popillia", which has the aim to develop these measures. The project elaborates socially acceptable and environmentally friendly control measures, which are suitable for application in many different types of infested habitats, and at the same time meet the requirements for containment of a regulated quarantine pest.

Keywords: *Popillia japonica*, Integrated Pest Management, containment measures

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## OC251. How reachable is Europe for the Japanese beetle: tracking planes, trains and trucks to inform surveillance strategies

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The Japanese beetle (*Popillia japonica*) is a polyphagous insect listed as priority pest by the EU phytosanitary legislation. The beetle was first detected in Continental Europe in 2014, in the Italian regions of Piedmont and Lombardy. Since then, it has quickly invaded a large portion of Northwestern Italy and Southern Switzerland, despite the eradication effort of regional phytosanitary services. Furthermore, several interceptions of living adults have occurred in distant locations as a result of unintended passive transport of the beetle. Indeed, it is well established in the literature that Popillia japonica is capable of being dispersed over large distances via passive human transportation (hitchhiking behavior). In this work we analyzed how the invaded areas of Northern Italy and Switzerland are connected to the rest of Europe via three main transportation networks: planes, trains and trucks. We built reachability maps from the invaded zone highlighting sites that are likely to act as stepping stones or entry points for the beetle, both close and further away from the currently infested zone. Combined with a suitability map, this allows to prioritize sites for early-detection surveillance based on how likely they are to be reached, as well as their potential for being a future outbreak of infestation. This is crucial, as experiences in North America proved that early detection followed by effective eradication protocols can prevent the establishment of the beetle.

**Keywords:** *Popillia japonica*, connectivity, dispersal, human transports, early detection, hitchhiking

## OC252. Tracing the dispersal route of the invasive Japanese beetle *Popillia japonica*

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The Japanese beetle, *Popillia japonica*, is a highly polyphagous Scarabaeidae native to Japan that has colonized the North American continent and the Azores Islands in the last century and has recently been found in Italy and Switzerland. Given its economic impact on the horticulture and turfgrass industries, this species was ranked within the EU priority pests list in 2019. According to the EU Convention on Biological Diversity (SCBD), identifying of invasion routes is a pivotal aspect of an effective management program to control invasive alien species. To reconstruct the origin of the introduction of this pest, we investigated the genetic variability of *P. japonica* in its native and invaded areas worldwide by analyzing microsatellite and mitochondrial data. In Japan *P. japonica* is structured into two populations one located in the southern and another in the northern-central region of the archipelago. A limited area within central Japan was identified as the putative source of the US outbreak. The two European populations originated from independent introductions: the Azorean outbreak occurred approximately 50 years ago and originated from the south-eastern region of US while the more recent Italian introduction originated from northeastern region of US. The Swiss outbreak, instead, can be considered as an extension of the Italian one.

**Keywords:** phylogeography, insect pest, Azores, grub, Japan

## OC253. Draft *de novo* assembly and annotation of the nuclear genome of *Popillia japonica* from the invasive Italian population

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*Popillia japonica* (Coleoptera: Scarabeidae) is a beetle native to Japan that has become an invasive species in many parts of the world, including North America and, more recently, Europe. The beetle feeds on a wide variety of plants, including turfgrass, flowers, and fruit trees, and can cause significant damage to crops and gardens. In order to provide solid knowledge to understand the drivers of *Popillia japonica* population development, study its route of invasion and transcriptomic profiles under controlled conditions of management, we have sequenced and assembled its nuclear genome using a *de novo* approach. Predicted genome length is about 578 Mb with a N50 statistics of 0.89 Mb. BUSCO completeness was high when compared to the Endopterygota database: ~96% of complete genes with low duplication rate. Transposable Elements were masked, annotated and their distribution evaluated in comparison with other insect species. A preliminary structural and functional annotation predicted 31,724 genes of which 15,146 with an identifiable PFAM domain. Initial results are shown from a comparative genomics investigation focusing on sex-related genes, odorant receptors and genes involved in detoxification processes.

**Keywords:** pest insect, genomics, transcriptomics, WGS, Transposable Elements, sex-related genes

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## OC254. Searching for the role of the microbiome in the population growth of *Popillia japonica*

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*Popillia japonica* invaded Terceira Island of the Azores archipelago in the sixties of the last centuries and subsequently spread to 7 other islands. Despite it has established in very similar ecosystems, the rate of infestation is quite different among the islands. To investigate what role the microbiome might play in insect populations, we compared the soil and gut microbiome of *P. japonica* larvae from two islands with different infestation rates. We concluded that the two soils did not differ significantly in microbial composition, although we found bacterial biomarkers in the less representative families. On the other hand, significant differences were found between the associated microbiomes of larvae collected from the two sites, with higher diverse composition in larvae from high densities. Functional analysis based on whole-genome sequencing also allows identification of metabolic pathways and proteins enriched in the microbiome of larvae from high population density compared to the others. It is highlighted that larvae from the high population density showed an increased number of genes encoding enzymes involved in the deconstruction of complex carbohydrates, such as CAZymes, in comparison with the other microbiome. In addition, our analysis revealed a relationship between some soil composition parameters and bacterial markers of the microbiome of larvae from low densities. Overall, our results

suggest that the microbiome promotes or hinders the population success of P. japonica, opening new avenues for preventing the spread of this pest.

**Keywords**: *Popillia japonica*, soil microbiome, gut microbiome, CAZymes, bacterial biomarkers

## OC255. *Popillia japonica* infestation in Northwestern Italy: main damage in agricultural crops and challenges in pest control

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*Popillia japonica* Newman, the Japanese beetle, was detected in the Ticino Valley (Northwestern Italy) in summer 2014, and has since then continuously expanded its geographic distribution, affecting both landscapes plants and agricultural crops. The infestation was initially confined to a restricted area along the Ticino river, with limited damage to permanent grassland caused by larval populations often exceeding 200-300 larvae/m$^2$. The emerging beetles fed on wild plants (bramble, nettle, etc.) on the edge of the meadows, and to a limited extent on the leaves of soybean, the silks of corn cobs, and on ornamental and fruit plants in nearby private gardens. The progressive spread of the exotic beetle, about 7-10 kms/year, did not only lead to a spatial increase of the infested zone but also to the exposure of new agro-ecosysthems, and consequently to an increase of the number of plant species and agricultural crops affected. In Northeastern Piedmont, the first economic damages were reported on small fruits in lowland. The beetles not only skeletonize the leaves but also feed on the ripening fruits of blueberry, raspberry and other plants, seriously compromising the harvest. Furthermore, vineyards on hills near meadows and irrigated crops have faced increasing numbers of beetles year by year, with the risk of high defoliation levels if not controlled with insecticide treatments. Other crops, such as stone fruits, are also affected, disrupting I.P.M. strategies and compromising organic farming. In the last two years a severe drought, induced by climate change, is reducing the pest population.

**Keywords:** *Popillia japonica*, Japanese beetle, damages, control, vineyards

## OC256. Attract-and-infest strategy to biologically control Japanese beetles

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The Japanese beetle (*Popillia japonica*) is an invasive insect pest that is seriously damaging agricultural production with its extremely polyphagous lifestyle and fast invasion pattern. Only recently, the pest has established in northern Italy and southern Switzerland and is threatening to expand its range. To prevent this, we aim to implement an attract-and-infest strategy, where we use adult beetles as vectors to disseminate a fungal biocontrol agent in the adult and larval populations. With lab and field experiments, we tested whether infested adults (1) horizontally transmit the fungal inoculum to other adults and (2) carry the inoculum to the habitat of their offspring increasing larval mortality. Lab results show that inoculated beetles can indeed transmit the fungal spores horizontally and both donor and recipient beetles show increased mortality. Furthermore, inoculated beetles can carry the inoculum to their oviposition sites. This year, further experiments are carried out to investigate whether offspring in inoculated oviposition sites show increased mortality rates. Additionally, we will test the persistence of the control agent under field conditions. With our biological control concept, we aim to sustainably protect European agriculture from the invasion of this pest insect and to prevent its establishment in currently non-infested areas in northern Europe.

Keywords: *Popillia japonica*, entomopathogenic fungi, *Metarhizium brunneum*, invasive species, autodissemination, plant protection

## OC257. Invasion of the fruit fly *Bactrocera dorsalis* (Tephritidae), with a focus on the Indian Ocean Islands*,* a threat to Europe

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The polyphagous oriental fruit fly *Bactrocera dorsalis,* originating from Asia, started its invasion of Subsaharan Africa 20 years ago. Since then, it has spread very rapidly in continental African countries and has reached the Indian Ocean, becoming the main fruit pest in most countries. Several studies have been conducted in order i) to decipher the origin of invasive populations and ii) to understand the success of this spectacular invasion using ecological and genomic approaches. This was particularly studied in the context of successive invasions of fruit flies on the island of Réunion. On this island, after the invasion of *B. dorsalis,* a shift in the host range, spatial distribution and climatic niches for the generalist resident species, such as *Bactrocera zonata, Ceratitis quilicii* and *Ceratitis capitata* was demonstrated. Furthermore, field observations and laboratory experiments suggested the existence of apparent competition between the two *Bactrocera's* species via the parasitoid *F. arisanus,* which would have increased and accelerated the displacement of *B. zonata.* Regarding the origin of the invasion, using genome-wide SNP data and a multipronged approach, two independent invasion pathways were deciphered. A western pathway involving the migration of *B. dorsalis* from the east African coast into the Comoros, Mayotte and Madagascar. The Mascarene islands (Réunion and Mauritius) were colonized directly from Asia and formed a distinct cluster. The invasive population observed in the Mascarenes seems to have a greater impact on the resident species and on agriculture, so more attention should be paid to prevent any further spread of this new invader.

Keywords: *Bactrocera dorsalis*, competitive displacement, NGS analysis, genetic structuring, La Réunion, *Bactrocera zonata*

## OC258. Exploring the impact of changing climate and irrigation patterns on the potential distribution of organisms: Insights from fruit fly case studies

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Global change agents, including changing climate, land cover, and the rapid transport of people and goods, alter the distribution and abundance of many plant and animal species. Here, we explore how the changing climate suitability patterns and assumptions related to the distribution of irrigated areas impact the potential distribution of pests. We use CLIMEX combined with the CRU TS4 climate time series dataset to explore the temporal patterns of climate suitability for *Ceratitis capitata* (medfly) from 1970 to 2019 globally. At selected bellwether sites in Europe and North America, we found statistically significant trends in increasing climate suitability, as well as a substantial poleward (northward) expansion in the modelled potential range. To illustrate a new method for assessing the areas under irrigation, we use MODIS EVI (Enhanced Vegetation Indices) at 1 km spatial resolution and meteorological data from ECMWF to identify irrigated crop areas. The outputs are compared with CLIMEX-derived soil moisture data and demonstrated as an input to a CLIMEX environmental suitability model of *Bactrocera tryoni* (Queensland Fruit Fly) in southeastern Australia. Recent climate changes

reveal discernible fingerprints in the changing distribution of invasive fruit flies. Hence, models based on out-of-date climate data can misrepresent risks. Including an accurate representation of irrigation areas is important for understanding stepping-stone invasion risks.

### OC259. Innovative sexing systems for insect pest control through Sterile Insect Technique (SIT) within the REACT program

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Among numerous Sterile Insect Technique (SIT) programs to suppress insect pest populations and eradicate or contain invasive species, the Mediterranean fruit fly (*Ceratitis capitata* or medfly) control program has emerged as a model due to the development, testing, and optimization of efficient sexing strains over the past three decades. These genetic sexing strains (GSS) in medfly enable the production of male-only populations, based on selectable markers such as the phenotypic marker 'white pupae' or the temperature-sensitive lethal (tsl) mutation. In GSS, females exhibit white pupae and are temperature-sensitive, while males display wild-type brown pupae due to a Y chromosome-linked rescue of the *wp* gene. This enables sexing via pupal color. Furthermore, heat-shocking GSS embryos result in female lethality, with only male individuals hatching due to a wild-type rescue allele linked to the Y chromosome. In medfly GSS, heat shock generates a male-only population, and pupal color serves as a quality control parameter during the pupal stage.Although the medfly GSS's phenotypes are known and visible, the system's genetic background and underlying mechanisms remain incompletely understood. Recent breakthroughs have shed light on the *wp* and *tsl* genes responsible for the white pupae phenotype in *C. capitata* and other Tephritid flies. The current objective is to explore the feasibility of creating a genetic sexing system using the newly identified selectable markers. This approach aims to establish a universal strategy for generating sexing strains in multiple pest species for future applications and is part of the EU-funded collaborative program REACT.

**Keywords:** fruit flies, Diptera, Tephritidae, invasion, eradication, containment

### OC260. Response of adults of geographically distant populations of the Mediterranean fruit fly to different temperature regimes

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The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Diptera:Tephritidae), is an important invasive species and devastating pest for fresh fruit production worldwide. Medfly can prolong its developmental duration inside host fruits such as apples and accomplish overwintering in cooler temperate areas of the world. However, whether it can withstand winter conditions in cooler areas as an adult has still to be elucidated. The response of adult medflies at constant temperatures has been studied extensively for laboratory adapted populations. However, there are not comparative data for the response of geographically distant populations of medfly to different temperature regimes. In this study, we used three medfly populations originated from Crete, Volos (Greece) and Dubrovnik (Croatia) to assess survival, fecundity and fertility of adults under constant and fluctuating temperature regimes in laboratory conditions. Cages of 15 females and 15 males were kept at four constant temperatures (6°C-15°C-20°C-25°C) and three different thermocycles (6:15°C-6:20°C-6:25°C_3:1 days). Our study shows the effects of the tested factors (population, treatment and sex) on survival, fecundity and fertility of medfly. Population and treatment were significant predictors of survival and fecundity, while fertility was affected only by treatment. These results are expected to contribute towards understanding invasion dynamics of medfly and the biological traits that influence invasion success, with emphasis on range expansion to northern, more temperate areas of Europe.

Keywords: Tephritidae, Diptera, invasion, demography, geographic origin, temperature regime

### OC261 Effects of thermal history and ambient temperature on the flight performance of the Mediterranean fruit fly

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In recent years, due to climate change, invasive species such as the Mediterranean fruit fly (*Ceratitis capitata*) have extended their geographic range to new environments. The flight traits of insects are strongly influenced by the thermal environment. It is not only the current, ambient temperature that can affect the flight performance of insects but also past temperatures (thermal history). To better understand the dispersal capacity and invasion to new environments, we explored how thermal history and ambient temperature affects the flight performance of *C. capitata* adults. We used the $F_3$ laboratory reared progeny of a wild *C. capitata* population originated from the area of Volos Greece. We used flight mills to test the performance of sexually mature *C. capitata* adults that were acclimated at either 20, 25 or 30°C for 48 hours. The tethered flight was recorded for 2 hours at 20, 25 or 30°C and the wing morphology of tested adults depicted. Higher ambient temperatures favored the speed reached by the adult flies with females being potentially faster than males regardless the ambient temperature. Acclimation temperature did not affect the flight ability of adults while heavier flies had a better flight performance regarding speed and distance. The importance of these findings to predict dispersal potential of *C. capitata* in relation to climate change is being discussed.

Keywords: tethered flight, acclimation, flight capacity, temperature, *Ceratitis capitata*

Acknowledgements: This work was carried out in the framework of the FF-IPM Project (Horizon 2020. GA818184).

## OC262. Temperature-induced plasticity on the thermal tolerance landscape in *Drosophila suzukii*

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Biological invasion is an important driver of global change, having detrimental impacts on human health, food security, and biodiversity. Invasive species also represent a key biological system to understanding the underlying mechanisms associated with rapid adaptation to new environments. An excellent model to study these mechanisms is the spotted wing fly, *Drosophila suzukii*, which is a native species from Asia and is currently distributed in Africa, Europe, and the Americas. An important mechanism involved in invasive success is phenotypic plasticity, which has been considered a key factor to explain the invasion, establishment, and ecological success of *D. suzukii*. Here we explored how different thermal conditions induce phenotypic plasticity on the thermal tolerance landscape in *D. suzukii*. To answer this question, we measured the heat tolerance of fly adults developed at two different temperatures: 18 and 25ºC. We measured heat tolerance at three different static temperatures (36, 38, and 40ºC) to estimate the plasticity thermal-dead-time curves, a unifying approach to account for heat stress intensity and duration. We found that warm-developed flies exhibited a higher thermal tolerance than cold-developed flies at 36 and 38ºC but not at 40ºC. This heat tolerance pattern revealed that thermal sensitivity was lower for cold-acclimated than for warm-acclimated flies because the latter decreased faster their thermal tolerance as thermal stress increased. We concluded that single measurements of heat tolerance are not enough to reveal the complex nature of thermal tolerance, which is key to understanding the ecological success and adaptation to global warming in invasive species.

**Keywords**: global warming, invasive biology, spotted wing drosophila, thermal tolerance

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## OC263. Thermal preference plasticity of a major pest: a key to its invasion success?

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Temperature is one of the most important abiotic factors affecting the physiology of ectotherms. Small ectotherms like insects rely on behavioral thermoregulation to maintain their body temperature within permissive range. The plasticity of preferred body temperature (Tp) contributes to an organism's adaptive capacity to environmental changes. It also plays a substantial role in the establishment of invasive species. Here we studied the Tp plasticity of a major pest, the fruit fly *Drosophila suzukii*. A high Tp plasticity allows for more precise behavioral thermoregulation, decreasing the selection pressure on thermal tolerance. We hypothesized that larvae are less plastic than adult because they are constrained by the fruit selected by the mother. Furthermore, we tested whether the endosymbiont *Wolbachia* played a role in the level of plasticity of Tp. We measured the response of Tp to developmental temperature using a thermal gradient apparatus in a controlled laboratory environment. We assessed the effect of variables such as developmental stage, age, sex, reproductive status, and the presence of

*Wolbachia* (developmental plasticity). We followed the flies for several generations to question a transgenerational effect on Tp.

All studied variables affected *D. suzukii*'s Tp, suggesting a high plasticity. The developmental temperature influenced the mean Tp of adults, but not larvae, highlighting the importance of the mother's temperature choice for its progeny. Females preferred higher temperatures than males, maybe to accelerate egg maturation. *Wolbachia* infected individuals had lower Tp than uninfected flies. The high plasticity of *D. suzukii's* Tp could have contributed to its invasion success worlwide.

Keywords: behavioral thermoregulation, development, ectotherms, transgenerational, *Wolbachia*

## OC264. How climate and invasion can shape the transmission of vector-borne diseases

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The ecology of mosquitoes and the pathogens they transmit is strongly affected by environmental temperature. For this reason, there is substantial interest in the extent to which climate change could alter patterns of transmission. Adding invasive mosquitoes into the mix could lead to dramatic changes in the dynamics and distribution of vector-borne disease. However, the patterns are potentially complex and uncertain. This is partly because transmission is determined by a suite of interacting factors and does not depend on environmental conditions alone. In addition, our current understanding of how temperature affects the various mosquito and parasite/virus life history traits that combine to determine transmission potential is surprisingly incomplete. Here I will highlight some of our research exploring the interaction between temperature and transmission, focusing on key invasive mosquitoes including *Aedes aegypti*, *Ae. albopictus* and *Anopheles stephensi*. One aim is to emphasize some priorities for future research to increase understanding of mosquito thermal ecology and improve predictions of disease risk.

**Keywords:** invasive mosquitoes, climate change, local adaptation, disease risk, malaria, dengue

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## OC265. Modelling how climate change will affect the distribution of arthropod-borne disease across Europe; a case study on West Nile Virus and the invasive vector *Culex modestus* in the UK

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Species distribution modelling of vectors and hosts is being used to determine areas of higher risk of vector-borne diseases, by combining models of both hosts and vectors it can highlight those areas which have the greatest risk of potential disease incursions. *Culex modestus* is a key vector of West Nile Virus as it regularly bites birds, humans, and horses. *Cx. modestus* was discovered to be established in Southern England in 2010, where it is considered to be invasive as there is no prior evidence of it in the UK. To improve our understanding of the potential spread of *Cx. modestus* in the UK species distribution modelling was used to map suitable habitat throughout mainland Europe. These maps were then overlapped with other vector (*Culex pipiens*) and host maps to highlight areas at greater risk of West Nile Virus, and where incursions were most likely to occur, both in the current climate and under different climate change scenarios. Overall, this study provides a model framework for using species distribution modelling to investigate the risks of vector-borne disease using the invasive *Cx. modestus* and West Nile Virus as a case study.

**Keywords:** vector, disease, mosquitos, climate change, species distribution modelling

## OC266. Trade-offs between desiccation tolerance and midgut permissiveness to arboviruses in *Ae. Aegypti*

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As the earth becomes hotter and drier, mosquitoes will adapt to evolving climates. An important physiological response to dry environments is desiccation tolerance. While various environmental factors such as temperature and the microbiome are known to influence mosquito – virus interactions, little is known about how variation in humidity levels, or importantly how adaptation to dry environments, alters midgut permissiveness to arboviruses. We have identified two genetically diverse colonies of *Ae. aegypti* with marked differences in both desiccation tolerance Zika virus susceptibility, where the desiccation tolerant line is more permissive to Zika virus infection. RNAseq analysis demonstrates that the genes differentially expressed in response to desiccation stress differ between desiccation tolerant and susceptible lines. The most highly expressed transcript under desiccation stress in the desiccation susceptible line encodes a peritrophin protein. Peritrophins plays a crucial role in peritrophic matrix formation after a bloodmeal. Knockdown of the peritrophin transcript by RNAi shows that expression of this peritrophin gene is required for survival of the desiccation susceptible line under desiccation stress, but not for the desiccation tolerant line. Following a bloodmeal, the desiccation susceptible line develops a thicker peritrophic matrix than the desiccation tolerant line and initial results suggest that knockdown of the peritrophin gene alters viral replication in midgut. Together these results suggest that desiccation tolerance is accompanied by changes in the midgut processes surrounding bloodmeal digestion which could have a potential role in viral replication in the midgut.

**Keywords:** *Aedes aegypti*, arboviruses, vectorial capacity, desiccation, climate change

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## OC267. Carry-over effects of different larval competition treatments on vector competence in three medically important mosquito species

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The circulation of mosquito-borne diseases (MBDs) depends on the presence of medically important mosquito species. In addition to the native common house mosquito (*Culex pipiens*), a vector for West Nile and Usutu virus, invasive species such as the tiger mosquito (*Aedes albopictus*) and Asian bush mosquito (*Aedes japonicus*) are spreading rapidly through Europe. They pose a major threat because of their high vector competence for viruses such as chikungunya, dengue and Japanese Encephalitis. These three species share a similar ecological niche, especially in the aquatic life stage, but the carryover effect from aquatic to terrestrial stage remains largely unknown. Especially the medical relevance of this ecological pattern needs further investigation.In this study, the context-dependency of intra- and interspecific larval interactions of *Aedes albopictus*, *Ae. japonicus* and *Culex pipiens* was examined. The development time, survival and energetic storage was measured in different European vector populations (Belgium, Germany, Italy) via density-response and replacement experiments at 20°C and 26°C. Selected larval density treatments were used to rear larvae until adulthood and the vector competence of these mosquitoes was studied. *Culex pipiens* was infected with West-Nile virus (WNV), *Ae. albopictus* with Chikungunya virus (CHIKV) and *Ae. japonicus* with Japanese Encephalitis virus (JEV). No infection with JEV was found, but for CHIKV and WNV higher infection rate and body titer per mosquito was observed if mosquitoes experienced interspecific encountering during their larval life history stages. This increased vector competence possibly means a higher risk of arbovirus transmission in co-occurring populations.

Keywords : *Culex pipiens*, *Aedes albopictus*, *Aedes japonicus*, arbovirus, infection rate, vector competence

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## OC268. Investigating the presence of three sympatric Phortica *spp*. in the Latium region (Manziana, Rome, Italy)

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Drosophilidae family flies are widely studied for their ability to spread, rapidly reproduce, and expand across countries. However, information on the worldwide distributed Steganinae subfamily genus *Phortica* is still limited. The biology of *Phortica* spp. is known only under laboratory condition, though an association with oaks forests have been hypothesized in natural conditions. Five species have been reported in Europe and Middle East. *Phortica variegata* (Fallén, 1823), the only confirmed biological vector of the nematode *Thelazia callipaeda* in Europe, and *Phortica semivirgo* (Máca, 1977) that are broadly distribute. *Phortica erinacea* (Máca, 1977) and *Phortica goetzi* (Máca, 1987) which had been sporadically reported in Bulgaria and Turkey. Finally, three specimens of *Phortica oldenbergi* (Duda, 1921) were collected, between 1908 and 1929 in Germany. After almost 100 years, we present the discovery of a *P. oldenbergi* population in Italy, more precisely in the Macchia Grande Oaks Forest (Manziana, Rome, Italy). During a three-year sampling period (from 2018 to 2020), a total of 5018 *Phortica* spp. flies were collected during their activity period (April-October), and both morphologically and molecularly identified. Our results reveal the stable presence over the years of *P. oldenbergi* (9%) in sympatry with *P. variegata* (90%) and *P. semivirgo* (1%). The presence of this poorly known and probably Afro-tropical species in Italy represents a red flag for the diffusion of a potentially invasive vector species in our continent. It also suggests the existence of opened gateways for the introduction of African alien species, probably due to the fruits trade.

Keywords: *Phortica variegata*, *Phortica semivirgo*, *Phortica oldenbergi*, simpatry, vector, alien species

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## OC269. The Growing threat of invasive ticks in a changing world

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Hard ticks (Ixodiadae) are vectors of important diseases to animals and human, and climate change combined with international trade of domestic and wild animals can increase the adverse effect of these pests on food safety and human and animal health. Little monitoring has been done regarding the introduction of nonnative ticks, their establishment and diseases they can transmit. Rise of temperature creates new zones of establishment for ticks and gives them the chance of surviving overwintering with much higher numbers. Invasive ticks can establish easily in urban areas and thus be a serious health problem in cities. In addition to the above, increasing development of resistance of ticks to acaricides make their control increasingly more difficult.

*Rhipicephalus annulatus* and *Rhipicephalus microplus* (Cattle Fever Ticks) and *Haemaphysalis longicornis* (Asian Longhorned Tick) are good examples of this situation.

Resistance of Cattle Fever Ticks to acaricides in the US has become a real threat to the cattle industry and the annual damage could be as high as $2.5 to 3 billion a year because of meat and hid yield losses. *R. annulatus* is native to southeast Europe and *R. microplus* native to southeast Asia.

The Asian Longhorned Tick is native to eastern Asia and was detected for the first time in 2017 but might have entered the U.S. around 2010. Developing solutions to this growing problem is a high priority for the U.S. and the EBCL is collaborating on this AND Cattle Fever Ticks work.

Keywords: Ticks, climate change, invasive, resistance

## OC270. Climate change and adaptations: presence and trophism of sandflies on winter in Sicily

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The sand fly is the main vector of leishmania, the causative agent of human and animal leishmaniasis. Leishmaniasis is a zoonosis caused by infection by one of the numerous species of parasitic protozoa of the genus Leishmania, which carries out its life cycle within the digestive system of the vector and is transmitted during the trophic activity of the female sandflies. The biological cycle, as with many other arthropods is closely linked to the climate. From observations in nature and from studies on laboratory colonies it appears that the average duration of the development cycle of the species widespread in Italy can vary from 45 days to two months. Their activity in Sicily, as well as in the rest of Italy, was recorded between May and October. Recently, in the period between 2019 and 2022, we have extraordinarily detected the presence of adult sandflies and their trophic activity in the regional hinterland even in territories with altitudes well over 500 m above sea level, in the coldest months between October and April. The unusual presence of sandflies during the winter months is due both to climate change and to the dangerous ability of these arthropes to adapt to adverse conditions. Furthermore, trophic activity indicates the need to strengthen prophylaxis plans and, with a view to a One Health approach, intensify entomological surveillance to contain the risk of contracting this zoonosis and other Vector-borne Disease.

**Keywords:** climate change, sandflies, winter, One Health

# Session 11:
## Biodiversity and Conservation

## OC271. Outcomes from the European project SPRING: capacity building in pollinator taxonomy

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The skills for pollinator identification, specially bees and hoverflies, are restricted to a small number of people and not covering homogeneously the European territory. There is a strong need for increasing the number of people that will be able to identify the samples coming from the future EU Pollinator Monitoring Scheme, particularly at the species level. The objective of European project SPRING is to strengthen taxonomic capacity in EU Member States with regard to pollinators, and support preparation for the implementation of the EU Pollinator Monitoring Scheme. A series of trainings have been organized to connect the people working on bee identification across different geographical scales; and to increase the taxonomic skills of the local people across the European territory. The trainings aimed at 4 different levels: (i) identifying large pollinator groups, (ii) identifying bees and hoverflies to genus level, (iii) identifying bees or hoverflies to species group level, (iv) identifying bees or hoverflies to species level. Trainings at level (i) were developed at the national scale; levels (ii) and (iii) were covering multiple countries; and level (iv) considered the whole Europe. Moreover, the project created a series of e-learing materials on pollinator identification at different levels, focusing on the needs of people involved on the monitoring. All this material will be open to the public on the online portal 'Pollinator Academy', that will act as a hub to centralise information about already available tools related to pollinator identification (articles, online keys, books, interesting links, other courses, etc.).

**Keywords:** SPRING project, capacity building, taxonomy, pollinators, trainings

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## OC272. Toward a unified view of insect distribution

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Insects are the center of an ever-growing body of data and literature on potential declines, but to know even where something lives is no simple task when studying invertebrates. Consequently, some estimates of decline may overstate the seriousness of the situation, obscuring those real declines that are almost certainly taking place for some groups in some areas. Focusing primarily on insects and especially bees, I will discuss several recent published and ongoing studies via which we are slowly bettering our knowledge of insect distribution.

**Keywords:** conservation, biodiversity, mapping, insect decline

## OC273. Beelibre: Towards the development of Luxembourg's first on-line repository of local wild bee content

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Wild bees are fundamental insect pollinators for flowering plants. Therefore, developing effective conservation strategies targeting regional wild bee biodiversity has become a matter of utmost concern in many European countries aiming for sustainable development. Some wild bees can thrive in diverse environments, but others have specific habitat requirements. Thus, the success of conservation strategies relies heavily on our understanding of the taxonomy and ecology of local species. Properly curated reference material is vital for both morphological and molecular taxonomic tools to provide accurate biodiversity assessments. Currently, reference data of all kinds for the wild bee fauna of Luxembourg are scattered across multiple sources and can be hard to obtain. These obstacles restrict our capacity to propose well-informed conservation initiatives. In the context of the European Commission's "New Deal for pollinators", the 'beelibre' project aims to develop an open-access, user-friendly online database for national wild bee data. Grounding on the metabarcoding results of the "Atlas of the wild bee fauna of Luxembourg" and the Hymenoptera collection of the National Museum of Natural History Luxembourg (MNHNL), this project attempts to build a multi-lingual website collating relevant content concerning local wild bee identification, DNA barcodes, nationally relevant publications and descriptions of plant-pollinator interactions found in the region. Moreover, 'beelibre' aims to act as an instance of mediation between researchers, stakeholders, and the general public in Luxembourg, socializing scientific knowledge to a broader audience. Our project's final goal is to raise awareness regarding pollinator biodiversity at the national science-public-policy interface – and beyond.

**Keywords:** Apiformes, conservation, online repository, Luxembourg

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## OC274. Could we identify bumblebees from their buzzes? A proof of concept combining acoustic tools and artificial intelligence

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While the importance of pollinators in the maintenance of wild, ornamental and crop plant communities is widely acknowledged, there is clear evidence of a global pollinator decline. However, the extent of the decline remains to assessed for many species (in terms of both species distribution and population trends). Traditional methods for monitoring bumblebees (e.g. visual counting, sweep netting) and associated ecosystem services such as pollination are laborious, thus limiting our ability to monitor over large spatial scales and long temporal periods. Thanks to recent technological and methodological advancements, the use of acoustic sensors with artificial intelligence (AI) offers exciting developments for future monitoring programmes of bumblebees but have not been fully explored yet. The primary aim of this work was to create and develop a new tool for surveying European bumblebee species in a cost-effective and non-invasive way. The objectives were twofold: (i) create an open-access reference library of buzzing sounds from common bumblebee species occurring in the United Kingdom and France, and (ii) develop, train and test AI algorithms for automatically recognizing bumblebee species and behaviour from their buzzes using acoustic sensors. We will present the key results of this work as well as the bioacoustic tool that is under development. Finally, we will discuss whether and how acoustic monitoring of bumblebees has the potential to be transformative for bee ecology and conservation.

Keywords: bioacoustics, bees, machine learning, monitoring

## OC275. Environmental variables and wild bee species traits as drivers of change in pollination spectrum in agricultural landscapes: a pollen DNA approach

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Wild bees (or non-*Apis* bees) are an essential group of insects not only from a biodiversity but also from a society perspective, as they provide a key ecosystem service, pollination. In fact, 80% of the crops worldwide are pollinator dependent. However, pollinators, together with the rest of insect groups, are currently facing declines of their populations. These declines are mainly due to human-driven changes and one important threat to their biodiversity is the use of chemical pesticides in agriculture. Therefore, there is an urge to modify agricultural landscapes in a manner that crop yield and populations of pollinators are both maintained or increased. The first step for these strategies to work is to acquire knowledge of pollinator's foraging requirements. In this study, our main aim was to characterize the pollination spectrum of wild bees, or the community of pollinated flowering plants they visit within an intensive agricultural landscape. To do this, we focused on sunflower crops, in Nouvelle Aquitaine (France), and used a pollen environmental DNA (eDNA) and metabarcoding approach. Our experimental setup allowed us to infer the environmental variables and wild bee species-specific traits, such as sociality and body size, which were responsible for changes in these plant-insect interactions. Whether wild bee species-specific traits were drivers of change of the pollination spectrum of wild bees was another objective of our study. Unravelling plant-insect interactions and their threats in a changing world is key to conserve pollinators and the ecosystem services they provide.

Keywords: wild bees, conservation, agricultural landscapes, pollen DNA, metabarcoding

# OC276. A DNA barcoding library for exploring the evolution and diversity of European ants

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Ants are the most species-rich and ecologically diverse group of social insects and are important components of ecosystems. Europe hosts approximately 630 ant species, and this number continues to grow each year as new species are discovered, and alien species are accidentally introduced. Comprehensive sequencing of mtDNA and construction of DNA barcoding libraries provide useful tools for the detection of potential new species and invasive ones, as well as for studying ecological and evolutionary processes.We assembled the first DNA barcoding reference library based on COI for European ant species, both native and alien, and we assessed their intraspecific variability. We obtained a 78% taxon coverage (488 species) and ~5,800 sequences (~3,800 newly sequenced and ~2,000 retrieved). The dataset covers 37 European countries and 61 islands.We found striking differences across groups in terms of DNA barcoding performance. We also estimated the percentage of sequences with stop codons as a proxy of pseudogene incidence and compared it across genera. We produced species-level maps illustrating the genetic variability. We found high intraspecific genetic variability and, by applying different species delimitation methods, we found a large fraction of putative cryptic species. Genetic results allowed us to report new faunistic records both at the European and country levels. Remarkable phylogeographic intraspecific patterns in a number of species suggest the effect of glacial cycles in combination with a geographical framework in shaping the observed differentiation.

**Keywords:** cryptic diversity, phylogeography, Formicidae, Hymenoptera

# OC277. Capture efficiency of pitfall traps based on sampling strategy and the movement of arthropods

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Pitfall traps are frequently used to capture ground-dwelling arthropods, particularly beetles, ants and spiders. The capture efficiency of a pitfall trapping system strongly depends on the number and opening size of traps, how traps are distributed over the sampling area (spatial arrangement) and the movement characteristics of arthropods. We use numerical simulations for a single species to analyse the trap count patterns that emerge in terms of these variables. Arthropod movement of individuals is modelled as correlated random walks, with multiple traps placed over an area, and catches are simulated as individual interactions with traps. We consider four different types of spatial arrangements of traps across a homogeneous landscape: grid (i.e., rectangular array), transect, nested-cross and randomised. We place our results in ecological context by considering the movement capabilities of *Pterostichus melanarius*, a highly active carabid beetle often serving as a biocontrol agent for the suppression of pests and weeds. We show that for a given movement model (i.e., species) there is an optimal inter-trap separation distance (trap spacing) that maximises captures, expressed using exact formulae in terms of trap opening sizes, sampling area and trap number. Moreover, for the grid and nested-cross arrangements, larger trap spacing to maximise spatial coverage over the whole sampling area is sub-optimal. Also, we find that over a large sampling area, there is a hierarchical order for spatial arrangements in relation to capture efficiency: grid, randomised, transect, followed by the nested-cross. However, over smaller sampling areas this order is changed as the rate at which trap counts accumulate with trap number varies across arrangements- eventually saturating at different levels. In terms of movement effects, capture efficiency is maximised over a narrow diffusive range and does not depend much on the type of spatial arrangement- indicating an approximate optimal mode of arthropod activity i.e., rate of spread. Our approach simultaneously considers several important aspects of pitfall trapping providing a basis to optimise and adapt sampling protocols to other types of traps to better reflect their various purposes, such as biomonitoring, conservation, or pest control.

**Keywords:** arthropod movement, capture efficiency, diffusion, inter-trap spacing, random walk, pitfall trapping, sampling strategy, spatial arrangement

## OC278. Biodiversity discovery with the DiversityScanner and the Entomoscope

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Expensive microscopes and custom camera setups are typically utilized to capture images of invertebrates, particularly for the purpose of discovering new species. However, the expense associated with imaging large, ethanol-preserved insect samples from Malaise traps limits the accessibility of biodiversity research, particularly in countries in the Global South where the majority of species reside. Moreover, identifying species and specimen abundances in Malaise trap samples often necessitates the involvement of multiple specialists to handle different taxa. To address these challenges, we have developed two novel instruments: the DiversityScanner and the Entomoscope. The Entomoscope is a do-it-yourself macroscope that captures high-resolution, focus-stacked images of invertebrates preserved in ethanol, while the DiversityScanner is a robot-assisted macroscope that can sort species into multiwell plates for sequencing. In a proof-of-principle study, we have shown that both systems can classify insects (primarily by Linnean “families”) with over 95% accuracy. The classifier can be customized and expanded to suit specific research needs. All of the resources required to construct the systems, as well as a manual, are available as open-source files, enabling anyone to construct their own apparatus.

**Keywords:** taxonomy, biodiversity, smart macroscopy, smart microscopy, deep learning

## OC279. Biodiversity drivers of arthropod pest regulation services in tropical agroecosystems of La Réunion island, a first systemic insight

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Natural pest regulation is a key ecosystem service to achieve agroecological intensification. However, its routine integration to pest management strategies is pending because of its unpredictability, hampering the trust of producers to rely on this service. To reduce this knowledge gap and increase foresight of natural pest regulation in production systems, we studied its drivers in vegetable crop fields in La Réunion, a tropical island in the Indian Ocean. We selected and characterized 20 fields representing the diversity of the grown crops, surrounding habitats, management practices and seasonality of the island. We then assessed the plant and arthropod communities associated with the crop and its margins using quadrats and non-selective interception and pitfall traps. We also observed the presence of pests and their natural enemies on the crop, whilst measuring crop damages and yield. Finally, the surrounding landscape was described at the short scale (within 500m) through GIS. Results demonstrated the strong relation between plant diversity and arthropod diversity, with subsequent consequences on pest abundance and pressure. Effects of the management practices and the landscape on these interactions were highlighted and discussed. Aside increased knowledge concerning systemic pest regulation mechanisms, this study opens the discussion on the utility of the development of bioindicators of these services. Such tools are expected to help stakeholders to further mobilize and integrate these services in their management strategies.

Keywords: biological control, natural enemies, biodiversity, multitrophic interactions, agroecology, vegetable crops

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## OC280. Conservation of farmland biodiversity in Mediterranean agroecosystems

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The Mediterranean basin has been recognized as a global biodiversity hotspot. Cyprus, the third largest Mediterranean island, is characterized by high rates of endemism. A large percentage of the farmland on the island is a mosaic of small agricultural fields interspersed within a matrix of (semi-) natural areas and they can be characterized as High Nature Value Farmland, important for biodiversity conservation, providing feeding and nesting resources to many different species . To better understand factors affecting biodiversity conservation in agricultural fields, biodiversity was monitored in vineyards and carob groves over consecutive seasons. Our results suggest that landscape features, such as stonewalls, non-productive bushes and trees, as well as flower rich margins promote biodiversity conservation. In addition, low input agricultural management practices are beneficial for the conservation of species. The results of the current work were used to develop a user-friendly Biodiversity Metric Tool for vineyards. The Tool enables farmers quantify the impact of management practices on biodiversity, with the potential to promote their products based on the perceived value for conservation.

**Keywords:** Cyprus, farmland, conservation, biodiversity metric tool

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## OC281. Cover crops enhance ecosystem services and increase biodiversity in pear orchards

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Croplands and pastures are nowadays one of the largest terrestrial biomes on Earth. The transformation of natural environments into cropland and the intensification of agriculture have led to a biodiversity crisis that urgently needs to be reverted. Because of the great extension of cropland, green infrastructures, such as cover crops, may contribute to restore ecosystem services and biodiversity in agricultural areas. The aim of this research was to assess biological pest control and biodiversity in pear orchards with and without cover crops. The abundance and diversity of arthropods in pear trees and the cover were assessed by periodical sampling in spring during three years. Cover crops significantly reduced the abundance of ants (namely, *Lasius grandis*) and increased the abundance of spiders on pear trees. The response of other important natural enemies (i.e., predatory mirids) was variable and other groups of predators were little abundant. The abundance of aphids on pear trees was significantly lower in the presence than in the absence of cover crops. In contrast, the response of the pear psyllid (*Cacopsylla pyri*) was variable. Sweep net sampling between the lines of pear trees showed a much higher number of species of hymenopterans, true bugs, beetles and spiders in the presence than in the absence of cover crops. In addition, their diversity and abundance in the cover increased between two to six-fold over the three years of the research. Cover crops are presented as an option to enhance biocontrol and the maintenance of biodiversity in agricultural landscapes.

**Keywords:** biological control, green infrastructures, ants, spiders, predatory mirids, pear psyllid

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## OC282. Floral resources for beneficial arthropods in apple orchards

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Floral resources such as flower strips are increasingly being used to support natural enemies of insect pests, pollinators as well as to increase biodiversity in general. Effects of flower strips and hedgerows were investigated in a series of field studies, with focus on beneficial arthropods in organic apple orchards. In the apple orchards, presence of a flower strip promoted abundance of natural enemies of the rosy apple aphid *Dysaphis plantaginea*, and the natural enemy abundance and diversity was found to be positively affected by proximity to the flower strip. A hedgerow also had some positive effect, and promoted additional groups of predators compared to the flower strip, further supporting the natural regulation of pests. Apple pollinators also responded positively to flower strips, with effect on abundance of flower visitors and pollination. In these field studies, a highly diverse mix of native, perennial flower species was used. Findings indicate that distance to flower rich habitats, the area with flowering resources and the diversity and quality of habitats for beneficial arthropods are important considerations for orchard (re)design and can contribute to integrated pest and pollinator management.

**Keywords:** predators, *Dysaphis plantaginea*, pollinators, flower strip, functional diversity

## OC283. Wild vegetation and marketable habitat enhancement plants support pollinator diversity in Moroccan farmlands

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Several management practices have been suggested to mitigate the global pollinator decline in agro-ecosystems, including wildflower strips and Farming with Alternative Pollinators (FAP). FAP proposes to dedicate 25% of the field area to seed Marketable Habitat Enhancement Plants (MHEP) around the main crop, occupying 75% of the field. However, wild pollinators may not rely fully on the resources that fields provide due to differences in flying period and host- plant preferences and need additional resources from wild flowering plant communities. Here we aim to compare wild pollinator communities between FAP fields, monoculture of pollinator dependent crops and the nearby wild flowering plants. We developed two experimental trials with two main crops (faba bean and eggplant) in 16 fields in North-West Morocco and we com- pared wild pollinator richness and wild pollinator specialization between FAP fields, control fields and the nearby wild flowering plants. We recorded a significantly higher pollinator richness in FAP fields compared to wild flowering plants and monoculture. Pollinator specialization index (i.e. degree of interaction specialization at the species level) did not differ significantly between the three treatments in faba bean trial (i.e. FAP, control and wild plants), whilst in eggplant trial, wild plants harboured signifi- cantly more specialist species than FAP fields. Yet, no significant differences in pol- linator specialization index were reported between the other treatments in eggplant trial (i.e. FAP vs. control and control vs. wild plants). Moreover, 28% of the pollinator species collected, were only observed on wild plants, particularly thistles. These re- sults highlight the potential of FAP approach as a tool for pollinator conservation in farmlands. However, the FAP approach alone is not sufficient to cater the diverse pol- linators present in the agro- ecosystem, and hence, the maintenance of the surround- ing wild flowering plants is necessary to support pollinators in farmlands.

**Keywords**: eggplant, faba bean, specialization, wild flowering plants

## OC284. Use of hedgerows with aromatic plants as conservation practice for natural enemies of pests in orange orchards

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The present study examined the ability of four Mediterranean aromatic plant species, i.e., oregano, rosemary, sage, savory, established in hedgerows in orange orchards to support natural enemies of citrus pests in comparison to field margins with bare soil or weed vegetation which is the commonly used management practice. The assessment was based on the abundance and diversity of parasitoid wasps, insect predators, spiders, and predatory mites in the field margins and on the orange trees for two growing seasons. The impact of aromatic plant hedgerows on natural enemies varied with the aromatic plant species and the natural enemy group. Savory plants had a positive effect on the abundance of parasitoids and arachnid predators; sage and oregano flowers favored predatory insects; aromatic plants hosted more spiders than the weed vegetation in the (second) year of their full growth, and the rosemary hedgerows served as the most suitable habitat for spiders and predatory mites. The similarity of natural enemy communities on the field margins and on the orange trees increased with time, indicating the insects' dispersal from the field margins in the orchard. The results support the use of the tested aromatic plant species in conservation schemes of targeted beneficial arthropod groups in orange orchards considering also the exploitation of suitable wild flowering plants of the weed flora.

**Keywords:** aromatic plants, conservation, natural enemies, parasitoids, predators, weed flora

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## OC285. Birds consume brown marmorated stink bugs, *Halyomorpha halys* (Hemiptera: Pentatomidae) in orchard agroecosystems

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In agroecosystems, brown marmorated stink bugs (*Halyomorpha halys*) (Hemiptera: Pentatomidae) are polyphagous pests that cause significant economic losses to a variety of crops every year. Synthetic, chemical-based arthropod control and management practices are common but face immense pressure to respond to rapidly evolving pest populations. Insectivorous birds may provide a means of sustainable, non-chemical management of invasive pests, such as *H. halys*. In forest margins surrounding peach, pecan, and mixed peach-pecan orchards, we monitored *H. halys* populations with pheromone-baited traps, mist netted birds, and collected avian fecal samples for molecular gut content analysis in southeast Georgia, USA. We screened 278 fecal samples from 19 bird species for presence of *H. halys* to determine whether birds provide biological control on this pest. Overall, we found evidence that three species consumed *H. halys*, including Northern cardinal (*Cardinalis cardinalisis*), Tufted titmouse (*Baeolophus bicolor*), and Carolina wren (*Thryothorus ludovicianus*). *H. halys* captured in traps increased over time but did not vary according to orchard type. Although incidence of predation was low, this may be an underestimate as a result of our current avian fecal sampling methodology. Because birds are members of the broader food web, future studies are needed to understand avian pest control services in southeastern agroecosystems to determine whether birds eat other pests in addition to *H. halys.*

Keyword: biological control, integrated pest management, molecular gut content analysis, arthropod prey, pecan orchard, peach orchard

## OC286. LIFE SOS Crau Grasshopper: habitat management, breeding programme and translocation strategy

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The pseudosteppic grasslands of the Crau plain in southern France are known for their high biodiversity. Grazed by sheep, they are part of the Natura 2000 network. Just over half of the remaining dry grassland areas are strictly protected by a national nature reserve (7 400 ha).

The Crau Plain Grasshopper, *Prionotropis rhodanica*, is endemic to this habitat with only three remaining subpopulations. Therefore, it is listed as "Critically Endangered" on the global IUCN Red List of Threatened Species. In 2014, a conservation strategy was elaborated by the IUCN SSC Grasshopper Specialist Group, the IUCN SSC Conservation Planning Sub-Committee and the CEN PACA.

Based on this strategy, the LIFE project "LIFE SOS Crau Grasshopper" (2021-2025) coordinated by CEN PACA aims to improve the conservation status of the species.

The project has four key objectives:

- Increasing favourable habitat through adaptive grazing management,
- Reducing predation by large insectivorous bird species,
- Improving breeding success *in situ* and starting a translocation programme,
- Raising awareness among local stakeholders and the general public.

The breeding programme of *P. rhodanica* started in 2015 and will be improved and expanded within the project. A translocation strategy has been developed in 2023 with the aim to test a first translocation in 2024. The methods used and developed for population monitoring, breeding and translocation can also be applied to other *Prionotropis* and Orthoptera species. Cooperation with European experts and the IUCN SSC Grasshopper Specialist Group enables the exchange of experience and replication of methods.

Keywords: *Prionotropis rhodanica*, conservation, breeding, translocation

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## OC287. Phylogeography and conservation of *Melitaea diamina* (Lepidoptera, Nymphalidae): southern relict populations trapped by climate change

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*Melitaea diamina*, commonly known as the false heath fritillary, is a butterfly species found mainly in temperate areas of Europe and Asia. The species has a patchy distribution, and its populations, especially the southernmost ones, are often scattered on mountain areas and isolated from one another. This suggests that they represent relicts of the species' more extensive range during the last cold phase. Previous studies using mitochondrial DNA data indicated the presence of several lineages in Europe, with the notable differentiation of certain geographically isolated Iberian populations. In this study, we investigated European populations of *M. diamina* in order to unravel the origin of the multiple Iberian lineages and find possible explanations. To achieve the desired level of detail, we generated a ddRADseq dataset, which we subsequently used to perform various analyses, ranging from phylogenomics to model and non-model based population genomics, and demographic analyses. The results follow a pattern similar to what has been found in other Lepidoptera species, showing limited genetic diversity across the majority of the species' range, but with a gradual increase towards the Iberian Peninsula. The analyses indicated the presence of at least three different lineages within the peninsula, with minimal genetic admixture between them. Some of these lineages correspond to genetically unique and geographically isolated populations, that are restricted high altitude areas and therefore would be threatened by further temperature raise brought by climate change. These findings highlight the importance of preserving these newly discovered evolutionary significant units and their corresponding habitats.

**Keywords:** population structure, isolation, altitude

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## OC288. Forest edge effects on termites in a neotropical rainforest

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Termites dominate tropical soils and play a major role in organic matter decomposition, especially in rainforests. However, tropical forests are heavily fragmented which implies a loss of surface as well as an increase in forest edges. The latter are characterized by different environmental conditions from the interior of the forest. Both factors —fragment area reduction and forest edge increase— are hard to study independently. Here, we investigated the occurrence and depth of edge effects on the termite assemblage in an otherwise continuous neotropical rainforest. Three sites were sampled in the Amazonian rainforest in French Guiana along the almost straight edge of a 90-m wide powerline clearing. Whereas edge effects on other insects such as ants and dung beetles have been reported up to 100-300 m into the interior, neotropical termites were not significantly affected. Soil feeder frequency was not lower near the forest edge although they have an allegedly thinner cuticle and require moist and soft soil. Finally, no variation in beta diversity was found near the edge either. Overall, termites appear as resilient insects against forest edges as long as they do not suffer from fragment size reduction. However, a threefold increase in frequency of an opportunistic pest species (*Heterotermes tenuis*) was found near the edge. We hypothesize that *H. tenuis* good adaptability and dispersibility may have allowed it to quickly colonize and dominate this environment before more cryptic species, as has been shown for ants.

**Keywords:** termites, forest edge, beta diversity, fragmentation, Amazonian rainforest, biodiversity

## OC289. Historical changes in bee species richness in two areas with contrasting land use patterns in Cyprus

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Wild bee populations are reported to be declining in Europe, yet only a few research studies have evaluated historical changes in bee assemblages to date. Here, we used a unique dataset compiled by the late bee taxonomist George Mavromoustakis covering a 30-year period beginning 100 years ago, as well as contemporary biweekly samplings over two years (2016-2018) to analyze changes in wild bee species richness in the insular ecosystems of Cyprus. Wild bees collected recently were sampled using insect-net biweekly and coloured pan traps once per month. We focused on two areas with contrasting land use patterns, one with mainly agricultural land use (Cherkes Chiftlik-Asomatos) and one dominated by semi-natural habitats (Akrotiri). Changes in land use were minor and relatively similar in the two areas. We detected a substantial species turnover, with 43 species collected only in historical samples, 77 only in recent samples, and 42 in both periods. The richness of plants visited by bees was higher at the area dominated by semi-natural habitats, with a significant relationship between plant richness and bee species richness. We hypothesize that the main drivers for species extinctions are the loss of habitat and floral diversity because of the expansion of agriculture and urban development. Thirteen bee species were recorded from Cyprus for the first time and three species are new to science. Our findings highlight the importance of historical data in the study of bee diversity, and the need to establish a wild bee monitoring and conservation program on the island.

**Keywords:** wild bees, landscape characterization, historical sampling

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## OC290. Protecting Farmland Pollinators: Whole Farm Scorecard

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Protecting Farmland Pollinators is about identifying small actions that farmers can take that will allow biodiversity to coexist within a productive farming system. Farmers in Ireland recognise the importance of pollinators, but farmland has experienced wide-scale loss of wild pollinators over the last fifty years.By working closely with 40 farmers, management practices that benefit bees and hoverflies on Irish farmland were identified, and a whole farm pollinator scoring system was developed. Using a whole farm pollinator scorecard, farmers receive ‘pollinator points’ each year based on the amount and quality of pollinator friendly habitat maintained and/or created and, each year, farmers receive a results-based payment that relates to the points. Irish farms have great potential to improve both the quantity and quality of biodiversity friendly habitats without negatively impacting on farm productivity. Thirty-one farmers increased their score between year one and year three of the results-based payment. The median whole farm pollinator score for the 40 farms increased 56% from year one to year three. Each farm type (beef, dairy, mixed and arable) increased their median score over the three years and dairy and arable farms showed the largest increase. Pollinator species richness and abundance varied across farm type.This project has helped farmers better understand and engage with nature on their land and has created a measurable system for improving habitats for biodiversity on farms. Investigations are ongoing to determine if those farms that have a higher pollinator score do have more pollinators and more biodiversity.

**Keywords:** biodiversity, farmland pollinators, results based payments

## OC291. A Conserving insect on Mediterranean islands

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Mediterranean islands are significant hubs of insect biodiversity and endemism, a result of their relative isolation from the neighbouring land masses. For instance, Cyprus with ca. 0.002% of the area of the EU, hosts ca. 20% of the wild bee (Hymenoptera: Apoidea) fauna of the Union. Historically recognized pressures on insect populations include land use change, habitat fragmentation, agricultural intensification as well as climate change. On Mediterranean islands the pressures on insect populations are magnified because of the small surface area, which severely limits the availability of undisturbed sites and increases the probability of several stressors acting together on a population. Relying on a decade of field work in agricultural and natural ecosystems, we discuss the potential impacts of human induced pressures on insects and propose effective conservation measures in agricultural and natural ecosystems. We found that low-input vineyards host ca. 50% of the butterfly species of the island. In addition, we detected that wild bee and butterfly diversity in vineyards surrounded by natural areas was similar to that of vineyards surrounded by low input agricultural areas. Another study showed that areas affected by high intensity agriculture and urban development lost wild bee species at twice the rate of areas unaffected by high intensity agriculture and urban development. We highlight the importance of low intensity agricultural ecosystems for insect diversity, and the importance of (semi-)natural areas with high floral diversity for wild bee species conservation.

**Keywords:** wild bees, vineyards, historical data, pollination

## OC292. Conservation of insect diversity needs long term commitment of various land use stakeholders – four-year results of the EU LIFE-Project "Insect Responsible Sourcing Regions"

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In order to reverse recent trends in insect diversity development, employees from nature conservation organizations and food companies have been establishing seven large-scale landscape units distributed throughout Germany, known as "insect responsible sourcing regions". In these regions, as many land-use stakeholders as possible are to make a joint effort to conserve and promote insects and come together to form an alliance for insect conservation. In order to provide these project goals, various methodical approaches were chosen. These range from the establishment of a network of agricultural demonstration farms, support for municipal administrations in the implementation of insect protection concepts, information and advice for the general public, and the implementation of training courses to promote populations insect competence. Our project shows that the protection of insects is hindered by various factors. These factors can only be overcome if they are differentiated by habitat. In agricultural contexts, efforts to protect biodiversity depend in particular on the provision of adequate monetary compensation. In urban contexts, the public promotes insects, but these efforts are subordinated to traditional ornamentally design option and individual well-being. Especially in public forests, conservation strategies, ecological standards but also the consequences of climate change lead to a steady improvement of habitat conditions for especially deadwood-dwelling insects.

Based on our experience, we conclude that the added value of insects is still not sufficiently internalized on a large part of the population. Regrettably, this also applies to land use groups whose existential basis is very much based on the occurrence of insects.

**Keywords**: insect conservation, land use stakeholders, agriculture, communities, forestry

## OC293. Drivers and Repercussions of UK Insect Declines (DRUID): Artificial Neural Networks predict species distributions for a wide range of insect taxa

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We show how an artificial neural network (ANN), given some novel specific developments, can capture the relationship between species response traits and environmental drivers to predict the probability of occurrence and therefore individual species distributions. Using our ANN architecture, we present community-wide occupancy predictions at the species level for the whole of the UK from both strategic monitoring networks and a citizen science data platform. Within our analyses we consider morphological (wing indices etc), physiological (voltinism) and ecological (number of host plants and type etc) traits as well as proportions of different landcover types, weather (temperature, rain, wind etc) and environmental (altitude, slops, aspect etc) covariates. The model is trained with long term data for butterflies (UK Butterfly Monitoring Scheme), moths (Rothamsted Insect Survey) and citizen science carabid, dragonfly & damselfly, grasshopper & cricket data from the National Biodiversity Network (NBN). Species-specific, UK-wide maps will be presented for these communities. Our approach explains the abiotic filtering behind our predictions, through the identification of the key environmental drivers of species niches, as well as the trait-mediation at the core of our model. We demonstrate that we can build on the high predictive performances of ANNs, while retaining the explainability that is key to species distribution models. For example, we highlight significant effects of wind exposure on flying insect communities, but also the importance of temperature and habitat, particularly broad-leaved woodland and arable land, across some taxa studied.

**Keywords:** Orthoptera, Odonata, Lepidoptera, Carabidae, NE/V00686X/1

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## OC294. Diversity of insects associated to systemic integration of millet and shrubs (*Faidherbia albida* and *Guiera senegalensis)* in Niakhar area Senegal

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Insects are an important part of ecosystems. They contribute to the dispersal and exploitation of organic matter as well as in the production of ecosystem services. This study deals with the diversity of insects in farms where millet is associated with fertile woody plants (*Faidherbia albida* and *Guiera senegalensis*). Carried out in September 2021 and January 2022 in two stations (Sanghaie and Sob), its objective was to analyze the composition and functional role of captured insects. Sampling was done using six barber traps placed at 0.5m and 20m from six pairs of *Faidherbia albida* (pruned/not pruned) and six others that have Guiera residues or litter inputs. After 48 hours of trap expositions, 49 insect species (7 orders, 18 families) were recorded. The Shannon H' diversity, Pielou evenness, and Jaccard similarity indices show that the catches in Sob-Faidherbia and Sanghaie-Guiera stations are quite close, equitable and more diversified than that of Sanghaie-Faidherbia. Five beneficials (*Scarites senegalensis, Crematogaster sp., Camponotus maculatus*, *Pimelia senegalensis* and *Heterogamodes sp*.), two polyphagous species (*Monomorium areniphilum*, *Zophosis trilineata*), a phytophagous species (*Carpophilus hemipterus*) and a decomposer (*Musca domestica*) were constantly observed. Hymenopteran (Formicidae) are prédominant under pruned *Faidherbia albida* with significatif effects of sites, seasons and their interactions (respective p-value of 2*10-16 and 0.0036). Coleopteran (Scarabaeidae and Tenebrionidae) were predominant in rainy season (82%) in plots amended with *Guiera senegalensis*. Pruning *Faidherbia albida* and use of *Guiera senegalensis* in crop amendments increase insect diversity and could contribute to natural pest regulation and seed dispersal in agroforestry systems.

**Keyword:** diversity, insects, millet, *Faidherbia albida, Guiera senegalensis,* Senegal

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## OC295. EU LIFE Programme funding direct action to protect Europe's threatened insects

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The European Union protects more than 27,000 natural areas of special European importance. The network, known as Natura 2000, is set up to safeguard Europe's most valuable and threatened species and habitats. Since 1992 the EU has provided co-funding, through the LIFE Programme, to support direct action aimed at the protection and restoration of Europe's threatened habitats and species; and halting and reversing biodiversity loss. The decline of insect diversity and abundance across the world is well documented in the scientific literature (Sánchez-Bayo and Wyckhuys, 2019) and evidence of similar trends have been reported in Europe (Seibold et al. 20196 and Hallmann et al. 2017). The losses, and the potential impact on ecosystem services, are becoming better understood leading to responses such as the EU Pollinators Initiative. The LIFE Programme has played a part in seeking to address these declines and has supported over 220 projects across Europe which benefit threatened invertebrate species listed in the EU Habitats Directive (HD) and/or European Red Lists. However, of the insect species listed in the HD, less than half have been targeted for conservation action by LIFE projects, and few projects focus on European Red Lists species. There is therefore a recognition of the under-representation of actions for insects in LIFE projects in general, as well as a bias towards the same insect species (Neemo, 2020). The contributions of the LIFE Programme to the conservation of endangered insects as well as the opportunities that the programme currently offers must be more widely disseminated.

**Keywords:** funding, conservation action, biodiversity loss, pollinators, insects

### OC296. Movement ecology of Hermit beetles (*Osmoderma eremita*) in Eastern Romanian Carpathians

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Saproxylic beetles are fundamental components of forest ecosystems, contributing to nutrient cycling and decomposition processes, but Romanian forestry practices aim to decrease the presence of saproxylic species because they are considered pest species. Movement ecology can help protect saproxylic beetles by identifying the key habitat features that they require for movement and dispersal. For example, saproxylic beetles may require specific types of deadwood, such as logs or snags, for feeding or breeding, or they may need certain microhabitats within deadwood, such as moist or rotting areas. By understanding these requirements, we can develop conservation measures that aim to improve the protection of these species. Between 8 July and 29 August 2022, we monitored the movements of 28 Hermit beetles (*Osmoderma eremita* species-complex) using miniaturized VHF transmitters (Advanced Telemetry Systems T15). The study was carried out in an old-growth Beech Forest nearby Lepşa village (Vrancea county, SE Romania), on the right bank of the Putna River. Beetles were captured using flight intercept traps baited with Decalactone (Synergy Semiochemicals, Burnaby, Canada). We searched for individuals with transmitters three times a day using homing radiotracking and recorded their location, position, and behaviour. The results showed that the beetles moved between 6 and 405 meters (median = 21.43), with some remaining static or moving short distances. Individuals who remained in the same position moved vertically or were static in cavities.

Keywords: animal tracking, saproxylic species, movement ecology, habitat selection

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### OC297. LIFE BEETLES (LIFE 18 NAT/PT/000864) – The first insect conservation project in the Azores archipelago

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The LIFE BEETLES project- Bringing Environmental and Ecological Threats Lower to Endemic species, is a project coordinated by the Regional Secretariat of Environment and Climate Change, co-financed by the LIFE programme, that aims to improve the population size, distribution area and conservation status of tree endemic beetle species: Ironclade beetle (*Tarphius floresensis*) on Flores island, Laurocho (*Pseudanchomenus aptinoides*) on Pico Island, and Ground beetle (*Trechus terrabravensis*) on Terceira Island. These species' populations have been declining and they are now classified as endangered (*T. terrabravensis*) and critically endangered (*T. floresensis* and *P. aptinoides*). In order to reverse this trend and mitigate some of the threats to these beetles (fragmentation of habitat, invasive plant species, changes in land use and climate change), the project has been working since 2020 on different actions: i) Improvement of habitat by removing invasive plant species; ii) Re-naturalization of planted *Eucalyptus sp* forests; iii) Reconversion of pastureland to natural forest; iv) Creation of green corridors using nature based solutions; and IV) Environmental awareness, through land stewardship, volunteer works and

environmental education actions for schools and general public.So far, the project has intervened in more than 35 ha controlling invasive plant species, planted 3160 endemic species, removed 5 ha of eucalyptus forest, promoted about 25 volunteering actions and general public activities reaching 450 people, and performed 76 school actions, reaching 1217 students.With this project we hope to increase the awareness for the conservation of endemic beetle species, and their importance towards a sustainable future.

**Keywords:** beetles, endemic species, conservation, environmental awareness

## OC298. Drivers and Repercussions of UK Insect Declines (DRUID): Updating a 30-year dataset to a 48-year dataset alters trend direction

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The Rothamsted suction-trap network has run a number of 12.2m traps around the UK since 1964. The traps monitor aphid migration, with daily samples of bycatch stored since 1974. Previous work developing a long-term dataset on aerial insect biomass at four UK sites from 1974-2002 (Shortall *et al.* (2009) Insect Conservation and Diversity, 2: 251-260) found a significant decline at one of the sites – mainly driven by the decline of one species *Dilophus febrilis* (L.), with the other three showing no significant trend. We show that updating this dataset to 2020 for three of the sites results in the previously declining site now showing no significant change while one site now shows a significant increase in biomass. *Dilophus febrilis* shows no change in abundance over the post-1990 period indicating it does not have a role in more recent changes in biomass. We report the taxonomic breakdown of the samples post-1990 and investigate potential drivers for these changes.

**Keywords**: biomass, long-term monitoring, suction-trap, Diptera, NE/V00686X/1

## OC299. CZ-SK SOUTH LIFE: Optimalization of Natura 2000 sites management delivery in the South Bohemia Region and the territory of South Slovakia (LIFE16 NAT/CZ/000001)

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Ongoing threats to habitats and insect species in 55 Natura 2000 networks sites targeted by this project include the abandonment of traditionally managed pastures and meadows and their overgrowth with dense vegetation; the spread of invasive species; changes to water regimes in wetland and peat bog habitats caused by drainage, the intensive forest management and the removal of old trees. This transboundary LIFE project (CZ-SK) has been improving the conservation status of EU priority woodland and grassland habitats to benefit the priority species *Osmoderma eremita* ond of EU priority raised bog and bog woodland habitats for *Carabus menetriesi pacholei* through actions to improve the hydrological function of bog habitats, to restore mowing and grazing regimes on grasslands, to treat old cavity trees and restore pollarding, to plant tree species, and remove invasive species. An important part of the project is also raising the awareness regarding of the public and stakeholders by the organization of community engagements, presentations, excursions, workshops, etc.

**Keywords:** LIFE, Natura 2000, *Osmoderma eremita*, *Carabus menetriesi pacholei*

## OC300. Citizens Science campaign – new approach to knowledge in forgotten beetle

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In 2019, the organisers of the Citizen Science campaign "Krešo the Firefly", with the help ofthe media, called on citizens to find out if there really is a decline in fireflies in Croatia. Reports of fireflies, including pictures and videos, were collected from all over Croatia via emails, Facebook and Instagram pages. The campaign relied heavily on the use of media (radio, print, and online) to promote the campaign. The media was used to educate citizens about firefly biology and data collection. Citizen scientists reported the occurrence of fireflies during the spring and summer of 2019-2021. In total, over 1700 reports were collected and processed from all over Croatia during the campaign and over 400 photos and videos were analysed. During 2022, a detailed analysis of the data from the project was conducted and four species of fireflies were identified based on the colour of the emitting light and the appearance of the luminescent organ. These species are: *Lampyris noctiluca, Lampyris germariensis, Lamprohiza splendidula, Luciola italica*. Their morphological characteristics and identification features will be presented at the symposium, as well as their spatial distribution and occurrence. Due to the general decline of firefly populations in recent years, this project represents a valuable source of information for the scientific community, especially for the field of entomology, conservation biology and ecology. Future work will build on the success of the Krešo the Firefly project and move to formal scientific surveys of identified species and population genetic analyses.

Keywords: lampiridae, citizen science, luminescence, light pattern

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## 0C301. Climate Change Impact on Damselfly-parasite Interactions

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Climate change is one of the major causes of biodiversity loss. Insects are particularly vulnerable to climate change because of their short life span, ectothermic nature and dependencies on diverse habitats and other organisms. One-way in which climate change can cause or contribute to extinction is by facilitating the spread of diseases and modifying host-pathogen interactions in a way that is detrimental to hosts. Therefore, understanding how climate impacts host-pathogen interactions is crucial. Here, we studied latitudinal variation of parasitism in damselflies to determine how climatic factors impacts host-parasites interactions. We studied seasonal variation of parasite prevalence (i.e., the proportion of damselflies parasitised), and parasite intensity (i.e., the number of parasites per infected damselfly) across Germany using *Coenagrion puella* as a study system and across Australia Australia in *Ischnura heterosticta* damselflies. We found that parasitism varies across latitudes and there

is a positive correlation between climatic factors and parasitism across latitudes. Our study suggests that parasitism is most likely to increase due to global warming, which will decrease insects' fitness and contribute to global insect decline.

**Keywords:** global warming, host-pathogen interactions, extinction risk, diseases ecology, Odonata

### OC302. Small-scale richness of pollinator species in a threatening world. Lessons from LIFE PollinAction

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The conservation of pollinators in agricultural landscapes is threatened by several factors, including landscape simplification, loss of native plant species, pesticide use, and introduction of invasive species. Even former hotspot communities for pollinators typical of agricultural landscapes, such as extensively managed grassland communities, have been increasingly simplified by more intensive agricultural practises or their abandonment. Understanding the relative contribution of all these factors to small-scale pollinator richness is therefore important for developing effective conservation and restoration measures.

In this study, we investigated which factors most influence small-scale richness of wild pollinator species in managed grassland communities in the eastern Po Valley, one of the most human-modified landscapes in Europe. We selected 16 grasslands differing in area, management intensity, richness of animal-pollinated plants, abundance of flowers of native and alien species and presence of beehives, and investigated their influence on small-scale richness of wild pollinator species.

Small-scale richness of wild pollinator species showed an humpshaped relationship with the abundance of flowers of native plants and a negative relationship with the average number of honeybee visits per flower. The results highlight the importance of the presence and abundance of native plants for the conservation of wild pollinators, which should therefore be prioritised when creating or improving habitats for pollinators. They also highlight the threat posed by beekeeping. The foraging behaviour of honeybees and the competitive interactions they develop with wild pollinator species should be considered in pollinator conservation, which requires an urgent solution to reconcile beekeeping and wild pollinator conservation.

**Keywords:** honeybees, wild pollinators, pollination, pollinator conservation

## OC303. Promoting pollinator protection among farmers in the LIFE4pollinator project

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Pollinators are threatened by various adversities such as land use change, pollution and climate change, but the agricultural environment is undoubtedly the one where they face the greatest threats; this is mainly due to intensive farming and spread of monocultures, and to the use of pesticides.
LIFE4Pollinators (LIFE18/GIE/IT000755) is a *Governance and Information* project aiming at spreading knowledge on the importance of pollinators and promoting good practices for their conservation. The actions are addressed to different categories of stakeholders, but a specific "pilot project" has been dedicated to agricultural sector, to drive farmers towards a pollinator friendly farm management.
The pilot project is performed in one Italian region (Emilia Romagna) and involves the following actions:

- training on pollinator-friendly farm management, addressed to farmers and technicians of agricultural organizations;
- distribution of a technical manual on pollinator-friendly farm management;
- definition of pollinator-friendly practices to be proposed to the regional administration for inclusion in the guidelines for integrated agriculture;
- promotion of a "Code of conduct" containing indications on pollinator-friendly practices, the application of which allows to obtain a certificate of "pollinator-friendly farm";
- development of an indicator based on the diversity of bees ("bee-indicator") for evaluating the effectiveness of the project actions and of the CAP green measure and eco-schemes.

All these actions carried out in collaboration between researchers, farmers, technicians and institutions, should lead to the application of pollinator-friendly measures in line with the CAP objectives and meeting the interests of all the stakeholders involved.

**Keywords**: pollinator-friendly measures, CAP, agriculture

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## OC304. Terrestrial islands: how habitat fragmentation shapes wild bee diversity and threatens the conservation of our most important pollinators

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A major factor of bee decline is anthropogenic habitat loss, resulting in habitat fragmentation, the isolation of populations, and subsequent regional or global extinctions. A comprehensive understanding of the driving factors of wild bee community structure in combination with intensive, structured surveys, especially in under-investigated regions of Europe is essential for current and future conservation of wild bees. Therefore, the primary objective of our project is to assess the effect of fragmentation and

isolation on wild bee communities along a gradient of potential suitable habitat. We aim to integrate measures of species and functional diversity, community structure, and co-occurrence patterns, in order to better characterise how wild bee communities are affected by isolation in anthropogenic landscapes. To achieve this goal, we surveyed wild bee communities on 32 locations in two major cities and in large-scale agricultural fields in Czechia from April to August 2022 using standardised field methods. The 16,000 collected specimens were identified to species level and assigned a functional niche based on relevant traits. The results show that the amount of suitable habitat surrounding a location affects both species richness and functional diversity and is an important factor in local wild bee community structure. Future work on the genotypic diversity of these specimens, as well as fine-scale landscape connectivity measures will be integrated to more fully assess the effects of fragmentation on wild bee community structure, specifically for conservation purposes.

**Keywords:** biodiversity, bees, fragmentation, habitat, pollination, community

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### OC305. Supporting biodiversity restoration in an urban environment: Uniting insects and citizens

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Recent studies reveal that insect abundance and diversity are decreasing worldwide. Various anthropogenic factors are implicated in these declines, including urbanization and attendant habitat loss. This led us to re-design urban green space to broadly facilitate insects. At the same time, we aimed to involve citizens in implementation, management and monitoring of this green space to increase nature connectedness, which is considered an important factor in supporting biodiversity. We present our City Oasis concept, based on mini-biotopes and insect-plant relationships. We also present our first insights into insect monitoring, using a vacuum-sampling technique to collect insects from vegetation. This method proved to be highly effective in sampling insects that are often overlooked, yet have important ecosystem functions, including aphids. Our findings confirm that insect abundance can be boosted in a densely population urban environment by increasing vegetation diversity.

**Keywords:** biodiversity, plant-insect relationships, citizen science

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### OC306. Grand challenges in entomology: priorities and actions for the future

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Both entomology and the global scientific community are faced with a gripping number of challenges affecting how people and insects survive and thrives in a changing world: climate change, increasing vector-borne disease, how to sustainably feed a growing world's population, and the causes and effect of increasing biodiversity loss. Through an inclusive and intensive consultation of its members, the Royal Entomological Society has proposed a set of grand challenges to address the intersection of insect and human suffering, but to influence policy change and public engagement at a national and international scale, a balance needs to be struck between conveying the real threats posed by inaction with stories of success which highlight the hope and inspiration that can be provided by insect scientists. Global collaboration between entomologists and the entomological societies will be key for addressing the challenges ahead.

**Keywords:** challenges, society, policy, biodiversity, collaboration

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## OC482. Ant community dynamics on small Corsican islands over a 37-year period

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MacArthur & Wilson (1967)'s dynamic equilibrium theory states that immigration rate is equal to extinctions, resulting in a steady species richness and a turnover in the composition of fragmented communities over time. However, the equilibrium theory is today questioned and there is an urge to define a new paradigm and to assess dataset with various temporal and spatial scales. The aim of this study is to assess ant community dynamics on small Corsican islands over 37 years. Ants, considered as ecosystem engineers, are great biological models as they are ubiquitous on islands and easy to sample. Moreover, no study, that we know of, used these organisms over such a long period of time. Ants will be surveyed during spring 2023 on 45 small islands around the main island of Corsica. Islands range between 0.01-ha and 22-ha and will be prospected randomly using hand-collecting method similarly to the 37-year ago sampling. Effects of time and island spatial characteristics on species richness and community composition will be assessed. Previous studies realized by Morrison (1998, 2002, 2010) showed a low turnover rate in archipelagos of the Bahamas over a 17-year period, despite the high hurricane activity over the archipelago. As social insects, ants have many ecological characteristics explaining that they experience relatively low turnovers. Thus, we expect that species turnover will still be low even if the study period is twice as long, and that smaller and more isolated islands will be more prone to extinctions. Results will be further described and analyzed.

**Keywords:** islands, ants, equilibrium theory, Corsica, turnover

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# Session 12:
## Social Insects and Apidology

## OC307. Unveiling the use of Precision Apiculture Systems Usage by European beekeepers

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In the context of growing ecological concerns surrounding honey bee colony mortality, Precision Apiculture Systems (PAS) emerge as promising monitoring tools to safeguard bee health and improve beekeeping practices. These systems offer critical insights into colony dynamics, aiding decision-making processes in beekeeping. Despite a surge in relevant academic research, knowledge on actual PAS usage and preference among beekeepers remains limited. To fill this gap, a standardized survey was conducted amongst beekeepers in France, Germany, and Greece. This survey aimed to determine the extent of current and potential PAS adoption, the motivations behind usage or avoidance, and ascertain future preferences. Among the 538 respondents, 45% reported utilizing some form of PAS, with beekeeping scales being the most commonly used. The study revealed a positive correlation between the use of PAS and a) the number of hives managed, as well as b) with the distance of the apiary from the place of residence. 90% of respondents expressed satisfaction with their use of PAS, although the high costs of these tools was identified as a primary obstacle to broader adoption. About 48% of the beekeepers preferred non-invasive systems with external sensors, highlighting a clear demand for health-related colony information. The advantages fostering PAS usage included support in decision-making and improved efficiency in monitoring colony growth and honey productivity. These findings can assist in making PAS more accessible to beekeepers, with the aim to enhance colony performance and decision-making capabilities, thus addressing honey bee colony mortality.

**Keywords:** colony monitoring, survey, honey bee, Information and Communication Technologies, Precision Apiculture Systems

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## OC308. Climatic conditions, geographic features, land cover, genetic background, and profile of beekeeping operations contribute to honey bee winter losses

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Honey bee winter losses are a significant concern for beekeepers and the agricultural industry worldwide, and they could have severe consequences for ecosystem services and food production. Colony losses have been previously related to parasites, pathogens, pesticides and poor nutrition (the 4 P's). Although those contributing factors are spatially widespread and consistently prevalent over the years, winter losses show significant variation and heterogeneity across time and space. Moreover, the relationship between winter losses and various factors, such as yearly weather, long-term climate, deviations from climatic averages, geography, land use patterns, genetic background or hybridization status of native colonies, and the profile of beekeeping operations remains poorly understood. Here, we surveyed 392 stationary beekeepers using only the local stocks coming from a background of 5 native subspecies in Türkiye (*Apis mellifera anatoliaca*, *A. m. caucasica*, *A. m. syriaca*, *A. m. meda* from the O-lineage, and the C-lineage ecotype from Thrace). By utilizing generalized linear mixed models fitted to observed winter losses for 7 years since 2016, we show that those factors influence losses to a certain extent with varying weights. We also make predictions on the short, medium and long-term impact of climate change (from 2030 to 2090) on winter losses under various Shared Socioeconomic Pathways scenarios. Our findings have significant implications for beekeepers and policymakers around the world in developing strategies to reduce honey bee winter losses and ensure the sustainability of honey bee populations.

**Keywords:** honey bee winter losses, beekeeping operation profiles, genetics, land cover, weather and climate change, sustainability

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## OC309. Nutrition of the honey bee parasite *Varroa destructor*, from behavioural observations to molecular considerations

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*Varroa destructor* is one of the major threats to the Western honey bee *Apis mellifera*, causing colony weakening in many countries. Relying mostly on olfactive cues, the mite is able to detect suitable host stages to pierce their cuticle and feed on their body fluids. Yet, in artificial environments with few or no odours coming from the bee, *V. destructor* is still capable of finding a synthetic food source. In our study, we explored the behaviour of mites in these artificial control environments. We demonstrated that even deprived from olfactive information, the mite assesses the shape of its substrate to find a proper feeding site. A 3D rounded shape has an arrestant effect on the mite, probably because it is close to the shape of larvae or pupae. Based on these observations, we developed a feeding device using 3D tube-shaped Parafilm dummies. When honey bee larval hemolymph is included into the dummy, the parasite feeds and survives for weeks. Even once cleared from its cells, larval hemolymph contains all the essential nutrients for *V. destructor* survival. Proteomic analyses ran on these hemolymph samples allowed the identification of crucial candidate proteins required in the mite's physiology. Altogether, our result shed light on the foraging and nutritional requirements of this major honey bee parasite. Further analyses are still needed to explore the link between nutrition and other important biological functions such as reproduction.

**Keywords**: *Varroa destructor*, honey bee, feeding, shape, hemolymph, nutrients

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## OC311. Effects of lactic acid on *Varroa destructor* grip skill: fitness cost under artificial conditions

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*Varroa destructor* is one of the major parasites threatening the eusocial species *Apis mellifera* if not managed. Yet, sustainable treatments are rare due to the difficulty to impact the mite without disturbing the host. One current semiochemical used by beekeepers is lactic acid, although its way of action on the parasite remains poorly understood. We undertook the first investigation about lactic acid on *V. destructor* at the individual level under artificial conditions. We found that lactic acid does not kill *V. destructor* but rather interfere with its attachment skills. In particular, we focused on its arolia, mite's soft pads, and highlighted shape and fluid modifications induced by lactic acid. We also analysed the fitness cost of impaired attachment due to lactic acid on mites by assessing a reduced locomotion but not nutrition, as well as a potential lower reproduction. Even if conducted under artificial conditions, our results significantly improve our understanding of lactic acid effect on the parasite's biological abilities. They also provide new perspectives to apply differently this semiochemical in the field against *V. destructor*.

**Keywords:** parasite, organic acid, host attachment, bees' pest, *Varroa* mite, behaviour

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## OC312. Evaluation of the efficacy of essential oils against *Varroa destructor* on *Apis mellifera macedonica*

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The improper use of synthetic acaricides has led to the differentiation of resistant varroa populations. With the purpose of developing alternative and environmentally friendly techniques to control *Varroa destructor*, a study on the biological activity of *Geranium robertianum* L., *Melaleuca leucadendron* L. var. *cajeputi* R. and *Lavandula officinalis* Chaix essential oils (EOs) was carried out. The study, conducted at the Institute of Apiculture in Nea Moudania (Greece), included *in vitro* tests on varroa adults and larvae of *Apis mellifera macedonica* (Ruttner), cage trials on adult bees, and field trials on *V. destructor*. In the *in vitro* tests, 10 varroa and 10 *A. m. macedonica* larvae (5 replicates) were used and the EOs were

tested at 1.5; 2.0; 2.5; 3.25; 5.0µL/127.17 mL Petri dish. The tests indicated that the maximum mortality of the mites was observed, at different concentrations of the three oils, 21h after their administration. Oxalic acid (OA) induced the highest honeybee larval mortality. The cage trials demonstrated that EOs induced an adult bees mortality not different from that recorded in controls. For the field trials, 20 hives were utilised (5 per EO and 5 mineral oil) and EOs were tested as a 4 ml mineral oil:EO solution (1:1). Results revealed that EOs solutions induced a significantly higher mean mortality of mite than that obtained in the control and comparable to that of OA. Similar results were obtained controlling *V. destructor* on *A. m. ligustica* Spinola. EOs could be an effective and sustainable tool to control *V. destructor*.

**Keywords**: toxicity test, LD50, geranium, lavender, cajeput

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## OC313. Challenges facing beekeeping in Europe through the lens of stakeholders and beekeepers

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Challenges facing beekeeping in Europe have been studied through the lens of stakeholders and beekeepers within the B-GOOD project (EU H2020, No. 817622, 2019-2023, b-good-project.eu). This contribution presents the main findings, conclusions and recommendations from the socio-economic research activities of B-GOOD. Using a mixed methods approach, a SWOT-analysis was conducted and insights were gained into the role of climate change, the diversity among beekeepers, and their interest in digital hive monitoring technology. Data was collected through stakeholder interviews (n=41) and Europe-wide surveys of stakeholders (n=504) and beekeepers (n=844). First, SWOT-analysis identified five strengths, five weaknesses, nine opportunities and nine threats for beekeeping in Europe. Their combination resulted in 18 attention points for policy development. Second, the role and perceived impact of climate change was examined, as it emerged as an external SWOT-element that can be perceived either as a threat or an opportunity, depending on the context. A multinomial logistic regression analysis using the beekeeper survey data revealed significant regional differences in perceived climate change impacts with southern European beekeepers experiencing a 10-fold likelihood of being heavily impacted compared to northern European beekeepers. Third, the diversity among beekeepers was explored through two-step cluster analysis using beekeepers' affective/natural orientation versus utility/business orientation towards honeybees and beekeeping as segmentation variables. This analysis resulted in five types of beekeepers profiled in terms of personal, operational and performance characteristics, such as management practices, honey yield and winter colony loss. Finally, stakeholder views and beekeepers' interest in adopting digital monitoring and its determinants were analysed.

**Keywords:** apiculture, climate change, precision beekeeping, segmentation, survey, SWOT

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# OC314. Optimizing Italian honey bee (*Apis mellifera ligustica* Spin.) density in caged laboratory trials

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Laboratory trials (LT) with honey bee (*Apis mellifera* L., HB) can avoid the environmental factors affecting the *in-field* trials. Among the experimental conditions, for practical and scientifical reasons the HBs' density (HBD) per unity of cage volume can be considered relevant in LTs. To try optimizing the HBD in LTs, a test under controlled conditions (34.3 ± 1.0 C°; 72.0 ± 1.5 % RH) with increased HBD was performed. A four bees' densities trial, in triplicate, was set up with 20 (D1), 30 (D2), 40 (D3) and 50 (D4) 0-24h post-emergence *A. m. ligustica* Spin. workers (11.9, 17.8, 23.8, 29.7 bees/100 $cm^3$) by using 168 $cm^3$ plastic cages. Bees were supplied *ad libitum* with 50% w/w sugar syrup (SS50), tap water and Haydak solid diet. Daily, for 15 days, deceased HBs were recorded, and daily individual consumption of water and SS50 (µg/HB) as well as solid diet intake (mg DM/HB) were calculated. D2 showed the lowest ($p<0.001$) mean mortality (19.9 ± 3.9 %) compared to other densities; in addition, it showed the slowest ($p<0.01$) daily mortality rate if compared to D3 and D4. The D2 individual consumption of SS50 was lower (15.0 ± 6.7 µg/HB) compared to D3 ($p<0.05$) only. The individual consumption of Haydak diet was density-dependent, with a significant difference ($p<0.01$) between D4 and D1. D2 and D4 consumption of water results lower ($p<0.01$) than D1 and D3. Our test suggests, also for the easier management, that D2 (17,8 bee/100 $cm^3$) can be the optimal density for LTs.

**Keywords:** caged honey bees, crowding, artificial nutrition, protein diet, bees' mortality

## OC315. The hidden diversity of Asian honey bees

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The currently recognized species of cavity-nesting honey bees are the Asian *Apis cerana*, *A. koschevnikovi*, *A. nuluensis* and *A. nigrocincta*, and the western *A. mellifera*. Our phylogeographic study of all the cavity-nesting species (except *A. nuluensis*) using genomic Single Nucleotide Polymorphisms (SNPs) showed that (1) *A. mellifera* is sister to the Asian cavity nesting species; (2) *A. koschevnikovi* is sister to the other Asian cavity-nesting species; (3) *A. nigrocincta* and "*A. cerana*" from the Philippines are reciprocally monophyletic sister groups (each other's closest relatives); (4) both mtDNA and genomic data show that the "black hill" and "yellow plains" *A. cerana* of India are genetically distinct and not each other's closest relatives; (5) the yellow Indian bees are a distinct lineage, sister to the *A. cerana* of Mainland Asia (including the black Indian bees) and Sundaland, which includes Indonesia, Malaysia and the southern part of Thailand. Our data also indicate that (6) the Mainland and Sundaland *A. cerana* populations are each other's closest relatives, and that they too may be genetically distinct from one another. We propose that the Philippine cavity-nesting bees, the yellow plains bees of India and Sri Lanka, and possibly the Sundaland *A. cerana* populations are distinct species. While this is interesting from the standpoint of the evolution and phylogeny of honey bees, these results also contribute to understanding the evolution and variation of behavioral and physiological traits such as host-parasite relationships, nest defense and critical thermal limits.

Keywords : *Apis*, phylogeography, critical thermal limits, nest defense, *Varroa*

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## OC317. The colony environment and brood care interact to influence circadian brain gene expression in *Apis mellifera* and *Bombus terrestris*

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Circadian rhythms are important for timing many biological processes. However, some animals naturally switch between activity with or without circadian rhythms, and the underlying mechanisms are unknown. Social insects provide an attractive model system for studying natural plasticity in circadian rhythms because the presence of circadian rhythms is influenced by task performance and the social environment. We tested the influence of social contexts on brain circadian gene expression in two highly social bee species with different levels of social complexity, the honey bee *Apis mellifera* and the bumble bee *Bombus terrestris*. Whole-brain mRNA was collected in time-series from four groups of bees: foragers (colony, no brood care), nurses (colony and brood care), solitary bees (no colony or brood care), and solitary bees with brood (brood care, no colony environment) to isolate the effects of brood care and colony environment on circadianly expressed genes (CEGs). As expected, we found significantly more CEGs in foragers compared to the other groups, but surprisingly, the solitary with brood group had the second highest number of CEGs in both species. Additionally, in both species, we found significantly more CEGs in colony-reared bees than in solitary bees. These trends, along with further differences in CEG numbers and phases between groups suggest that the colony environment increases circadian gene expression while caring for brood may decrease some circadian processes, while increasing others in *B. terrestris* but not *A. mellifera*.

**Keywords:** circadian rhythm, division of labor, social synchronization, non-photic entrainment

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## OC318. Ontogeny of superorganisms: Social control of queen specialization in ants

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The functioning of biological systems relies on the cooperation of specialized components. Understanding the processes that produce such specialization - the ontogeny of biological systems - is a major challenge in biology. Although biological systems exist at multiple phenotypic scales, most studies of their ontogeny have focused on multicellular organisms. In my research group, we aim to expand our understanding of the ontogeny of biological systems to insect societies (i.e., superorganisms). We use the black garden ant (*Lasius niger*) as a primary study system to investigate how founding queens, the earliest developmental stage of ant colonies, transition from expressing a diverse repertoire of behaviors to being strictly specialized in egg production. We demonstrated that the presence of workers is sufficient and necessary to inhibit the behavioral pluripotency, initiate the specialization of queens, and thus establish division of labor. We showed that this specialization is reversible, as queens revert to expressing behavioral pluripotency upon removal of their workers, even after having been specialized for several years. We found a similar social control of queen behavior in other species, suggesting that it may be common in ants and regulated by ancestral mechanisms. Finally, we are in the process of investigating the epigenetic, transcriptomic, and hormonal bases of the social control of queen specialization. By investigating a prevalent, yet underappreciated flexibility within the queen caste, our research may improve our understanding of division of labor in insect societies.

**Keywords:** social insects, division of labor, colony foundation, brood care

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## OC319. Royal protein signature in termite:chemical profiling and behavioral bioassays

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Social communication in insects mainly involved semiochemicals like cuticular hydrocarbons and volatile mediators. Proteins are also known to be involved in social interactions but mostly in mammals and aquatic species with only very few descriptions in insects and scarce experimental demonstrations. In terrestrial environments, proteins are usually deposited on substrate, which limits their dispersion and therefore their perception in open areas. Consequently, proteins are believed not to fit with the communication systems of insects. Yet, eusocial insects are frequently in contact with each other usually in closed structured habitats. Sexe and caste variations have been identified in a wasp, the honeybee and termites suggesting a potential implication of the proteins in caste and reproductive signaling.Hence, we explored the diversity of the cuticular protein compounds and tested the behavioral response of the subterranean termite *Reticulitermes flavipes* with polar extracts. Characterizations of the cuticular protein compounds were done using MALDI-TOF mass spectrometry on reproductives and workers of both sexes. Analyses revealed a rich protein diversity in reproductives compared to workers and a sex-specific composition exclusive to reproductives. Cuticular polar extracts of reproductives and workers were tested in behavioral bioassays by measuring elicited body-shaking events of workers. Body-shaking is a vibratory behavior involved in reproductives recognition in subterranean termites. Only extracts of reproductives trigger body-shaking (in the same way as the presence of reproductives does). These results show that royal polar extracts are involved in social interactions, opening new perspectives on the function of proteins in insects' communication.

Keywords: social insects, reproductive recognition, protein profiling, vibratory behavior, mass spectrometry, termite

## OC320. Ecological drivers of sociality in *Xyleborinus saxesenii*, a widely distributed ambrosia beetle

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Altruism evolves when indirect fitness benefits are high enough. Ecological factors are important to explain the evolution of social life. For ambrosia beetles, the nest is the most valuable resource: it provides food, a stable environment and protection against parasites and predators. Tunneling into wood may be the key innovation leading to the evolution of sociality in ambrosia beetles.

The cooperatively breeding ambrosia beetle *X. saxesenii* is an ecologically generalist, it colonizes several species of host plants. While it is able to colonize a broad range of hosts, its performance differences across these hosts are unknown. Generalist should have low peak performance and a large performance breadth, but performance trade-offs can be difficult to detect because they involve different dimensions. In ambrosia beetles it is particularly interesting whether different hosts influence fitness through effects on the beetle's social farming behavior and productivity of their fungal symbionts. We investigated how the host-plant species impacts the social behaviour and life-history of *X. saxesenii.* We also investigated the impact of the host-plant on the symbiont community.Our results show that *X. saxesenii* follows a true generalist strategy, performing equally in different host-plants. We found no evidence for trade-offs between different life-history traits, suggesting that this species only uses one biological strategy regardless of the specific conditions the individuals encounter. We posit that such a generalist species has the potential for several speciation events, each one giving birth to a specialized species. Detailed effects on social behaviors and symbiont communities will be discussed too.

**Keywords:** cooperative breeder, evolution of sociality, ecological factors, ambrosia beetle, *Xyleborinus saxesenii*, symbiont community

## OC321. The effects of juvenile hormone on long and short-term memory in a social bumble bee (*Bombus terrestris)*

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Juvenile hormone (JH) is a gonadotropin regulating fertility and behavior in the bumble bee *Bombus terrestris*. Our recent transcriptomic analysis suggests that it mediates a physiological reproduction/maintenance tradeoff. JH upregulates the expression of genes associated with high metabolic activity in the fat body, whereas in the brain it down-regulates translation related processes, and up-regulates lysosome biogenesis and autophagy. Given that long-term memories (LTM) require protein biosynthesis, we hypothesized that bees with high JH levels and active ovaries will show impaired long- but not short-term memory . In order to address this hypothesis we first established and validated a new Y-maze based visual learning and memory system. We will present our system as well as results testing the influence of JH-III supplementation on long- and short-term memory.

**Keywords:** *Bombus terrestris*, juvenile hormone, translation, long-term memory, Y-maze

## OC322. Solitary bees (*Osmia* spp.) as model organisms in ecotoxicology studies

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In agricultural environments, bees are simultaneously exposed to a variety of chemicals at low concentrations. Usually, the effects of these low multi-residue exposure are sublethal, and therefore are not easily detected in the field. Most information on the sublethal effects of pesticides on bees comes from studies with the western honey bee, *Apis mellifera*, a highly social species. However, most bee species are solitary and have life history traits that widely differ from those of honey bees. For example, solitary bees lack colony resilience and are known to be more sensitive than honey bees to certain pesticides. For these reasons, the European Food Safety Authority recommends the inclusion of solitary bees (*Osmia* spp.) in risk assessment schemes. The life history traits of *Osmia* spp. pose a number of challenges, and at the same time offer some opportunities, that need to be addressed to develop appropriate methods to detect sublethal effects in these species. This presentation will provide an overview of ongoing ecotoxicological studies with *O. cornuta*, *O. bicornis* and *O. tricornis* at our laboratory. We will describe and validate several laboratory methods and demonstrate their ecological relevance through field studies analysing nesting performance and reproductive success. We will also demonstrate the use of these methodologies in studies addressing the combined effects of pesticide exposure and other stressors, such as climate change and nutritional deficits.

**Keywords:** mason bees, ecotoxicology, sublethal effects, pesticide exposure, climate change, nutrition

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## OC323. Pesticide loads in food provisions of the solitary bee *Osmia cornuta*: effects of landscape composition and management practices

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Bees in agricultural landscapes are exposed to a variety of pesticides. To mitigate the impact of this multiple exposure on bee populations we need to understand how local and landscape factors affect environmental pesticide load and composition. In this study, we analyzed the pesticide contents of pollen-nectar provisions of the solitary bee *Osmia cornuta* in fourteen apple farms in northeastern Spain. We collected provisions from nests produced in the first and second halves of the flowering period, respectively, and submitted them to multi-residue analysis screening for more than 450 pesticides utilizing GC-MS/MS and LC-MS/MS analysis. We detected more than 45 active ingredients, including insecticides, fungicides and herbicides. The insecticide Flonicamid was found in all of the samples, with a maximum concentration of 2.9 ppm. The fungicides Captan and Cyprodinil were also found in all of the samples, with a maximum concentration of 79.3 and 4.2 ppm, respectively. We used the amounts of residues found in the provisions and the reference toxicity value (median lethal dose, LD50) of each compound to calculate an additive pesticide risk index for each farm. Pesticide composition and risk index were analyzed based on management practices (organic vs IPM), local non-apple floral resources and landscape composition.

**Keywords:** pesticide residues; larval exposure; pesticide risk index; apple orchards; orchard management; landscape effects

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### OC324. Multiple exposures to a neonicotinoid pesticide reduces longevity of bumblebee queens

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Although impacts of pesticides on bees are increasingly understood, most research focuses on workers Queen bumblebees can be exposed to pesticides at various life stages, but the most vulnerable time may be when they are foraging just before and after diapause (hibernation) when they lack the support of a colony. This study investigates how single (pre-diapause) or multiple (pre and post diapause) exposure to field relevant doses of a fungicide (prothioconazole), insecticide (acetamiprid) or a combination of both affects bumblebee diapause survival, nest initiation and colony development. Although fewer fungicide-exposed bees survived diapause, insecticide or a fungicide exposure pre-diapause had no detectable impacts on queens during colony initiation and development. However, multiple exposures pre- and post-diapause to an insecticide reduced queen longevity, while multiple exposures to the fungicide-insecticide combination may reduce the likelihood of nest initiation. This indicates that multiple exposures to pesticides, both individually and in combination have the potential to be harmful to bumblebee queens during their most vulnerable life stages, which is currently overlooked in pesticide research and risk assessment. As queens are crucial to colony founding and success, any impacts on this life stage could have important ramifications to bumblebee population stability and deserve further attention.

**Keywords:** queen rearing, overwintering, synergistic, antagonistic, fungicide, insecticide

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### OC325. Short-term exposure to elevated temperatures affects learning and memory in bumblebees

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Global warming has been identified as a key driver of bee declines around the world. While it is clear that elevated temperatures during the spring and summer months – the principal activity period of many bee species – is a factor in this decline, exactly how temperature affects bee survival is unknown.

In vertebrates, there is clear evidence that elevated ambient temperatures impair cognition but whether and how heat affects the cognitive abilities of invertebrates remains unclear. Cognitive skills in bees are essential for their survival as, to supply the hive with nutrition, workers must be able to learn and remember the location of the most rewarding floral resources. Here, we investigate whether temperature-related cognitive impairments could be a driver of bee declines by exploring the effect of short-term increases in ambient temperature on learning and memory in bumblebees. We found that, in comparison to bees that were tested at 25°C (a temperature that they would typically experience in summer), bees that were exposed to 32°C (a temperature that they will becoming increasingly exposed to during heatwave events) were significantly worse at forming an association between a coloured light and a sucrose reward and that their capacity to remember this association after just 1 hour was abolished. This study provides novel experimental evidence that even just a few hours of exposure to heatwave-like temperatures can severely impair the cognitive performance of insects. Such temperature-induced cognitive deficits could play an important role in explaining recent and future bee population declines.

**Keywords:** behavior, *Bombus terrestris*, cognition, global warming, proboscis extension reflex, stimulus

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## OC327. Diversity or redundancy of stingless bee resin sources in tropical forests: Resin chemistry and their spatial distribution in lowland moist dipterocarp forests

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Resin is an important tropical forest resource for the majority of stingless bees, which use resin for nest construction, physical and chemical defence, and inter-/intra-specific communication. Moreover, chemicals derived from dipterocarp resin collected by stingless bees were found to be associated with their cuticular chemical profiles and in nest material, underscoring the close association between stingless bees and resin. In Southeast Asian forests the Dipterocarpaceae are the dominant resin producers, yet their resin chemistry has been little investigated as has the overall availability of resin in primary rainforests. Previous research indicates there could be greater intra- than inter-specific differences in resin chemical profiles among dipterocarps, which hints towards redundancy in resin chemicals available within the family. Chemical redundancy in the dipterocarps, i.e. similar chemicals found across many dipterocarp species, would enable resin-generalist stingless bees to obtain necessary resin chemicals from individuals of different species. This would facilitate resin foraging in environments where encountering individuals of the same dipterocarp species is low due to high tree species diversity and low individual density. We analysed the resin chemical profiles from dipterocarps in two Smithsonian Institution Forest Dynamics Plots (Brunei Darussalam and Peninsular Malaysia), in order to assess overall resin availability and to establish if there is resin chemical redundancy among the dipterocarps. We conclude that resin chemical redundancy present in dipterocarps is a boon for stingless bees in the light of the often unpredictable and highly patchy resin availability of dipterocarp forests.

**Keywords:** stingless bee, resin, foraging, resource, dipterocarp, redundancy

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## OC328. The effect of sublethal imidacloprid and clothianidin on cognitive learning in the visual domain of honey bees

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Honeybees serve as the world's most crucial pollinators and have a monumental impact on the agricultural industry of up to $577 billion. However, their population is declining due to various factors, with pesticides being one of the most detrimental. This project investigated the effect of neonicotinoids imidacloprid and clothianidin on the positive transfer of learning in the visual domain of honeybees, a higher-order cognitive function correlated to foraging behavior. Additionally, the project identified differences between varying dosages of these insecticides. Employing a self-designed y-maze paradigm, three experiments were systematically designed including the control, imidacloprid, and clothianidin group. Experiments were conducted in a free-flying setting, simulating a real-world environment and eliminating external stressors. Each experiment consisted of training, in which bees were conditioned with the rule of delayed matching-to-sample (DMTS), followed by a positive transfer test from pattern to color stimuli. Results of the control experiment indicated that positive transfer was successfully completed, as there were no significant differences in performance between training and testing ($p=0.1453>0.05$). In imidacloprid experiments, it was concluded that medium-term memory was negatively impacted due to decreased transfer test performance ($p<0.05$), while short and long-term memory remained intact. However, clothianidin experiments impacted both training and transfer tests ($p<0.0001$). It had stronger effects than imidacloprid and it was concluded that long and likely short and medium-term memory were negatively impacted. The project emphasized the destructive effects of neonicotinoids on honeybees and stresses the need for environmental change.

**Keywords:** positive-transfer, delayed-matching-to-sample, cognitive, neonicotinoids, memory, learning

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## OC329. Paving the way for marker-assisted selection in beekeeping

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In this lecture we give an update of the progress made in the field of marker-assisted selection. In 2019 our team discovered that a model of 8 genetic variants (single nucleotide polymorphisms; SNPs) was associated with the 'suppressed mite reproduction' phenotype or SMR (Broeckx et al., 2019 in *Scientific Reports*). SMR is seen as an important lever for achieving resilience against the ectoparasitic mite *Varroa destructor*. The varroa-mite is now considered as the most important biotic threat of the honey bee. Efforts to screen colonies for SMR by microscopic examination has been hampered by several

practical obstacles and an alternative approach is required. One of these consists of using the genetic variants found as markers for the trait and making the selection depend on genotyping in a molecular lab. In the past few years our team has explored this further. The allele frequencies of the protective wild or variant type mutations was determined. And it was evaluated whether the 8 SNP model, that originally was discovered in a single hybrid colony, hold stand in the complexity of the Flemish honey bee population with its different races, beekeeping management and environmental circumstances.

**Keywords:** honey bee, selection, varroa-resistance, suppressed mite reproduction

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## OC330. Exposure to realistic levels of pesticide mixtures affect nesting performance, longevity and reproductive success in a solitary bee

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In agricultural environments, bees are routinely exposed to mixtures of pesticides at low concentrations. For the most part, this kind of exposure does not result in acute lethal effects, but we know very little about potential sublethal effects and their consequences on reproductive success and population dynamics. In this study, we orally exposed newly emerged females of the solitary bee *Osmia cornuta* to environmentally realistic levels of acetamiprid (a neonicotinoid insecticide allowed for field use in Europe) and tebuconazole (an EBI fungicide), singly and in combination. Following exposure, females were individually marked and released into field oilseed rape cages to monitor their nesting performance and assess their reproductive success. The amount of feeding solution consumed during the exposure phase was highest in bees exposed to the neonicotinoid, and lowest in bees exposed to the pesticide mixture. The nesting performance and reproductive success of bees exposed to the insecticide or the fungicide were similar to those of control bees. By contrast, bees exposed to the pesticide mixture showed lower establishment, decreased longevity and reduced fecundity, which compromised female net reproductive rate. The interaction between the insecticide and the fungicide on survival probability was synergistic. We found no evidence of any effects of the pesticide mixture on nest provisioning rate, offspring body weight or sex ratio. Our study provides insights on the mechanisms through which sublethal multi-residue exposure affects the dynamics of solitary bee populations in agricultural environments.

**Keywords:** solitary bees, pesticide mixture, realistic levels, synergistic interaction, nesting performance, reproductive success

## OC331. Evaluation of environmental stressors on the learning behaviour of honey bees

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In agricultural areas, insect learning and memory can be affected by environmental stressors, such as exposure to insecticides. The proboscis extension reflex (PER) is a widely applied technique to evaluate learning ability of the honey bee, *Apis mellifera*. In order to train honey bees and measure their learning response, it is necessary to handle insects with care and to apply the desired stimuli at the right time, according to PER protocols. Here, we provide an overview of our ongoing research activities. In laboratory conditions, specially developed PER protocol and apparatus were used to train honey bees to associate a specific odour with a sucrose reward. Bees were collected from hives located either in agricultural areas or in natural areas. Results show that, although with some exceptions, there was no difference in the PER response between hives from agricultural and natural areas. However, a certain variability was observed between the hives, suggesting that it is important to keep them in as homogeneous conditions as possible. In a second experiment, honey bees were exposed to a small dose (LD20) of a commercial formulation of the natural insecticide Spinosad, which is known to be highly toxic to honey bees. The PER protocol was used to assess possible associative learning deficits. Compared to the control group, LD20 adversely affected associative learning of treated honey bees. Our results provide relevant information on the neurotoxic effects of a natural insecticide on *A. mellifera*.

**Keywords:** *Apis mellifera*, spinosad, sublethal effects, proboscis extension reflex

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## OC332. Assessing bee exposure to pesticide residues in pollen from crop flowers, and bee collected pollen

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In an agricultural environment, where crops are treated with pesticides, bees are likely to be exposed to various chemical compounds through multiple routes and the extent to which different bee species may be affected mainly depends on the levels and type of exposure. Here, we sampled 12 sites in Ireland to quantify the presence of selected pesticide compounds in the pollen of two different crops, oilseed rape (*Brassica napus* L.) and broad bean (*Vicia faba* L.), and then compared it with the corbicular pollen of honey bees and bumble bees simultaneously foraging on those crops. Most detections originated from compounds that were not recently applied on the fields, and various detection patterns were observed for each compound. Highest mean concentrations of the total residues detected were observed in crop pollen, followed by honey bee and bumble bee collected pollen. Crop pollen was contaminated only with fungicides, honey bees collected pollen mainly with fungicides, while bumble bee pollen had more insecticide detections. The highest number of compounds and most detections were observed in bumble bee collected pollen, where all the five neonicotinoids assessed (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam) were detected, despite the European ban and no recent application on the studied fields. Several compound mixtures were identified, while azoxystrobin, boscalid and thiamethoxam was the most frequently detected combination. Our results raise concerns about potential bee exposure to multiple residues and whether honey bees are suitable surrogates for pesticide risk assessments for all bee species.

**Keywords:** pollinators, fungicides, insecticides, risk assessment, pollen contamination

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## OC333. Differences in sensitivity between Africanized *Apis mellifera and Melipona scutellaris* after exposure to fungicide pyraclostrobin

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The honey bee (*Apis mellifera*) is the main crop pollinator managed worldwide; in contrast, the stingless native species (*Melipona scutellaris*) is vital for maintaining native flora. However, the use of pesticides associated with other stressors is a threat to the populations of these bee species. Therefore, the present study aimed to evaluate the biological effects of pyraclostrobin on an individual and tissue level scale of Africanized *A. mellifera* and *M. scutellaris*. Foragers of both subspecies were collected at the Brazilian apiary during summer. The bees were kept in different experimental cages (20 bees in five replicates) in an incubator under controlled temperature and humidity. They were subjected to oral exposure for five days to three residual concentrations of fungicide pyraclostrobin [0.125, 0.025, and 0.005 ng a.i./mL]. Additionally, solvent-control and untreated-control groups were established. The parameters observed were survival rate, qualitative morphological and semi-quantitative analyses, and histochemical analyses of their midgut. The results showed reduced longevity ($P < 0.05$- *A. mellifera* and $P < 0.01$- *M. scutellaris*), an increase in the total lesions index, and a decrease in the labeling intensity of macromolecules such as proteins, neutral polysaccharides, and glycoconjugates, in the midgut of both subspecies. Extensive morphological and biochemical changes were observed in *M. scutellaris*. Due to exposure to pyraclostrobin, individuals of both species have been affected. However, as a consequence of the characteristics of the colony of these species, the adverse effects appear to be more challenging to recover in stingless bees than in honey bees.

**Keywords:** biomarkers, honey bees, midgut, morphophysiology, residual concentrations, stingless bees

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## OC334. Multi-omics approach to assess honeybee adaptation in threatening habitats

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One of the major scientific challenges for the years to come will be to find effective means for preserving pollinator populations from the many consequent threats of climate change, such as the reduction of food resources. To mitigate resource fluctuations, beekeepers have already multiplied the transhumance journeys of their hives to safeguard their bees and ensure profitable honey production. But while these multiple movements could guarantee the food security for bees and the pollination services for farmers, they expose bees to additional threats such as agro-pesticides and the spread of varroa mites. In this multiple environment field study, hives were closely monitored during transhumance journeys towards potentially threatening habitats to better understand the consequences of these habits on bee health. Notably, the ecological niches of the different habitats crossed by the bees were characterized through honey metagenomics technique. Thus, the interactions between the intrinsic hive variables were associated with the fluctuations of the forage resources and the composition of honeybee microbiota to reveal the effectiveness and the limitations of such movements for honeybee long-term preservation.

**Keywords:** foraging behaviour, plant diversity, gut microbiome, honey metagenomics, bee health

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## OC335. Climatic and geographic drivers of intra-specific turnover in honey bee subspecies compositions across space and time: insights from Gradient Forests and Generalized Dissimilarity Modeling

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Anthropogenic factors, such as queen/colony trade and migratory beekeeping practices, can significantly influence gene flow between honey bee populations and impact genetic structure in space and time. However, there is limited knowledge about how environmental features often continue to shape the distribution of native honey bees. Here we show the relative significance of geographical and climatic features on the distribution of 5 honey bee subspecies in and around Türkiye (*Apis mellifera anatoliaca*, *A. m. caucasica*, *A. m. syriaca*, *A. m. meda* from the O-lineage, and the C-lineage ecotype from Thrace). To this end, we employed Gradient Forests (GF) and Generalized Dissimilarity Modeling (GDM) to explore the patterns of intra-specific turnover across environmental gradients and quantify the relative importance of geographical and climatic factors. We sampled 691 stationary colonies across the country and two reference populations (*A. m. carnica*, *A. m. caucasica*) and genotyped them at 30 microsatellite loci. Our results identify the core zones where subspecies are found in unmixed form, the transition areas where they exchange genes, and the critical boundaries where sudden changes occur in subspecies compositions. Spatio-temporal analysis of genetic offsets using future climate projections provide essential information and valuable insights for policymakers to improve the management and protection of honey bee populations in Türkiye and other regions of the world. Overall, the study's innovative use of GF and GDM represents a significant advancement in our understanding of the factors driving intra-specific turnover and helps us better predict the impacts of climate change on biodiversity.

**Keywords:** distribution of honey bee subspecies, gene flow, anthropogenic factors, environmental features, Gradient Forests and Generalized Dissimilarity Modeling, climate change

## OC336. Biogeography and ecology of European bees

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Bees are among the most important pollinator groups. They are threatened by global changes and knowledge of their distribution and ecology at the national and continental levels is needed to implement efficient conservation actions, but this knowledge is still fragmented and/or difficult to access. As a step forward, we present here an updated list of >2,000 European bee species, including their distributional status at the national level (in the form of present, absent, regionally extinct, possibly extinct or non-native). This work was attainable by incorporating both published and unpublished data, as well as knowledge from a large set of taxonomists and ecologists in both groups. After providing the first National species lists for bees for many countries, we examine the distributional patterns of these species and designate the countries with highest-level of species richness. We also show that many species are recorded in a single European country, highlighting the importance of articulating European and national conservation strategies. Finally, we will give a global overview on the knowledge on important morphological and ecological traits. We will discuss how the data provided here can be combined with future trait and Red List data to implement research that will further advance pollinator conservation.

Keywords: pollinators, conservation, spatial distribution, species list, ecological and morphological traits, Europe

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## OC337. The use of RFID technology in the study of solitary bees- on the case of *Osmia bicornis*

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So far the radio frequency identification (RFID) technology has been successfully employed to study the nest-drifting behaviour of paper wasps and the decision-making behaviour of ants. In the case of bees RFID systems have been mainly used to examine ecology, behaviour and the effects of pesticides in honeybees and bumblebees. We assumed that applying this technology on solitary bees is feasible and can provide a lot of novel, so far unachievable information about their biology. To test our assumptions, we have designed and built-up a special nesting construction with RFID system (i.a. antennas network) for cavity nesting bee species – *Osmia bicornis*. It was set in an early spring in an area rich in bee-friendly plants. Female and male bees were tagged with individually coded special miniaturized passive RFID tags (Hitachi®, Japan).The experiment was successfully conducted, and huge amounts of data has been received from RFID system throughout the 24 hours/day, 7 days/week until the natural death of tagged *O. bicornis*. On a basis of the conducted experiment, it is possible to draw conclusions on the circadian cycle of *O. bicornis* females. Moreover this also shows that it is possible to conduct further future basic research on other species of cavity nesting bees with the usage of RFID technology. This study was

possible through implementation of the research project No. 2019/33/N/NZ8/02864, entitled: “Keeping track of solitary red mason bees flight performance using miniaturized system of radio frequency identification (RFID)“ financed by the National Science Centre, Poland.

**Keywords:** behaviour, circadian activity, mason bee, monitoring

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## OC338. Phylogeny and the evolution of *Nomada* cuckoo bees

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We reconstructed the phylogeny of the most speciose genus of cuckoo bees, genus *Nomada* Scopoli, 1770, using hundreds of species from throughout its distribution. Ultraconserved elements were used for phylogenetic reconstruction. We combined published and new data to construct robust phylogenetic estimates. Development of knowledge about species groups and subgeneric classification is overviewed. By integrating extensive information on *Nomada* host records, we present macroevolutionary patterns of host associations, transitions, and phylogenetic conservatism. Using Bayesian divergence time estimates, we assess the historical biogeography of the genus, focusing on the West Palearctic. Our results show that *Nomada* likely originated in the Eastern Mediterranean and Near Eastern region, and likely expanded its range to a near-global distribution from there. We recovered long-standing phylogenetic conservatism in the host usage of *Nomada* and provide strong statistical evidence for an ancestral host association with *Andrena* and its most recent common ancestor. However, host transitions occurred multiple times independently in the natural history of *Nomada*, and species of the genus are brood parasites in at least five genera and four different families of bees in the Old World. We systematically revise the taxonomy of the Old World *Nomada* by integrating morphological study with our well-supported phylogenetic estimates.

**Keywords:** UCE, host association, biogeography, taxonomy, behavior

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## OC339. Bee and non-bee pollinator importance for food security

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Animal-mediated pollination provides critical service for wild plant communities and agriculture. Bees are the most recognized functional group of pollinators, which provide critical pollination services with numerous benefits to human well being such as enhancing crop production. Yet bees are not the only species visiting crop flowers. While the role of bees for crop production has received a lot of attention since bee population are declining worldwide, knowledge on the role of non-bee pollinator for crop production and food security are still scattered. This talk aims at first exploring the public's knowledge of pollinator diversity beyond bees, based on a citizen science survey. Then, I will present the diversity, conservation status, and role of non-bee pollinators in 83 different crops described either as important for the global food market or of local importance. Overall, non-bee pollinators are not recognized by public and are neglected in current research. Thus, integrating non-bee pollinators into scientific studies and conservation agenda is urgently required for more sustainable agriculture and safeguarding food security for both globally and locally important crops.

**Keywords:** agriculture, biodiversity, ecosystem services, food production, pollination

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## OC340. Spatio-temporal dynamics of wild pollinator diversity and activity in Avocado orchards

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Avocado is a worldwide economically important crop, whose productivity is highly dependent on biotic pollination. European Honeybees, the globally main managed crop pollinator, fail to provide sufficient pollination services to Avocado in many cases, due to low attractivity of Avocado (some varieties) to bees, and high pollination requirements. Diversifying Avocado's pollinators may enhance the overall pollination services delivered and increase its productivity. To apply this approach, we need to understand habitat requirements of the different wild pollinators of Avocado, and manage orchards accordingly. As a first step towards this goal, we examined the pollinator communities in five Avocado orchards and their semi-natural surroundings (SNS) in central Israel. We sampled three habitats (SNS adjacent to Avocado orchard, the orchard margin and the orchard interior) over the flowering season of 2021. The main functional visitor groups were honeybees, wild bees, syrphid flies, and other flies (OF). The pollinator community in the SNS was highly diverse, and the wild bee fauna was abundant, whereas inside the orchard Avocado visitors were mainly flies, syrphids in particular. Wild bees didn't visit Avocado flowers at all. Syrphids abundance and Avocado flower visitation rate declined as the season progressed, in oppose to honeybee's increased activity along the season. OF's activity was lower than that of Syrphids and honeybee and remained steady throughout the season. Syrphids activity patterns indicate they may be adapted to the conditions characteristics of Avocado orchards; their high activity early in the season might provide complementary pollination services while honeybees prefer different floral resources.

**Keywords:** pollination, avocado, wild pollinators, syrphid, honeybee

# OC341. Ecology and management of pollination services in Korla fragrant pear in China

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Insect pollination is critically important to Korla fragrant pear (Pyrus sinkiangensis), a premium species of pear that is widely cultivated in Korla, Xinjiang, China. There is little information on the wild pollinator community and its role in pollination. We therefore conducted a four-year study to quantify pollination of Korla fragrant pear and assess the relationships between pollinator abundance, diversity and the availability of floral resources in the landscape. We also studied the contribution of honeybees and the potential effect of an artificial bee pheremone, viz. Nasonov gland pheromone, on bee abundance, flower visitation and pollination services. Pan trapping showed that the five most common wild bee species in pear orchards were *Andrena yammagishii*, *Lasioglossum pseudannulipes*, *Lasioglossum niveocinctum*, *Andrena gelriae* and *Halictus pulvereus*. These species were not often observed visiting pear flowers. Wild bee abundance was higher in mixed pear-apricot orchards than in pear monoculture orchards. We found that there is a pollination deficit of 89% when there are no beehives near the orchard, and that this deficit can be mitigated by using managed honeybees. Introducing the attractant with Nasonov gland pheromone on pear flower increased honeybee abundance, flower visitation and pollination services to pear flower. Honeybees and use of the Nasonov gland pheromone can strengthen pear pollination in this intensive agricultural system while flowering resources can enhance wild bees.

**Keywords:** pollination deficit, wild bee diversity, fruit set, landscape, flowering resource

# Session 13:

## Toxicology and pesticide resistance

## OC342. The evolution of resistance to natural and synthetic xenobiotics in the aphid *Myzus persicae*

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The green peach aphid, *Myzus persicae*, is a globally distributed highly damaging crop pest. This species has demonstrated an exceptional ability to evolve resistance to both synthetic insecticides used for control, and natural insecticides produced by certain plants as a chemical defense against insect attack. Research on the biochemical and molecular basis of resistance in *M. persicae* has uncovered at least eight genetically independent mechanisms of resistance, making it an important case study for molecular evolution in insects. Over the last decade, understanding of the genetic architecture that underpins the key adaptations that allow *M. persicae* to effectively tolerate natural and synthetic insecticides has advanced considerably. In this this talk I will present recent research on this topic and discuss the insights these studies have provided into long-standing questions of both evolutionary and applied importance.

**Keywords**: aphid, resistance, insecticide, *Myzus persicae*, genomics

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## OC343. Molecular mechanisms of resistance to complex II inhibitors in *Tetranychus urticae* populations from Türkiye

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Complex II inhibitors are one of the most recently developed acaricidal mode of action with high efficacy against every developmental stages of *Tetranychus urticae*. Although there are four compounds within this group (cyflumetofen, cyenopyrafen, pyflubumide, cyetpyrafen), only cyflumetofen is currently registered in Türkiye. Still, high levels of cyflumetofen resistance were detected only three years after the registration of cyflumetofen. In this study, a new mutation located in a conserved site of subunit B was uncovered in a cyflumetofen-resistant *T. urticae* population. The population displayed cross resistance between all complex II inhibitors with $LC_{50}$ > 5000 mg a.i./L. The novel mutation was validated establishing near isogenic lines by marker assisted backcrossing. Furthermore, the mutation was introduced into a susceptible population using CRISPR-Cas9 genome editing. The obtained data confirms that the mutation, uncovered in an unexpected region of the target-site compared to other known mutations, confers very high levels of resistance to complex II acaricides.

**Keywords:** complex II inhibitors, spider mite, acaricide resistance, target-site mutation, CRISPR-Cas9

## OC344. Chromosome-level genome assembly of *Helicoverpa assulta* (Lepidoptera: Noctuidae) provides insight into the biology of the host-plant specialist

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*Helicoverpa assulta,* commonly known as the oriental tobacco budworm, is a major agricultural pest in many parts of Asia, including Korea, China, and Japan. The larvae feed on only a few plants of the Solanaceae family, causing significant damage to the crops such as hot pepper and tobacco. While the genomes of two congeneric generalist pests, *H. armigera*, and *H. zea*, are available in GenBank, no genome assembly of the specialist (*H. assulta*) has yet been reported. Here we present the chromosome-level genome assembly of a Korean strain of *H. assulta* (Pyeongchang strain, K18), which was obtained using a combination of the Nanopore long-read (ca. 78X) and Illumina NovaSeq short-read (ca. 54X) sequencings. The total assembly spans 424.36 Mb with an N50 length of 14.54 Mb and 35.44% GC contents. The final assembly (JAQOZT000000000, 424.36Mb) consists of 62 scaffolds, of which the largest 31 scaffolds account for 98.7% of the assembly, confirming the expected number of chromosomes (n = 31). Complete BUSCOs values were 99.0% and Repeat contents were 33.01%. Based on this assembly, genome annotation predicted 18,593 protein-coding genes. In addition to the host range difference, *H. assulta* acquires insecticide resistance at a much lower rate than *H. armigera* when exposed to the same insecticide. This draft genome of *H. assaulta* will shed light on various aspects of the biology of this specialist pest, including the host-plant interactions, chemical communications, insecticide resistance, and the evolutionary relationship of the genus Helicoverpa.

**Keywords:** *Helicoverpa assulta*, genome assembly, insecticide resistance, insect-plant interactions, host-range evolution, Nanopore sequencing

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## OC345. Molecular genotyping of mutations in the GABA-gated chloride channel in populations of *Euschistus heros* in Brazil

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The Neotropical brown stink bug, *Euschistus heros*, is an important pest of several agricultural crops in Brazil. This species is widely distributed in agricultural areas, commonly attacking soybean, corn, cotton and sunflower plants, resulting in up to 30% yield loss in soybean alone if not kept under damage thresholds. Ethiprole is the most recent and new phenylpyrazole insecticide registered for the management of *E. heros* in Brazil. Phenylpyrazoles are described as non-competitive blockers of the gamma-aminobutyric acid (GABA)-gated chloride channel, which is encoded by the rdl gene, mediating the inhibition of synapses in the central nervous system. The objective of this work was to sequence and characterize the GABA-gated chloride channel gene in *E. heros*. To do this, 12 populations of *E. heros* were collected in different regions of Brazil (crop season 2022/2023). To sequence the target region, the DNA was extracted from the insect legs. Several polymorphisms were detected in the receptor, including A301S known to confer high levels of resistance to dieldrin. Ongoing studies will verify the impact of A301S on the effectiveness of different insecticides acting on the GABA receptor. It is essential to evaluate the impact of polymorphisms at the rdl gene in *E. heros* to inform insecticide resistance management (IRM) strategies. Furthermore, we conducted population studies and ethiprole susceptibility monitoring, aiming to anticipate and prevent possible cases of resistance in E. heros populations in Brazil.

Keywords: Neotropical brown bug, *Euschistus heros*, ethiprole, insect resistance management

## OC346. Combatting pesticide resistance in insects using botanical bio-synergists

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Over 600 species of pests have developed some level of resistance to pesticides, rendering many products ineffective and reducing the options for pest management. With increasing restrictions on use and withdrawal of active ingredients, together with the decline in the rate of new insecticide discoveries, options need to be explored to extend the useful life of products currently available. Plant derived compounds have been shown to interfere with the activity of enzymes that detoxify insecticides and are involved in metabolic resistance in insects. The use of such compounds will increase insecticide efficiency thereby reducing application rates and/or counteract metabolic resistance, allowing effective control to be restored. Botanicals have been tested in combination with selected plant protection products and efficacy compared to single product applications, using standard laboratory efficacy testing methods. Data shows that the efficacy of pyrethroids against the cabbage stem flea beetle (*Psylliodes chrysocephala*), which have developed resistance against this pesticide group, can be restored when used in combination with botanicals. Similarly, efficacy of Spinosad against cabbage root fly (*Delia* radicum) can be enhanced when used in combination with botanicals, reducing the amount of product required for effective control. Enzyme activity assays have also indicated that some of these botanicals interfere with the function of certain enzyme groups that detoxify insecticides, which correlates with the mortality data from the bioassays. Botanicals may counteract the metabolic resistance of some insect pests and thereby restore efficacy and/or allow the reduction of pesticide required for effective control.

Keywords: metabolic resistance, cabbage root fly, cabbage stem flea beetle, pyrethroids, enzyme inhibition

## OC347. High-resolution genetic mapping of dicofol resistance identifies the glutamate-gated chloride channel as the mite-specific target-site of diocofol, bromopropylate and chlorobenzilate

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The acaricide dicofol has been widely used in phytophagous mite control for over half a century. Today, it is still effectively used in various regions in the world to control the two-spotted spider mite *Tetranychus urticae*. Surprisingly, although dicofol is chemically related to the voltage-gated sodium channel modulator DDT, its mode of action has remained elusive. Here, by using a population-level, high resolution bulked segregant analysis (BSA), two QTL loci were identified to be significantly related with dicofol resistance. Interestingly, the identified QTLs overlap with a previous study identifying loci associated with abamectin resistance, and harbor glutamate-gated chloride channels containing the known abamectin resistance mutations G323D in *TuGluCl1* and G326E in *TuGluCl3*. The role of G326E in *TuGluCl3* in resistance against dicofol and chemically related compounds bromopropylate and chlorobenzilate was validated by functional expression in *Xenopus laevis* oocytes followed by two-electrode voltage-clamp electrophysiology. Together, this work elucidates the mode of action dicofol, bromopropylate and chlorbenzylate as mite-specific agonist of GluCL channels.

**Keywords:** *Tetranychus urticae*, dicofol, resistance, bulked segregant analysis, glutamate-gated chloride channel, electrophysiology

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## OC348. Oviposition avoidance against systemically applied imidacloprid in the Colorado potato beetle

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Rapid evolution of insecticide resistance threatens sustainable global food production. Despite employing biochemical and/or physiological mechanisms of resistance, agricultural pests can develop behavioral resistance to insecticides via choosing to feed or oviposit exclusively on insecticide-free hosts. As young offspring have relatively low mobility, oviposition preferences of female adults may play a critical role in shaping the evolutionary trajectory of pest populations. While oviposition avoidance of insecticide-treated hosts was found in different agriculture pests, it remains unclear whether female adults actively choose to occupy insecticide-free hosts. To address this question, we investigated feeding and oviposition preferences to imidacloprid in the Colorado Potato Beetle (CPB, *Leptinotarsa decemlineata* (Say)), a major potato pest. First, we performed toxicity assays on five different strains of CPB using the neonicotinoid insecticide, imidacloprid. Then we investigated feeding and oviposition preferences using behavioral choice assays. We identified a strain that chooses to feed more from the insecticide-free plants than the insecticide-treated plants. We found that the females of this strain prefer to lay more eggs on the insecticide-free plants. Identifying the genetic basis of the avoidance behavior and examining its link to imidacloprid resistance will significantly advance the relatively unexplored field of behavioral resistance to insecticides. In order to do this, we generated a mapping population by crossing five CPB strains. Then we pooled individuals with extreme phenotypes for the traits of interest and sequenced the pools. We will use the cost-effective bulked segregant analysis approach to map the genomic regions associated with avoidance and survival traits.

Keywords: oviposition, avoidance, behavior, insecticide, resistance, choice

## OC349. Using genome engineering to characterize the molecular basis of insecticide resistance in mosquitoes and agricultural pests

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For mosquito and agricultural pest control programs to succeed, it is crucial to manage the increasing problem of insecticide resistance, and this can only be done if we understand the mechanisms that underpin the trait. Although high-throughput methods have identified a considerable number of changes across the genome and transcriptome of resistant insects and new resistance mechanisms are being proposed, in most cases functional validation of their role is lacking. Even for genes and mutations that have repeatedly been associated with resistance, their relative contribution to the phenotype remains elusive. We use functional genetics (Gal4-UAS system, CRISPR) to characterize the role of genes and their mutations in insecticide resistance in mosquitoes and agricultural pests. Through dose-response bioassays we quantify the levels of resistance conferred by single resistance mechanisms, but also by their combination, which can have a synergistic effect. Some representative case studies include: A) the functional validation through CRISPR of different voltage gated sodium channel (VGSC) mutations in *Anopheles gambiae* pyrethroid resistance and investigation of their combined effect with the over-expression of detoxification enzymes, B) investigation of the contribution of putative target site resistance mutations (on the Ryanodine Receptor, the VGSC and the ABCC2 transporter, associated with resistance against diamides, indoxacarb and Bt respectively) in major lepidopteran pests, through CRISPR induced mutations in *Drosophila*. These studies provide invaluable information both for better understanding the genetic basis of resistance and for practical applications, by determining the diagnostic value of genetic markers.

**Keywords**: functional genetics, insecticide resistance mechanisms, synergism, mosquitoes, lepidoptera

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## OC350. Characterization, impact and spread of ketoenol resistance in different whitefly species with special reference to mutations in acetyl-CoA carboxylase

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The control of whitefly pest species, such as cotton whiteflies *Bemisia tabaci,* greenhouse whiteflies *Trialeurodes vaporariorum*, and more recently cabbage whiteflies *Aleyrodes proletella*, largely relies on the application of synthetic insecticides. Among them particularly tetronic and tetramic acid derivatives such as spiromesifen and spirotetramat (ketoenol insecticides), acting as insect growth regulators targeting acetyl-CoA carboxylase (ACC). Reduced efficacy against *B. tabaci* of ketoenol insecticides at recommended label rates has been previously reported. More recently we also confirmed the presence of ketoenol resistance in samples of *T. vaporariorum* and *A. proletella* collected in different European countries. Transcriptome sequencing and assembly of ACC variants from susceptible and resistant whitefly strains revealed the presence of an A2083V target-site mutation in the carboxyltransferase (CT) domain of ACC in *T. vaporariorum* and *A. proletella* as previously described for *B. tabaci*. We developed molecular genotyping assays based on pyrosequencing diagnostics for all three species to investigate the geographic spread of the resistance allele in several European countries. We also conducted different functional assays to validate the mutation, incl. the recombinant expression of different whitefly ACC variants in insect cells. Crossing experiments revealed an autosomal dominant trait, i.e., heterozygotes express a fully resistant phenotype. The studies conducted over the last years confirmed the presence of target-site resistance against ketoenols (and other insecticides as well) in field-populations of three different whitefly species. This emphasizes the importance of implementing appropriate resistance management strategies to prevent or delay the spread of resistance through global whitefly populations.

**Keywords:** ketoenol insecticides, resistance, whiteflies, acetyl-CoA carboxylase

## OC351. Gene editing in thrips species to validate the molecular mechanisms of insecticide resistance

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Species of the order Thysanoptera (thrips) are considered to be among the most harmful insect pests, and are characterized by an exceptional economic importance. The frequent use of insecticides in thrips control, and some of their biological aspects favored the emergence of resistance to most of the classes of insecticides used for their control. Despite their status, molecular mechanisms of resistance in thrips have been poorly investigated. Resistance mutations in genes encoding the target-sites of insecticides have been infrequently reported for only few target-site genes and have never been directly validated in thrips. We here present an overview of known molecular mechanisms of insecticide resistance in thrips and we refine this information analyzing public databases, providing a clear overview of the incidence and molecular mechanisms of insecticide resistance in economically important thrips species. We subsequently developed a CRISPR/Cas9 knock-in protocol that can be used to directly validate resistance mutations in *Frankliniella occidentalis.* We focused our attention on the I1017M substitution in the CHS-1 gene, that has been described as the main factor involved in resistance to CHS-1 inhibitors in many arthropod species and that has been detected in natural populations of thrips. Gene editing of I1017M in a susceptible strain allows to investigate the phenotypic strengths of the mutation, in a first step towards devising a reliable molecular diagnostic marker.

**Keywords:** Thysanoptera, resistance mutations, target-site insensitivity, benzoylureas, chitin synthase, CRISPR/Cas9

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## OC352. Management of western corn rootworm with transgenic maize: current status and future prospects

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Western corn rootworm*, Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is among the most serious pests of maize in North America and is an invasive agricultural pest in Europe. In the United States, one of the primary tools used to manage western corn rootworm is transgenic maize that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt). The greatest challenge for the use of Bt maize in the management of this pest has been the evolution of Bt resistance. Approaches for delaying the evolution of Bt resistance by western corn rootworm have focused on the use of non-Bt refuges and the cultivation of Bt maize that produces a pyramid of multiple toxins that target this pest. In some regions of the United States, western corn rootworm have undergone sequential evolution of resistance to multiple Bt toxins in transgenic maize, which has compromised the extent to which Bt maize can reduce feeding injury. One important factor facilitating resistance evolution in western corn rootworm appears to be the non-recessive inheritance of resistance to Bt maize. Lessons learned from these cases of Bt resistance can be used to improve the sustainability of future varieties of transgenic maize that target western corn rootworm, and more broadly, the use of Bt crops for the management of other agricultural pests. Future management of western corn rootworm could be improved by increasing the planting of non-Bt refuges, using more diversified approaches for management, and developing Bt varieties that contain novel pyramids of transgenic insecticidal traits.

**Keywords**: agriculture, evolution, insect resistance management, transgenic crops

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## OC353. Characterization of resistance to *Bacillus thuringiensis* (Bt) Cry1A and Vip3A insecticidal proteins in Brazilian strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a highly-destructive moth pest native to the tropical and subtropical Americas that preferably feeds on corn plants. FAW pest management strategies largely rely on the use of synthetic insecticides and transgenic crops, e.g., corn expressing *Bacillus thuringiensis* (Bt) insecticidal proteins such as Cry1A.105, Cry1F and/or Vip3A. Transgenic corn (as well as cotton and soybean) expressing Bt proteins remain a cornerstone in FAW control in North/South America, although resistance is on the move. Resistance to Cry1F is rather widespread, and in some cases, cross-resistance has been described to Cry1A.105 and Cry1Ab, but not Vip3A. Here, we characterized the genetics of resistance to Bt insecticidal proteins in three different Brazilian FAW strains, all showing high levels of resistance against Cry1F, Cry1Ab, Cry1A.105 and Vip3A in diet overlay bioassays in comparison to a susceptible reference strain. Reciprocal crossing and backcrossing experiments combined with dose response bioassays revealed the genetics of resistance. Furthermore, we investigated differences between field populations as well as selected and unselected strains at the molecular level by transcriptome and whole genome sequencing approaches. Such approaches have been helpful in previous studies to map and characterize mechanisms of resistance at the molecular level. Finally, we discuss our findings in an applied context and how these could inform the implementation of resistance management strategies.

**Keywords:** Bt toxin, GM plants, pest, fall armyworm, cross-resistance

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## OC354. Fall armyworm invasion in maize fields in Israel – establishing an insecticide-resistance management plan

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Fall Armyworm (*Spodoptera frugiperda*) (FAW) is endemic to tropical and subtropical regions of the Americas. FAW larvae can cause significant yield losses to various important crops, such as maize, rice and cotton. In Israel, populations of FAW have been found since 2018 damaging many maize fields. Management of FAW relies mainly on the use of insecticides; however, this pest has evolved high resistance level to many insecticides, worldwide. We have established a baseline susceptibility of FAW larvae to insecticides such as diamides, IGRs and *Bt*. During the springs and summers of 2022 and 2023, we collected larvae of FAW from maize fields and they were assayed for their susceptibility to various insecticides. Hitherto, we found a medium level of resistance to indoxacarb and Dipel (*Bt*), and a low resistance level to chlorantraniliprole. Since its invasion, we have been conducting a long-term survey in maize fields to find out the effect of several agrotechnical factors on its populations, along with chemical treatments. Survey results in the maize fields showed that the sowing dates may affect FAW populations and the numbers of chemical applications; where at early sowing dated fewer chemical treatments were applied than at late sowing dates. In addition, more applications were applied in fields of sweet corn than in fields of silage maize. An outcome of this study is to form an IPM-IRM strategy that will have the ability to decrease FAW exposure to insecticides and to increase the use of other environmentally-friendly pest control practices.

**Keywords:** *Spodoptera frugiperda,* maize, agrotechnical factors, resistance management, IPM

## OC355. Development of N-arylamide insecticides to control hemipteran pests of agricultural systems

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Aphid-mediated transmission of plant viruses occurs during distinct feeding events, such as probing for non-persistent viruses or phloem feeding for persistent viruses. We have shown that initiation of aphid feeding, and thus transmission of non-persistent viruses, occurs between 4-6 minutes after landing on the plant. Thus, we aimed to develop novel chemicals capable of inducing rapid (<6 min) mortality or altering feeding behavior to reduce horizontal transmission of plant viruses. We screened a newly synthesized *N*-arylamide series for acute toxicity against two aphid pests, *Myzus persicae* and *Aphis gossypii*, through direct spray exposure and leafdip bioassays. Twelve molecules resulted in >90% mortality within 6 minutes of exposure at 200 ng/cm$^2$ and two molecules, *N*-(2,6dichlorophenyl)2,2,3,3,3-pentafluoropropanamide (GMR031) and *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide (GMR138), resulted in >90% mortality at 3-4 minutes at 50 ng/cm$^2$. Electrical penetration graph (EPG) technique was used to quantify changes to feeding behavior and an 8-fold increase in time-to-first probe, 5-fold reduction of time spent in probes, and significantly ($P < 0.05$) reduced ingestion of xylem and phloem were observed after foliar application of GMR031 and GMR138. Based on the changes to feeding behavior, assays to quantify changes to horizontal transmission of potato leaf roll virus (PLRV; persistent) and sweet potato feathery mottle virus (SPFMV; non-persistent) after foliar treatment with *N*-arylamides were performed. Data indicate significant reduction or inhibition of aphid-mediated transmission of both, PLRV and SPFMV, after foliar application of *N*-arylamides. Structure-activity relationships, preliminary mode of toxicity, and implications of these findings for aphicide development efforts will be discussed.

**Keyword:** aphids, stink bugs, insecticide, pathogen transmission, mode of toxicity

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## OC356. Molecular diagnostics for pesticide resistance monitoring and management of major agricultural pests

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Decisions on which pesticide to use in agriculture, are expected to become more difficult as resistance is spreading in various pest populations across the world and is threatening the efficiency of chemical control applications. For major pests for which a number of candidate markers for pesticide resistance are available, molecular diagnostics could support decision-making for the rational use of pesticides. A suite of twenty TaqMan assays was developed to assay target site mutations in *T. urticae* mites, *B. tabaci* whiteflies and *F. occidentalis* thrips, validated against Sanger-sequencing, and adapted for use with droplet digital polymerase chain reaction (ddPCR) in bulk pooled samples. The achieved LoD was as low as 0.1% (detection of a 1 mutant in a background of 999 wild type pests). The assay panels were assessed in terms of agreement with phenotypic resistance, through a pilot application in field populations, with strong correlation and thus diagnostic value of the molecular assays in some cases. It was also able to capture emerging resistance that was missed by phenotypic bioassays in other cases. By using bulk pooled samples ddPCR diagnostics can boost the practicality of monitoring by decreasing the resources required. At the same time, they provide extremely high sensitivity for detecting the early spread or re-emergence of resistance, through the detection of known, validated resistance mutations, at very low frequencies. Thus, they can prove a valuable tool to support evidence-based insecticide resistance management strategies. This work was supported by the European Union's Horizon 2020 research and innovation program (773902-SuperPests).

**Keywords:** ddPCR, agricultural pests, resistance monitoring, mites, whiteflies, thrips

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## OC357. Highlighting new toxicity biomarkers using volatolome deviation analysis in *Apis mellifera* chronically exposed to fipronil

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The assessment of xenobiotic disturbances involves the determination of toxicity biomarkers on ecosystem auxiliaries. Among the various "omics" methodologies that can be used in (eco)toxicology, volatolomics is in full development. It is based on Volatile Organic Compounds (VOCs) analysis which are mainly low molecular weight compounds. A previous study carried out on soil bacteria demonstrated that this method allows to highlight toxicity biomarkers to various phytosanitary products. *Apis mellifera* was chosen to validate the relevance of volatolomics in toxicological context. This work was performed on emerging bees and studied in pain-type cages. For the volatolomics analysis, five bees abdomens (cuticle, fat bodies, adherent tissues) without digestive tract were collected in each cages after 14 and 21 days of chronic exposure to 0.5 and 1 µg/L of fipronil. VOCs analysis was performed using HeadSpace-Solid Phase MicroExtraction/Gas Chromatography-Mass Spectrometry. A total of 281 features were extracted and tentatively identified in this work. No effect of fipronil can be observed on the volatolome after 14 days of chronic exposure. The volatolome deviation was obtained after 21 days of chronic exposure on bees exposed to both concentrations of fipronil. The study of this deviation allowed to highlight 11 volatile compounds whose signal abundance evolved during the experiment. Among these VOCs, 1 possibly act on the γ-aminobutyric acid receptors activity (GABA-R, the fipronil target) while the others possess semiochemical activities (pheromones, repellent agent, compounds related to Nasonov gland) leading to a potential impact on bee behaviour.

**Keywords:** volatolomics, pesticide, (eco)toxicogenomics, GABA modulator, volatile compounds

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## OC358. *Apis mellifera* CYP6AQ1 orthologous genes of four stingless bee species mediate flupyradifurone detoxification

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Flupyradifurone (FPF), a novel butenolide insecticide binding to nicotinic acetylcholine receptors (nAChRs), has been shown to be less acutely toxic to western honey bee (*Apis mellifera*) than other insecticides such as neonicotinoids sharing the same target-site. A previous study revealed that this is due to oxidative metabolism of FPF mediated by three cytochrome P450 monooxygenases, including CYP6AQ1. However, it is unknown whether functional orthologs of CYP6AQ1 in non-Apis bee species possess the same catalytic capacity to degrade FPF. Here we investigated the potential role of functional CYP6AQ1 orthologs in FPF metabolism in stingless bees (Tribe: Meliponini). To address this, we conducted a phylogenetic analysis on four stingless bee species including *Heterotrigona itama*, *Tetragonula carbonaria*, *Frieseomelitta varia* and *Melipona quadrifasciata* to identify CYP6AQ1-like functional orthologs. We identified candidate P450s with greater than 63% predicted protein sequence identities when compared to honeybee CYP6AQ1 and recombinantly expressed these genes in High Five cells. All expressed P450 enzymes were demonstrated to possess a coumarin substrates profile, indicating functional expression. Competition assays using fluorescent probe substrate indicated non-competitive inhibition of these enzymes by increasing concentrations of FPF. Furthermore, UPLC-MS/MS analysis revealed the capacity of all CYP6AQ1-like orthologs to metabolize FPF by hydroxylation *in vitro* at various levels. This research, employing a toxicogenomics approach, provides important insights into the potential of stingless bees to detoxify FPF, and highlights the significance of investigating the detoxification mechanisms of insecticides in non-Apis bee species by molecular means to inform risk assessment and conservation efforts.

**Keywords:** P450, pollinators, flupyradifurone, toxicogenomics, stingless bee

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## OC359. Investigating mortality caused by environmental doses of urban pollutants, the phthalates DnBP and DEHP, on the urban pollinator *Bombus terrestris*

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Cities are environments with severe environmental constraints (e.g. fragmented habitats, urban heat islands, air and soil pollutants) which can be detrimental to wildlife development. We focus our research on the impact of urban pollutant chronic exposure, such as phthalates, on pollinators. These ubiquitous emerging pollutants are endocrine disruptors known to affect invertebrates through immune stress, reproductive impairments, and developmental issues. In this study, we first evaluated phthalates exposure at atmospheric and cuticular levels on city-caught *Bombus terrestris*. Then, we tested under laboratory conditions the effects of two commonly found phthalates, DEHP (Di(2-ethylhexyl) phthalate) and DnBP (Di-n-butyl phthalate), on the individual and colonial health of *Bombus terrestris*. Our first results suggest that environmental level exposure to those chemicals can lead to mortality in *Bombus terrestris*. Faced with these results, it appears that there is a need for deeper investigations of the role of endocrine disruptors, such as phthalates, in the current global decline of insects, and particularly, pollinator populations.

**Keywords:** phthalates, endocrine disruptors, urban pollutants, bee health, mortality study

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## OC360. The effect of pesticides on ants

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*The side effect of pesticides on ants still remains poorly understood. This may be because ants do not represent economically important organisms like bees*. However, although ants are small animals, their distribution, species diversity, different life strategies, food specializations, interactions with organisms and above all their number affect entire ecosystems. We conducted a series of experiments to monitor changes in fecundity and mortality of *Myrmica rubra* and *Lasius niger* ant queens for a period of six weeks after the pesticide treatment. We found out that manufacturer-recommended doses of tested pesticides, and even their lower concentrations, cause sublethal or lethal effects. Queens of *L. niger* showed greater resistance to the tested pesticides than *M. rubra*, which explains its wider distribution. In both species, fecundity was reduced by pesticides, but in the case of *M. rubra* queens would not be able to establish a new colony under natural conditions. The results of different resistance to pesticides in these two common ant species found in agricultural areas raise following questions 1) How much pesticides affect other ant species; 2) How pesticides change the composition of ant species especially in agricultural areas; 3) How possible changes in ant species diversity affect local fauna and flora.

**Keywords:** ants, pesticide*, side* effect, mortality, fertility

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## OC361. Insecticide Resistance of *Anopheles maculipennis* and *Anopheles melanoon* (Culicidae: Diptera) Species in the Mediterranean and Aegean regions of Turkey

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Chemical insecticides are still widely used in mosquito control effort but Insecticide resistance development by mosquitoes, environmental pollution concerns and toxicity to non-target species are a public health issues link to these chemicals. In this study, we assessed the level of resistance of *Anopheles maculipennis* and *Anopheles melanoon* from malaria endemic regions of Turkey to four insecticide families and revealed possible molecular resistance mechanisms. Three thousand *An. maculipennis* individuals from 30 populations and 1600 *An. melanoon* individuals from 16 populations in the Mediterranean and Aeagean region of Turkey were examined using WHO's insecticide susceptibility bioassay tubes. Then, *kdr* mutation screening was performed to determine the level of resistance. 27 *An. maculipennis* and 16 *An. melanoon* populations sampled in the Mediterranean and Aegean Regions were used in molecular studies; *kdr* mutations was identified in 12 and 3 populations of these mosquitoes, respectively. These results indicate a low level of resistance of the species to insecticides at population level in general. This is the first report on the molecular basis of the resistance of Turkish *An. maculipennis* and *An. melanoon* populations. These results may help to fulfil the lacking information in the literature regarding insecticide resistance status and underlying mechanism of these important species and enables the development of new control strategies.

**Keywords:** Turkiye, insecticide resistance, vector, mosquito

# Session 14:
## Biological Control and Biopesticides

## OC362. Biological control in greenhouse crops: from releasing natural enemies to ecosystem management

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Biological pest control has been used successfully in greenhouse horticulture for many years. According to the Statistics Netherlands' database, almost 100% of the greenhouse growers use natural enemies for biological control. However, the greenhouse industry is facing major challenges due to the fast decrease of pesticide availability, pesticide resistance and the increasing number of invasive pest species. Here we will take sweet pepper as a model crop to illustrate how these challenges change biological pest control in Dutch greenhouses. For many years conventional sweet pepper growers have applied a rather simple – and until recently effective - IPM system based on introductions of predatory mites and *Orius laevigatus* combined with selective pesticides. However, aphids clones with resistance to the narrow range of pesticides still available became very abundant and are now the most challenging pest to control. In addition, the invasive southern green stink bug *Nezara viridula* has established in Dutch sweet pepper greenhouses since 2018, causing serious damage. For both aphids and stink bugs, new natural enemies have been selected that strengthen the preventive control strategies based in addition to currently used natural enemy species. These new developments show a clear shift to more diversity and complexity, requiring an ecosystem approach that considers the specific needs of the natural enemies in terms of food, shelter, oviposition as well as the more complex interspecific interactions. Here we show our attempts to build the biocontrol food web in pepper with functional biodiversity and enhance efficacy through species complementarity and niche differentiation.

Keywords: *Nezara viridula*, aphids, food web complexity, functional biodiversity

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## OC363. Exploring the relationship between *Nesidiocoris tenuis* and *Trichoderma harzianum* in tomato plants under water stress

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In Southern Europe and North Africa, *Nesidiocoris tenuis* is commonly used as a natural predator to control pests in tomato crops. However, *N. tenuis* can also cause damage to tomato plants when it feeds on them, leading to issues such as flower abortion and necrotic rings. Recent research has suggested that using certain endophytic fungi in tomato plants can help reduce the damage caused by *N. tenuis*. In this study, we investigated the effects of using the beneficial fungus *Trichoderma harzianum* and *N. tenuis* (both individually and in combination) on tomato plants under different levels of water stress. Our findings indicated that *N. tenuis* caused more damage as the water stress increased.

Furthermore, inoculating tomato plants with *T. harzianum* enhanced their resistance to water stress. Most interestingly, in plants where *N. tenuis* was established and *T. harzianum* was also inoculated, the incidence of damage caused by *N. tenuis* was lower than in plants without *T. harzianum*. These results highlight the potential benefits of combining these two biological control agents, especially in climate change, where managing water stress will become increasingly important.

**Keywords:** zoophytophagous predator, endophytes, biological control, abscisic acid, jasmonic acid, salicylic acid

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## OC364. Pronematus ubiquitus: a multitasking mite in greenhouse crops

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The tomato russet mites *(Aculops lycopersici)* and powdery mildew are two main bottlenecks in tomato cultivation for which no effective biocontrol solutions exist. Commercially available predatory mites come from a single family (the Phytoseiidae). These are usually a poor match for tomato plants as they are too big to navigate the glandular hairs (trichomes). To solve this issue we studied small mites from the superfamily Tydeoidea, in particular the species *Pronematus ubiquitus*. Being omnivorous, these mites can feed on small arthropods, pollen, fungi and plant sap. Due to this broad diet, the mites can be preventatively established using the pollen food supplement Nutrimite.
P. ubiquitous proved exceptionally effective in suppressing tomato russet mites. In addition, we noticed that plants inoculated with *P. ubiquitous* remained free of powdery mildew, while control plants in the same compartment suffered high mildew infestation. This makes *P. ubiquitous* the first reported example of an arthropod that concurrently controls a pest and a pathogen. This mite therefore represents a whole new category in biocontrol. Powdery mildew is of course also a major problem in a range of other crops, including strawberries, where it affects both the leaves and the fruit. In commercial strawberry production, weekly fungicide treatments are required to control this pathogen. Our trials showed that *P. ubiquitous* can build up large populations on strawberry, following a single introduction and biweekly feeding with Nutrimite™. The mite provided better protection than conventional or IPM strategies. *P. ubiquitous* could be a real game-changer in biological crop protection.

**Keywords:** biological control, disease control, powdery mildew, tomato russet mite, dual function

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## OC365. Smart parasitoids: applying parasitoid learning to increase the efficacy of mealybug biocontrol

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A vast body of theoretical and empirical literature indicates that parasitoid learning can play an important role in foraging behavior and success. ‘Training’ parasitoid wasps through exposure to a conditioning experience can result in efficiency gains in host searching and host accepting processes, depending on the training method and target host. Manipulating parasitoid foraging behavior by exploiting parasitoids’ learning ability could therefore enhance the efficacy of augmentative biological pest control. Despite this potential, few studies have concretely investigated the operational application of parasitoid learning for specific biocontrol systems. In this presentation we discuss the application of parasitoid learning in the biological control of mealybugs in greenhouse crops. The effects of different training methods on the host searching efficiency of mealybug parasitoids are presented, drawing on experimental work carried out in laboratory and greenhouse settings.

Keywords: biological pest control, parasitoid learning, mealybugs, greenhouse horticulture

## OC366. Application of alternative food can constrain biological pest control

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Biological control to suppress greenhouse pests has been studied extensively as it offers an alternative to chemical pesticide applications. Addition of alternative food for generalist predators generally results in increased control because it enables predators to persist if pest population densities are low. The effectiveness of alternative food to increase pest control is impacted by the short-term and long-term effects on the predators. In the short term, predators may become satiated due to the alternative food, thus feeding less on the pest. In the longer term, the addition of alternative food results in higher predator densities and better pest control. However, how predator densities are impacted by factors such as the quality of alternative food and the frequency of application is not broadly understood. Here we show how the dynamics of both pest and predator densities are impacted by alterative food quality and application frequency. Our findings show that alternative food of higher quality can result in not only higher predator densities, but higher pest densities compared to lower quality alternative food, suggesting reduced effectiveness in pest control. However, this is only the case when alternative food is added with high frequency. In systems where alternative food is added less frequently, alternative food of high quality provides more effective pest control; pest densities are similar to low quality-food, but predator densities are higher. These results highlight that both quality and application frequency of alternative food can impact effective pest control in greenhouses.

Keywords: population dynamics, satiation, transient dynamics, apparent mutualism

## OC367. Testing effective and sustainable management strategies for the control of the horticultural pest Lygus rugulipennis

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Mirid bugs, in Europe especially *Lygus rugulipennis*, pose considerable thread to vegetable production, attacking several greenhouse crops and causing significant economic loss. In search of alternative solutions to control pest populations, producers rely more and more on combined integrated pest management (IPM) strategies rather than single insecticide treatments. From earlier studies we know that existing individual management interventions have limited potential to keep the damage of *Lygus rugulipennis* below an acceptable threshold on their own. As a consequence, we investigated single and combined effects of the following management interventions for the control of *Lygus rugulipennis.* Competition with *Macrolophus pygmaeus*, light traps, entomopathogenic fungi and repellents were tested in their ability to reduce pest populations and crop damage in egg plants (*Solanum melongea*). In control and treatment greenhouse adult *Lygus rugulipennis* were released in known densities to mimic natural colonization of covered crops during early summer. Contribution to pest reduction of each intervention as well as final yield were assessed. The results allow to identify practically and financially viable management interventions for vegetable producers in Europe. Our results emphasize the conception and use of a more systemic and multi-angle approach in plant protection as effective insecticides are banned. Such approaches contribute to the reduction of synthetic pesticide use and to more sustainable agricultural production.

Keywords: mirid bug, solanum, agroecology, push-pull,

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## OC368. Improved fitness of artificially selected strains of *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) feeding on astigmatid mites

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Augmentative biological control relies heavily on omnivorous predators, whose continued presence allows for an early response to pest outbreaks. This continuous presence is made possible by their ability to feed on alternative foods, such as pollen and other artificially supplemented factitious foods. However, the biotic potential of biological control agents (BCAs) decreases when they are fed with suboptimal feeds, hindering their beneficial action. However, there is variability between individuals and populations within a species, which can be exploited through genetic improvement to obtain BCA strains better adapted to these alternative foods. *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) is one of the most widely used BCA in augmentative biological control programs. On the one hand, we have developed two strains of *O. laevigatus* showing better fitness feeding on pollen, a suboptimal food. On the other hand, astigmatid mites, known as stored food mites, are increasingly being used as a food supplement for predatory mites, but not for predatory insects. Our selected *O. laevigatus* strains show increased survival during nymphal development and higher fecundity when they feed on astigmatid mites. Therefore, these astigmatid mites could be used as a cost-effective supplementary food on crops to improve the establishment and survival of our improved strains as part of a biological control strategy.

Keywords: *Orius laevigatus*, biological control, alternative food, astigmatid mites

## OC369. Biological control of invasive pests in citrus: challenges and solutions

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The number of invasive pests in citrus from the Mediterranean basin has increased dramatically during the last 20 years. Developing biological control programs against invasive pests is a challenge, but also the most economical and environmental solution for stakeholders. Here, we will present three biological control programs to improve the management of invasive pests in citrus: the mealybug *Delottococcus aberiae,* the psyllid vector of the huanglongbing (HLB) disease *Trioza erytreae,* and the thrips *Chaetanaphothrips orchidii*. The mealybug *D. aberiae* has become a key pest in Spanish citrus. Our data shows that this invasive mealybug has established a mutualistic association with the native ant *Lasius grandis* that promotes the establishment and accelerate the invasion of *D. aberiae*. The exclusion of this ant species from citrus canopies improved the biological control and reduced the damages produced by the mealybug. The presence and spread of the HLB vector *T. erytreae* in mainland Europe is one of the main threats for Mediterranean citrus. The parasitoid *Tamarixia dryi* was introduced in fall 2019. The parasitoid established and, more importantly, reduced the abundance of *T. erytreae* two years after its introduction. The orchid thrips *C. orchidii* is now spreading in eastern Spain and damaging citrus at harvest. This thrips pupates in the soil, mostly in areas close to the watering system. IVIA is developing a strategy to apply entomopathogenic fungi in these areas and increase the mortality of the thrips.

Keywords: mealybugs, thrips, psyllids, *Trioza erytreae, Chaetanaphothrips orchidii, Delottococcus aberiae*

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## OC370. Proactive biological control of *Lycorma delicatula* (Hemiptera: Fulgoridae) in west coast of USA: from classical to conservation?

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Spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), native to China, is a polyphagous invasive pest of ornamental and forest trees and several specialty crops, including grapes. *Lycorma delicatula* is rapidly spreading on the east coast of the US, and it is anticipated that this pest will invade the western US and possibly European countries as well, in a manner similar to that observed for brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). In response to this anticipated invasion, a proactive biological control program targeting *L. delicatula* is being developed in California. Host range test results of the principal biocontrol agent candidate, an egg parasitoid, *Anastatus orientalis* (Hymenoptera: Eupelmidae), native to China, indicated that this parasitoid has a very wide host range, attacking hosts in at least two orders (Hemiptera and Lepidoptera) and at least seven families. A new approach to *L. delicatula* biological control is now underway - an assessment of levels of naturally occurring biotic resistance that *L. delicatula* could experience upon establishing in California. We investigated if *Anastatus* spp. native to USA can parasitize *L. delicatula* eggs. We tested three native Anastatus species against *L. delicatula* egg masses for their ability to successfully parasitize eggs of this pest. In addition, intraguild competition of the native Anastatus species and *A. orientalis* was investigated. An improved understanding of the biocontrol function that native Anastatus species could have on *L. delicatula* will help determine if resident parasitoid species will provide appreciable levels of naturally occurring biological control against this invasive pest. We have consulted the scientific committee and it seams that despite the fact that your presentation was accepted as an oral unfortunately there are no available slots to present it after the evaluation of the total of submitions you can still presnt it as a poster if you wish so

Keywords: biotic resistance, *Anastatus orientalis*, parasitoids, Anastatus, Spotted lanternfly

## OC371. The killer detects prey's odours: can fungi be attracted to *Halyomorpha halys* eggs by their volatiles?

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The brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), is an alien species native to East Asia and it is now invasive in Europe, where it is one of the main phytophagous pests in orchards. We hypothesized that Volatiles Organic Compounds (VOCs) may be present on *Halyomorpha's* eggs and we aim at understanding their role. Using Solid Phase Microextraction, we collected VOCs from freshly laid eggs and from older eggs in order to assess VOCs variations according to the egg's age. We identified Hexadecanoic acid and Phenethyl alcohol from new eggs and Butyrolactone from old eggs. These compounds are reported in literature as antimicrobial compounds and, for this reason, we hypothesized that the present VOCs in *H. halys* egg clusters may act as a protection from external infections. We carried out antifungal assays where we tested the growth of entomopathogenic fungi and fungi of plant diseases in the proximity to *Halyomorpha* eggs. By the measurement of fungi growth and the mortality rate of *H. halys* eggs we were able to show that fungi are attracted to eggs clusters ($p<0.001$) and that the hatching rate of nymphs was reduced in freshly laid egg clusters compared to the older ($p<0.001$). Our data indicate fungi's selective virulence on *H. halys eggs* in respect of their age and their presumptive attraction towards the eggs by their volatiles. These results are to our knowledge the first evidence on how egg odours may influence the growth of fungi and this might lead to new control approaches in biological protection.

Keywords: attraction, entomopathogenic fungi, fruit protection, antimicrobial assays, orchards

## OC372. Consequence of rearing temperature for several generations on the low temperature activity of the parasitoid *Aphidius matricariae* on the rosy apple aphid, *Dysaphis plantaginea*.

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Low temperatures in spring can limit the activity of natural enemies while their preys are often better adapted. This asynchrony complicates the development of biological control programs. This is the case of the rosy apple aphid, which is active at low temperatures in early spring in orchard, causing damage to the flower buds. In this context, we first analyzed the influence of low temperatures on the activity of one of its parasitoids, *Aphidius matricariae*. Then, to select a strain potentially more active at low temperature, we studied the consequences of rearing *A. matricariae* at 12°C compared to 20°C for 7 consecutive generations on movement capacities, aphid patch exploitation and parasitism rates at 12°C. All generations combined, walked distance and time in movement were similar according to rearing temperature with an average of 250.2 cm for rearing at 12°C and 253.4 for rearing at 20°C with respectively 15.0 min and 16.6 min in movement. Parasitoid velocity remained higher for parasitoids from 12°C rearing with a speed of 2.70 mm/sec than for 20°C rearing parasitoids (2.49 mm/sec, $p < 0.01$). Females from 20°C rearing showed higher oviposition number (27.1 ± 1.4) than females from 12°C rearing (21.3 ± 1.5). However, parasitism rate for females of the 12°C rearing reached 21.75% which is significantly higher ($t = 2.33$, $p = 0.025$) than those coming from 20°C rearing with a parasitism rate of 13.05%. The developmental temperature therefore impacts the capacity for parasitism at low temperature. The perspectives offered by these results are discussed.

**Keywords:** low temperatures, selection, parasitoid, aphid, parasitism rate

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## OC373. Management of entomopathogenic based strategy against the red palm weevil in Israeli date palm plantations

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Environmentally friendly management of wood borers is a challenge. Until lately, the control measures against the red palm weevil (RPW), Rhynchophorus ferrugineus (Olivier), a severe invasive pest of palm trees, relied solely on non-specific synthetic pesticides, a non-sustainable management presenting a compromise rather than positive valence. We evaluated the usefulness of entomopathogenic fungi (EPF) as preventative control measure and entomopathogenic nematodes (EPN) as a responsive means for RPW early colonized palm. Mostly, the EPF are applied against adults and the EPN target the penetrating larvae. The objective of the present study was to evaluate the strategy in which EPF are applied as preventative measure against the early life stages of the RPW – eggs and larvae. The strategy relies on the vertical transmission of EPF conidia by the female, taken from the treated tree surface to its progeny. Early colonization was constantly monitored by IoT based seismic sensors developed by Agrint® located on all palm trees during the whole experimental period. The crystalized environmentally friendly management was achieved by series of laboratory and field trials, performed under the framework of a national project encompassing researchers, R&D and pest control companies with tight collaboration the date palm growers. The main findings and insights will be elaborated with the emphasis on the vast impact of the new management of RPW specifically and the implications on the future prospective of sustainable management of other wood borers.

Keywords: microbial control, entomopathogenic fungi, entomopathogenic nematodes, seismic sensors, date palms, wood borer

## OC374. Biological programs against stink bugs in France: diversity of egg parasitoids and deployment strategies

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The Pentatomidae family (Heteroptera) includes many pests of various crops. Many countries around the world have to deal with the damage caused by Pentatomidae, and France is no exception. Chemical insecticides are no longer considered a reliable solution due to social and environmental pressures. Biological control therefore appears as an alternative strategy. To this end, academic researchers, R&D professionals and agronomic partners have joined forces in a consortium. Egg parasitoids appear to be the best candidates, both for classical and augmentative biocontrol. However, the introduction of exotic species for classical biological control requires prior assessment of potential side effects on native and resident communities (including Pentatomidae and other egg parasitoids). Our study provides an overview of Pentatomidae species of agronomic importance in France, including native (or introduced) species such as *Palomena prasina, Nezara viridula, Eurydema* spp. and invasive species such as Halyomorpha halys. To identify candidates for control of native pests and to prepare classical biological strategies against H. halys, extensive monitoring programs have been carried out in France since 2016. DNA barcoding was used to characterize the natural biodiversity of egg parasitoids associated with Pentatomidae. We will discuss the diversity of these parasitoids and present ongoing R&D strategies, including the use of *Trissolcus basalis*, the release of *T. mitsukurii* and the forthcoming introduction of *T. japonicus*.

Keywords: biocontrol, egg parasitoid, Scelionidae, classical biological control, *Trissolcus japonicus*, *Trissolcus mitsukurii*

## OC375. Mortality factors of the red-legged shield bug, *Pentatoma rufipes* (L.) (Hemiptera: Pentatomidae), an emerging pest in European fruit orchards

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The red-legged shield bug, *Pentatoma rufipes*, is a widespread species in forest habitats of Europe. However, with the expansion of agricultural and residential land, the bug has also spread to parks, private gardens, and fruit orchards, where it finds ideal conditions for reproduction. Likely due to climate change and the withdrawal of organophosphate insecticides, fruit growers have experienced increasing losses in pear and apple production in recent years. To better understand the population dynamics of *P. rufipes*, mortality factors for each life stage (eggs, nymphs, adults) were estimated by either exposing sentinel stages or collections of wild populations in southern Germany and Switzerland over a period of two years. These data were then used to construct a life table and estimate the contribution of parasitoids and predators to the generational mortality of *P. rufipes*. Egg masses of *P. rufipes* were primarily parasitized by Scelionidae, of which *Trissolcus cultratus* was the most abundant, with parasitism levels up to 59%. In addition, in some locations *P. rufipes* was also attacked by the adventive *T. japonicus* (up to 25%) which is used in Italy for classical biological control of the invasive brown marmorated stink bug, *Halyomorpha halys*. In contrast, adult parasitism by tachinids was usually less than 1%, and nymphs were not parasitized at all. Predation levels did not exceed 18%. Inundative releases of *T. cultratus* during the oviposition period of *P. rufipes* could be considered as additional tool of integrate pest management of the bug.

Keywords: shield bug, *Pentatoma rufipes*, life table, Scelionidae

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## OC376. Study of the parasitoid wasp *Campoplex capitator* in the vineyards of the Centre-Val de Loire Region in France for biological control of the grapevine moth *Lobesia botrana*

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The grapevine moth, *Lobesia botrana*, is among the most important insect pests in European vineyards. The caterpillars provoke direct damage to berries, but indirect damage is often even more problematic.

Indeed, the damage caused by the caterpillars promotes secondary infections by pathogenic fungi and facilitates *Drosophila* attack on berries thereby deteriorating the quality of the wine. The project and results presented here are placed in a context of sustainable protection of vineyards to reduce chemical treatments. The parasitoid wasp, *Campoplex capitator*, is the main natural enemy of *Lobesia botrana*, which acts by laying its eggs in the caterpillars. The main aim of this project was to evaluate the biological control potential of this wasp from three different angles. First, the natural ecosystem service provided by the wasp in vineyards was evaluated by measuring the parasitism rate of *Lobesia botrana* using a Cytochrome Oxidase I (*COI*) metabarcoding approach. Secondly, the presence of the wasp in the natural environment was also explored to determine if there are alternative hosts in refuge areas and, also, to evaluate whether certain plants may enhance the presence of parasitoids. Finally, we found that *Campoplex* wasps produce viral particles, which we characterized in order to understand the parasitic success of the wasp. This molecular characterization of the functional biodiversity of the parasite will contribute to the conservation of the ecosystem service of parasitoids. Within this context, the possibility of biocontrol by increasing wasp populations in vineyards is under evaluation.

Keywords: pest management, grapevine moth, *Lobesia botrana*, parasitoid wasp, *Campoplex capitator*, metabarcoding, viral domestication

## OC377. Effects of *Trissolcus japonicus* releases on the distribution and parasitism rate of *Halyomorpha halys* egg parasitoids in Emilia-Romagna region, Northern Italy

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Egg parasitoids are promising biological control agents for the brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stål), an invasive pest that has been causing severe damage to orchards in the USA and Europe. Approximately 140,000 *Trissolcus japonicus* (Ashmead) individuals were released between 2020-2022 in 300 sites across Emilia-Romagna within the framework of one of the largest classical biological control projects ever attempted in Italy. The aims of this study were to explore: i) the abundance and distribution pattern of BMSB egg parasitoids across years; ii) the impact of *T. japonicus* releases on BMSB and non-target species; iii) the influence of landscape structure on parasitoid assemblages. Field monitoring at both release and no-release sites was performed to collect egg masses naturally laid by BMSB and other stinkbugs. Only sites in which at least 7 BMSB egg masses had been collected (71, 68 and 58 sites in 2020, 2021 and 2022, respectively) were considered for data analyses. A significant increase in parasitism rate by *T. japonicus* was found across years and distribution maps showed a progressive range expansion, thus demonstrating that this species established and overwintered successfully. Although with decreasing impacts across years, the European native parasitoid *Anastatus bifasciatus* (Geoffroy) was found in most of the sites and exerted the overall highest parasitism rate on BMSB egg masses. The exotic species *Trissolcus mitsukurii* (Ashmead), whose adventive populations have been detected in Emilia-Romagna since 2019, showed a decline especially at sites where *T. japonicus* was released, possibly indicating interspecific competition in the field.

**Keywords:** classical biological control, brown marmorated stink bug, invasive species, *Trissolcus mitsukurii, Anastatus bifasciatus*

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## OC378. Biological characteristics of *Anagyrus aberiae* (Hymenoptera: Encyrtidae), a parasitoid of the citrus mealybug *Delottococcus aberiae* (Hemiptera: Pseudococcidae)

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*Anagyrus aberiae* (Hymenoptera: Encyrtidae) is a parasitoid of the South African citrus mealybug *Delottococcus aberiae* (Hemiptera: Pseudococcidae). It was imported in 2019 from South Africa to Spain as a control strategy against the mealybug that was causing significant damage to citrus by deforming the fruit and reducing its size. Because no biological aspects of this species of parasitoid have been described so far, several laboratory studies have been carried out since its introduction in order to learn about its behavior and suitability to Mediterranean environmental conditions. To assess the potential of the parasitoid as a natural enemy of *D. aberiae*, biological aspects such as the developmental time and longevity, offspring production and thermal requirements were calculated. The results obtained were compared to other species of parasitoids of citrus mealybugs found in the Mediterranean basin.Results show that temperature strongly affect development time and longevity of *A. aberiae*. It also has an impact on offspring production as well as the sex ratio of progeny. Based on the development data obtained, we were able to estimate threshold temperatures of parasitoid from egg stage to adult emergence, as well as the optimum temperature and the thermal constant. Finally, a comparative analysis of the data obtained with other species of mealybug parasitoids in citrus from the Mediterranean basin was made.

**Keywords:** mealybugs, parasitoids, *Anagyrus*, biological control

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## OC379. Risk assessment of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) on three nontarget tephritids in laboratory bioassays

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Biological control is one of the most effective and sustainable strategy for the insect pest management. To prevent negative side effects to the environment, regulations and guidelines have been issued to evaluate the risk associated with the release of allochthonous biological control agents (BCAs). For these reasons, laboratory bioassays have been carried out to evaluate the host specificity of *Diachasmimorpha longicaudata* (Ashmead), a parasitoid that has been used worldwide as a BCA of fruit flies of economic importance. This species could be conveniently released in Italy to control *Ceratitis capitata*, which causes damage to many host fruits and is not hindered by effective natural enemies in this country. Moreover, *D. longicaudata* could be used to manage the outbreak of *Bactrocera dorsalis* recently recorded in South Italy.Different series of choice and no-choice tests have been conducted using *D. longicaudata* females. Three nonfrugivorous tephritids have been used as non-target species: two gall forming tephritids, *Myopites stylatus* living on *Dittrichia viscosa* and *Urophora stylata* infesting *Cirsium vulgare*, and the leaf miner *Acidia cognata* whose larvae develop inside the leaves of *Tussilago farfara*. All the non-target species were tested as full grown larvae concealed within their host plant flower or leaf. Bioassays have been carried out in arenas or small cages. Depending on the bioassay type, the wasp behaviour (host rejection or acceptance) or the choice made, were recorded.The parasitoid has never shown interest for non-target tephritid flies, and no one female accepted nor oviposited on non-target larvae.

**Keywords**: *Ceratitis capitata*, nonfrugivorous tephritids, biological control agent, allochthonous species

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## OC380. Comparison of digital and manual traps monitoring against theoretical flights modelling of the European grapevine moth (*Lobesia botrana*) in France in 2022

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The damage caused by European grapevine moth *(Lobesia botrana*) can be very significant reaching losses up to 40% in the harvest as a result of direct damage to bunches and subsequent fungal infections. The moth has one to four generations a year dependant on environmental conditions. In order to time treatments and reduce crop damage, adults monitor using Delta traps with species-specific pheromone is one of tool used actually. In 2022, Syngenta tested in 2 south vineyards of France, in parallel of manual Delta traps, 22 digital traps equipped with a daily artificial intelligent recognition system to count the butterflies. A model based on logistic flight cumulative purcentage of flight realization estimated the theoretical beginning, peaks and end of flights generations was used. This algorithm using hourly minimum and maximum thresholder temperatures accumulations (GDD) was then validated on captures data. The good correspondence of modelling and flights trap (1.2 ± 8.4 days between flights beginning of G1 generation adults detecting and forecast, -1.6 ± 10.4 for G2 and 1.6 ± 9.6 for G3) encouraged us to developed a decision support system (DSS) to the winegrower next year using 10 days of weather forecast to better positioning their insect control applications.

**Keywords:** *Lobesia botrana*, trap, AI recognition, modelling, DSS

## OC381. Disentangling the effects of the invasive pest, *Dryocosmus kuriphilus*, and the introduced biocontrol agent, *Torymus sinensis*, on native parasitoids in an isolated insular chestnut-growing area

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Studying multitrophic interactions among an invasive alien pest, a non-native introduced parasitoid and the guild of native parasitoids in a newly invaded area allows to raise the question as to what is the main culprit for the alteration of the natural systems. This particularly applies to the debate about the risks linked to the introduction of a non-native biocontrol agent in an area invaded by an alien pest. *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae) was first recorded in Sardinia (Italy) in 2007. Two years later, a biological control program started based on the introduction of its non-native parasitoid, *Torymus sinensis* Kamijo (Hymenoptera Torymidae). Over a nine-year period (2009-2017), data on the relative abundance of *D. kuriphilus*, native parasitoids and *T. sinensis* were collected. The "isolated" geographic area where the multitrophic system was studied can be considered ideal to reveal the role of each participant. During the first years of *D. kuriphilus* colonization, the large availability of its galls caused a chaotic change in the composition and species abundance of native cynipid parasitoids exploiting the new host. After the establishment of *T. sinensis* on the invasive pest, which made *D. kuriphilus* unavailable to other parasitoids, the natural enemy complex returned to attack almost exclusively the native hosts. In fact, the successful control of *D. kuriphilus* by the introduction of *T. sinensis* counter-balanced the ecosystem alterations caused by the arrival of the invasive pest.

**Keywords:** Asian chestnut gall wasp, apparent competition, Cynipoidea, Chalcidoidea

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## OC382. The side effects of two *Providencia entomophila* strains isolated from olive tree insect pests on the egg parasitoid *Trichogramma oleae*

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Two *Providencia entomophila* (Enterobacterales; Morganellaceae) strains (IO-6 and IO-20) previously isolated from the olive moth *Prays oleae* (Lepidoptera, Hyponomeutidae) and the olive fly *Bactrocera oleae* (Diptera, Tephritidae), respectively, were previously found efficient against *B. oleae.* However, in order to be considered nature-respectful entomopathogens, their side effects on auxiliary fauna must be thoroughly assessed. In that regard, the thelytokous egg parasitoid *Trichogramma oleae* (Hymenoptera, Trichogrammatidae) was used as a test-organism, and essays were conducted on its pre-imaginal and adult stages. The used concentrations for both strains ranged from $10^5$ to $10^9$ cfu/ml. Results showed that none of these tested strains or concentrations harmed adults. However, all strain IO-6 concentrations caused detrimental effects on *T. oleae* larvae, while only the concentration $10^9$

cfu/ml could harm prepupae. Also, only the $10^6$ and $10^9$ cfu/ml concentrations of strain IO-20 caused increased mortality in prepupae and larvae, respectively. Still, none of the tested *P. entomophila* strains was found to be persistent even 2 days after treatment application. Nonetheless, some side effects on adults' appearance were perceived when treatments were applied during developmental stages, and led to the detection of brachypterous, male, and bisexual individuals within *T. oleae* population. Taking into consideration all the aforementioned parameters, only *P. entomophila* IO-20 at the concentration $10^5$ cfu/ml could guarantee entire respect to all *Trichogramma oleae* developmental stages and offspring appearance.

**Keywords:** *Trichogramma oleae*, preimaginal, adults, mortality, *Providencia entomophila*

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## OC383. Vermicomposted olive mill waste soil amendment as potential pest biological control promoter in olive

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Agro-food industries like olive mills produce huge amounts of waste. Once vermicomposted, microbe- and nutrient-rich olive mill waste can be reused as an organic soil amendment or biofertilizer for the olive grove. However, its potential role in plant-induced defence against herbivores, particularly emissions of volatiles attractive to natural enemies of pests, remains unexplored. Here we investigate the effect of this soil amendment on olive tree volatile emissions when we simulated the attack by the carpophagous generation of the olive moth *Prays oleae* Bernard (Lepidoptera: Plutellidae; one of the major olive pests in Europe, and yet poorly investigated experimentally), and its potential effect on the attraction of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) predator in dual-choice olfactometer assays. Vermicomposted olive mill waste was added to the plant rhizosphere in its natural state in one treatment, in which both the microbiome and the nutrients could play bottom-up roles. Sterilized vermicompost only providing nutrients, and unamended soil were used as controls. Only olive trees with the soil amendment in its natural state were able to promote *C. carnea* attraction. In this treatment, *C. carnea* was more attracted to attacked plants than to the non-attacked ones, and in case of attack, they preferred it to the unamended control. Volatile blend profiles highly correlated with *C. carnea* attraction. Our current ongoing research aims to identify specific *P. oleae*-induced molecular patterns and to unravel the bottom-up mechanisms by which this soil amendment boosts plant indirect defenses. [Acknowledgement: Junta de Andalucía project PY20_00139]

**Keywords:** olive, *Prays oleae*, *Chrysoperla carnea*, volatiles, organic soil amendment, bottom-up

## OC384. Indirect effects of entomopathogenic fungi in biological control

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A range of entomopathogenic fungal species within the order Hypocreales have for decades been investigated and developed for biological control of insect and mite pests. Applications of fungal biocontrol agents rely on their direct effects on the target host through infection, resulting in host mortality. However, several of these fungi can also interact with insects indirectly where a third organism is mediating the interaction. Such indirect interactions may have profound effects on the populations involved and hence also impact outcomes of biological control efforts. It is timely to revisit the ecological concept of indirect interactions and effects to evaluate how entomopathogenic fungi may enhance or hamper biocontrol of herbivorous insects and mites. The fungi can potentially cause effects mediated by natural enemy behaviour or by modification of host plant physiology. The latter phenomenon has recently been elucidated in detail, revealing complex belowground-aboveground interactions and surprising ecological consequences. Selected studies and insights will be presented and discussed in relation to how these interactions may impact biocontrol scenarios. Context and complexity of the cropping system are important aspects to consider. Ultimately, the entomopathogenic trait may play a minor role when fungi are involved in indirect interactions with insects.

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## OC385. Interactions between entomopathogenic fungi and entomopathogenic nematodes in microbial control

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Entomopathogenic fungi and entomopathogenic nematodes are globally distributed soil organisms capable of infecting and killing a wide variety of insects. Therefore, these organisms are frequently used as biocontrol agents in insect pest management. Both entomopathogenic fungi and nematodes share the soil environment and therefore can infest and compete for the same insect host. However, natural co-infections are found rarely due to the cryptic soil environment. Our current knowledge on their interaction within the host comes mainly from laboratory experiments. Due to a recent trend of combining biocontrol agents to increase their efficacy, many studies focused on the co-application of different species of EPF and EPNs against various insect pests with variable outcomes for instance, synergistic effect, additive effect, and antagonism. Also, the effect on the development and reproduction of each pathogen varied from normal reproduction to exclusion, and generally, the outcomes of the interactions were dependent on the pathogen and host species, pathogen doses, and timing of infection. The present review aims to summarize current knowledge on the interactions of entomopathogenic fungi and nematodes within an insect host and estimate the possible effects of the interactions on natural pathogen populations and their use in microbial control.

**Keywords:** microbial control agents, synergy, antagonism, biocontrol, effectiveness

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## OC386. When competitors join forces: using consortia of entomopathogenic *Pseudomonas* bacteria, nematodes and fungi for pest control

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Below-ground pests are difficult to control because either no effective control methods exist or suitable insecticides are or will soon be banned due to their negative effects on the environment. We evaluated the potential of disease-suppressing *Pseudomonas chlororaphis* bacteria with entomopathogenic activity for insect control. Moreover, we combined the pseudomonads with entomopathogenic nematodes (*Steinernema feltiae*) and fungi (*Metarhizium brunneum*) with the aim to increase reliability and efficacy of biocontrol measures. In a series of experiments ranging from the greenhouse to the field, *P. chlororaphis* emerged to be highly efficient in controlling the cabbage maggot *Delia radicum*, an important pest of Brassicacean crops. Furthermore, the triple consortium of *P. chlororaphis* with *S. feltiae* and *M. brunneum* increased the number of marketable radishes by 50% in a field trial. In several experiments, we observed increased pest control when combining the pseudomonads with the nematodes or the fungi. These synergistic effects were verified when applying the combinations against two further pests. The triple consortium was the most lethal and fastest killing treatment against *Pieris brassicae* and *Diabrotica balteata* larvae. In the early stages of the infection, all three agents established inside the larvae.Our results show that entomopathogenic pseudomonads, nematodes and fungi are compatible and could potentially be used to control a variety of below-ground insect pests.

**Keywords:** biocontrol consortia, insecticidal pseudomonads, entomopathogenic nematodes, entomopathogenic fungi

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## OC387. Entomopathogenic fungi are compatible with a parasitoid for *Spodoptera littoralis* (Boisduval) control while inducting resistance mechanisms in melon under greenhouse conditions

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Endophytic entomopathogenic ascomycetes are multipurpose microorganisms used for IPM but positively impacting crop production. This study explores the potential of *Metarhizium brunneum* and *Beauveria bassiana* applied to melon plants for *Spodoptera littoralis* control and their compatibility with the larval noctuid parasitoid *Hyposoter didymator* in a multitrophic system. First, progressive endophytic colonization of the melon plant with *B. bassiana* through its life cycle was ascertained, with even 20% of seeds from the new fruits being endophytically colonized. Also, it was recorded that treated plants reached a greater development than control plants, with significant differences in root, shoot and total weight reported, both in fresh and dry matter. The foliar application of *M. brunneum* caused both lethal and sublethal effects on *S. littoralis* with considerable reduction in their biological performance. In this sense, a significant lengthening of the larval development time, together with a reduction of the larval and pupal weight, were detected in the fungally challenged insects. Moreover, the simultaneous use of the fungal strains and the parasitoid was compatible, with mortality rates in the range of 36.1 to 77.7%, being the reproductive potential of *H. didymator* not affected in any case. By using qRT-PCR it was detected that genes involved in the ethylene signaling pathway, salicylic and jasmonic acid synthesis and related Induced Systemic Resistance (ISR) were up-regulated in shoot tissues at different sampling times. These results demonstrate the multifunctionality of entomopathogenic fungi not only as components of IPM programs but also as beneficial microorganisms for crop plants.

Keywords: endophytic colonization, multitrophic relationships, *Hyposoter didymator*, ISR-SAR related genes

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## OC388. Laboratory and field evaluation of entomopathogenic fungi, bioinsecticides and RNAi for Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera: Chrysomelidae) control

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Colorado potato beetle (CPB) is the most important insect defoliator of potatoes and can be controlled with insecticides based on plant extracts, entomopathogenic microorganisms and other substances that pose a low risk to human and animal health and the environment. Within the EU project ECOBREED (GA no 771367) and ARIS (GA no P4-0165 and P4-0431), laboratory and field trials were conducted from 2020-2022 to test four bioinsecticides (azadirachtin (product Neemazal), spinosad (Laser Plus), conidial suspension of two isolates of entomopathogenic fungus Beauveria bassiana (EPF), Bacillus thuringiensis subsp. tenebrionis (Btt; Novodor)) and RNA interference (RNAi) against CPB larvae. The EPF were also combined with different doses of azadirachtin and spinosad to explore potential synergistic interactions between EPF and bioinsecticides. Treatment efficacy was determined by CPB larval mortality and a decrease in potato leaf consumption. A mix of both EPF isolates outperformed individual isolates and, combined with a 2% recommended dose of spinosad or 100% dose of azadirachtin outperformed those bioinsecticides alone, in laboratory assays. In field experiments in both years, treatments with spinosad (full and 20% dose, and spinosad + EPF) and azadirachtin alone showed significant larval reduction. In contrast, EPF, Btt and RNAi did not significantly control CPB larvae. All treatments except EPF in both years and RNAi in 2021 significantly reduced defoliation of potato plants. Despite significant differences in defoliation and larval reduction observed on individual plants, no significant differences in tuber yield were observed between treated and untreated field experimental plots, after a single (bio)insecticide application.

Keywords: azadirachtin, biological control, *Beauveria bassiana*, integrated pest management, RNA interference, spinosad

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## OC389. Assessing interactions among *Beauveria bassiana,* western corn rootworm larvae and maize

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Western corn rootworm, *Diabrotica virgifera virgifera* (LeConte) (Coleoptera: Chrysomelidae), is a serious pest of maize. Western corn rootworm larvae reside in the soil where they feed on maize roots, which can substantially reduce yield. Because resistance by western corn rootworm to management tactics such as crop rotation, insecticides and Bt maize has been documented, additional management tools including the use of biological insecticides, should be assessed. Entomopathogenic fungi (EPF), such as *Beauveria bassiana*, are naturally occurring in the soil and have, in some cases, been shown to grow endophytically, within plant tissue. Due to its persistence in the soil and endophytic competence, *B. bassiana* may be a beneficial tool in the management of western corn rootworm. We studied the effects of two isolates of *B. bassiana* on the mortality of western corn rootworm larvae in bioassay environments that contained and lacked soil. Additionally, maize seeds were inoculated with conidia of *B. bassiana* and plants were tested to determine whether *B. bassiana* was able to establish endophytically and whether it was able to persist in the rhizosphere. Results from this research will provide a better understanding of interactions among EPF, crop plants and insect pests, and the potential uses of EPF in pest management.

Keywords: entomopathogen, Western corn rootworm, biological insecticide

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## OC390. The feasibility of using entomopathogenic nematodes for Mediterranean fruit fly control

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Mediterranean fly *Ceratitis capitata*, Medfly is an important pest of fruits such as citrus, peaches and apricots. The use of entomopathogenic nematodes for medfly control is not new, however it has been limited. We have systematically over the last years explored this medfly biocontrol solution by a combination of laboratory experiments and field trials. The latter are very limited and need to be expanded to further evaluate the nematode efficacy. The species *Steinernema feltiae* that is widely commercially available can be used in application scheme where a single, relatively low dose of nematodes in autumn, (off season) or spring (early season) is sufficient, targeting overwintering medfly larvae and therefore reducing substantially the number of adult medflies emerging later in the growing season. Here we 1) present results of small and larger scale field trials in citrus groves in Greece 2) we discuss the feasibility of the method and 3) examine factors that can improve its efficacy.

**Keywords:** biological control, entomopathogenic nematodes, *Steinernema feltiae,* medfly, field trials

## OC391. Using cover crops and landscape features to promote biological control in vineyards: an Australian study

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This talk will summarize the three components of an Australia project that aims to provide vineyard managers with practical, evidence-based strategies to suppress pests and provide additional benefits. The first component of the project assessed the value of nectar from a range of plant species to *Trichogramma carverae* and *T. pretiosum* that are natural enemies of the key vineyard pest, lightbrown apple moth (LBAM). Some plants that are potentially suitable for use as vineyard cover crops provided strong benefit to the lifespan, egg production and parasitising capacity of *Trichogramma* species and some of these were 'selective food plants' that denied benefit to LBAM adults. The second component of the project comprised vineyard trials of mid-row and under-vine cover crop species to assess ease of establishment and value to growers. Treatments, such as alyssum (*Lobularia maritima*), led to significant decreases in grape bunch damage by LBAM and this had the flow-on effect of reducing incidence and severity of botrytis bunch rot, a fungal disease that is exacerbated by feeding damage caused by LBAM. The third component of the project surveyed dozens of vineyards over two years to assess the strength of 'spillover' effects to grapevines from differing forms of nearby land uses. For example, areas of vineyards bordered by roadways tended to have higher densities of natural enemies, especially spiders, an effect potentially caused by the structurally complex, diverse, perennial vegetation beside roads that provides food resources and movement corridors linking vineyards with donor habitats elsewhere in the landscape.

**Keywords:** conservation biological control, habitat manipulation, cover crop, parasitoid

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## OC392. Functional Agrobiodiversity supports pollination and pest control in agricultural landscapes

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Agriculture heavily relies on ecosystem services provided by inhabiting species. The densities and diversity of these so called "functional groups", however, have shown strong declines due to the intensification of agriculture, compromising their vital ecosystem services. Here, we review scientific literature and field experience to evaluate the effects of targeted landscape elements with increased plant diversity to restore pollination and pest control. These elements include flower-rich field margins, woody landscape elements, as well as intercropping, strip cropping, and agroforestry. It is important to realize that promoting agro-biodiversity in general is not a guarantee that ecosystem services will be supported and it could even increase the risk of diseases, weeds, and pests. In previous studies, the untailored design of landscape elements may have resulted in variable effects on pest control and pollination services. By tailoring the species composition of landscape elements and the landscape as a whole to the requirements of the functional groups, the ecosystem services can be more effectively supported, a concept called Functional Agrobiodiversity. Functional Agrobiodiversity involves the following steps to systematically strengthen ecosystem services. (1) Identify functional groups that provide ecosystem services for relevant crops. (2) Investigate their needs (e.g., food, shelter, breeding sites). (3) Identify and quantify the constraints related to these needs in the current agro-ecosystem. (4) Implement supportive measures that effectively address these constraints. Through this targeted approach, we can increase both biodiversity and agricultural productivity, while avoiding possible negative effects of increasing plant diversity.

**Keywords:** Functional Agrobiodiversity, pollination, pest control, ecosystem services

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## OC393. European ants: friends or foes in the biological control of agricultural pest insects?

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Ants are among the most successful groups of insects, playing a prominent ecological role across terrestrial ecosystems almost everywhere on the planet. In agroecosystems, they often play a relevant role in processes such as bioturbation or soil enrichment, while interacting directly with other arthropods, fungi, cultivated plants, and weeds. Most of the over 14,000 known ant species are predatory, and some, such as tropical weaver ants, have been widely used as biocontrol agents of agricultural pests due to their ability to hunt phytophagous insects. At the same time, a large proportion of species engages in a mutualistic relationship with honeydew-producing hemipterans that feed on plants, including many agricultural pests among aphids and mealybugs, which are granted protection against predators and parasitoids. Relatively little is known on which services and disservices most of the about 630 European ant species may be responsible for due to their complex interactions with pest insects. We report the results of field and laboratory investigations on both antagonistic and mutualistic interactions between ants and insect pests. Our results reveal significant behavioral differences between different ant species. Not all European ants provide notable protection for their mutualistic partners against natural enemies, while many common species may act as effective antagonists of native and exotic pests. Ant behavior on plants can be manipulated to a great extent, and sometimes ant-hemipteran partnerships may be exploited as a monitoring tool. Effectively balancing these different aspects promises to offer new opportunities in the development of effective biological control strategies.

**Keywords:** Formicidae, agroecosystems, natural enemies, multitrophic interactions, trophobiosis

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## OC394. Pest and beneficial arthropod responses to hedging in southeast USA pecan systems

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Managing the vegetative canopy of perennial systems using pruning techniques may be important for light interception and other abiotic conditions. However, altering canopy architecture also has the potential to modify interactions in arthropod communities. Here, we examine effects of canopy on a suite of pecan pests and natural enemies in a multi-year study. Hedging decreased the abundance of some pests, increased pest pressure from others such as aphids. For natural enemies, parasitoids, predatory mites and soil-borne entomopathogen activity was not affected by hedging. And, parasitoids were more active in the upper canopies of the trees whereas aphid hosts and parasitism frequently occurred in the lower. Given the mix of positive and negative effects of hedging and inconsistent effects between years, further investigation is needed to isolate the mechanisms driving the variability.

Keywords: arthropod communities, spatial structure, pest-parasitoid interactions

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### OC395. Pre-designed cover crops in citrus to build up complex trophic webs: Implications on Conservation Biological Control

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The late season period (from mid-August to the end of winter) in Mediterranean citrus growing regions can be critical for natural enemies because of the lack of prey and alternative food resources for the nutritional sustenance of predators and parasitoids. Under this situation, natural enemies leave the orchards unprotected in early spring, a critical period for pest biological control. Cover crops are known to offer alternative food resources and shelter and, through well-managed, may represent a sustainable tool to avoid this undesirable biocontrol imbalance. In two commercial young citrus orchards, three types of the sown cover crop were tested in a randomized complete block design: forbs, grass, and alternate strip plots of forbs and grass. The species composition, relative abundance and coverage of the cover crops was also characterized. Natural enemies and potential alternative preys were counted and taxonomically identified. A monthly sampling of each plot replication was carried out from the beginning of summer 2022 to spring 2023 using a suction device. Our results evidence that different cover crop designs host very distinct arthropod communities, including a valuable number of predatory taxa and alternative prey throughout the critical periods of the year, thus supporting complex trophic webs inside the orchard. The selection of specific plant species combinations for citrus cover crops would consequently allow the maintenance of more stable and resilient natural enemy assemblages.

Keywords: predators, ecological infrastructures, aphids, flower strips, grass strips, parasitoids

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## OC396. Understorey biodiversity management in olive cultivation for integrated management of natural enemies

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The management of natural enemies of perennial tree crops and especially insects is performed with pesticides, which can negatively impact the quality of products, natural resources and biodiversity as well as the health of producers and consumers. An emerging trend focuses alternatively on the use of less or no chemicals and the management of crop pests with natural means. This trend is being promoted by both the EU and the new CAP. Olive cultivation is one of the most important permanent crops in the Mediterranean area. The most important pest in olive groves is the olive fly [*Bactrocera oleae* (Rossi) (Diptera: Tephritidae)]. In this study we investigate the relationship between the fly population and plant and insect diversity in 15 fields on Lesvos Island during 2021 and 2022. Specifically, we monitored the population of olive fly with McPhail traps and understorey insects with the use of pantrap and pitfall traps. We also recorded temperature and humidity in the tree canopy. These data are correlated with two different understorey treatments, undisturbed understorey with plant mixture and cleared by mechanical means. The results indicate that in olive groves with undisturbed understorey there is a greater variety and abundance of insects while at the same time the arthropod abundance has a negative effect on the population of olive fly. The results will be used to formulate guidelines in the context of integrated olive grove management systems.

**Keywords:** *Bactrocera oleae*, biodiversity, dacus, olive, pest management, sustainable agriculture

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## OC397. Pesticides increase food and macronutrient deficiency levels in beneficial carabid beetles

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Pesticide usage is claimed as the main cause of insect declines. Pesticides can directly eradicate beneficial predatory beetles, but they are also exposed to indirect pesticide effects arising from reduced prey (as well as plant-based food) availability and quality in agroecosystems. The main aim of the current study was to investigate if higher food and macronutrient deficiency levels in carabid beetles are related to conventional agricultural practices (especially pesticide usage). Individuals of different carabid species were collected by hand from 8 conventional and 8 organic oat fields scattered around Tartu, Estonia (three times). In the laboratory beetles were served with three semi-artificial diets: lipid-rich, protein-rich, and sugar-rich. Consumption of each diet was evaluated after 24 hours (=test1). Afterwards, beetles were fed ad libitum for a week until full satiation. Then the procedure of test1 was repeated (=test2). The differences of consumed diets among two tests showed the level of food and macronutrient deficiencies in carabids within assemblages. Separately, pesticide residue analyses in beetle bodies were performed.

Seven different pesticides were detected in beetles from conventional fields. Several carabid beetle species weighed less in conventional than in organic fields. Double-test experiment revealed that carabids tended to be more food as well as sugar and lipid deficient with the presence of neonicotinoid insecticide and less so in organic fields. Finally, beetles were less protein-deficient in organic fields. We conclude that organic farming and reduced pesticide usage would improve beneficial carabid nutrition and fitness thus leading to higher efficiency in providing biocontrol service.

Keywords: agroecosystems, biocontrol agent, macronutrients, nutritional ecology, predatory beetles, sublethal pesticide effects

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## OC398. Impact of a rich-nutrient floral food source on the main parasitoids of *Tuta absoluta* in tomato

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*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the main pests threatening tomato production worldwide. After its detection in the Mediterranean basin, it has been the subject of intensive research to reduce damage in tomato crops. Up to now, biological control is based on the polyphagous predators *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) which are very effective in preying on eggs of the pest but have little effect on *T. absoluta* larvae. To complement its poor action on larvae, studies were carried out to detect which species of larval parasitoids can use this host. The Eulophidae *Necremnus tutae* (Reuter) and the Braconidae *Dolichogenidea gelechiidivoris* (Marsh) are the most abundant among the identified parasitoids in the northeast of Spain. It is well-known that adding floral resources near the crops is an increasingly valued tool to provide high-quality food for natural enemies and enhance biological control. In previous studies, we have shown that the flowers of *Lobularia maritima* L. (Brassicaceae) are a reliable food source for increasing the survival and egg load of *N. tutae*. This and its long flowering period make this floral resource ideal for promoting these parasitoids in tomato crops. Our work aims to elucidate if the availability of *L. maritima* flowers affects the biological control of *T. absoluta,* enhancing the conservation of both parasitoids in tomato-producing regions. To confirm this, the behavior of both wasps, at low and high *T. absoluta* densities, have been evaluated in the laboratory.

Keywords: *Necremnus tutae*, *Dolichogenidea gelechiidivoris*, biological Control, Nutritional ecology

## OC399. Expanding the use of *Orius* predatory bugs for the control of foliar pests in floriculture

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*Orius laevigatus* (Hemiptera: Anthocoridae) is a key omnivorous predator of flower thrips released augmentatively in horticulture. While being a generalist predator, it does not have a strong effect on foliar pests such as *Echinothrips americanus* (Thysanoptera: Thripidae) and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), that are especially problematic for ornamental plants. In this project, we compared the within-plant preferences and pest control efficacy of *O. laevigatus*, and the indigenous species *Orius majusculus* and *Orius minutus* in gerbera. When predators were offered a diet of flower (*Frankliniella occidentalis*) or foliar (*E. americanus*) pests in laboratory assays, we found that the developmental rate of *Orius* predators was higher when fed with foliar pests. However, in greenhouse trials and when both flower and foliar pests were present, *O. laevigatus* had a clear preference for oviposition in the flowering plant parts and effectively controlled the flower thrips, while *O. majusculus* and *O. minutus* were mostly found lower in the plant canopy and strongly suppressed the foliar pests (*E. americanus* and *T. vaporariorum*). When *Orius* predators with different habitat preferences were combined they complemented each other, controlling effectively both foliar and flower pests. This work clearly shows that different *Orius* species have distinct spatial preferences, which directly translates to their effectiveness as biological control agents against different groups of pests.

**Keywords:** habitat preference, predator complementarity, *Frankliniella occidentalis, Echinothrips americanus, Trialeurodes vaporariorum*

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## OC400. Building a better banker plant by incorporating predatory pallidus beetles, *Delphastus pallidus* LeConte (Insecta: Coleoptera: Coccinellidae), into the papaya banker plant system

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A variety of banker plant systems have been developed against whiteflies. Genus *Delphastus* Casey is a small whitefly-specific predatory ladybird beetle from the coccinellid tribe Serangiini. Our field surveys and experiment results indicated one of its species, *D. pallidus* LeConte has tremendous predation potential against multiple pest whitefly species and could be the best candidate for a papaya banker plant system (PBS). The PBS has a papaya plant as a banker plant and hosts non-pest whitefly species, papaya whitefly, *Trialeurodes variabilis (Quaintance*) as prey to rear natural enemies. The primary function of the PBS is to sustain and deliver natural enemies to control pest whiteflies in greenhouses and fields. The initial quantities of prey and natural enemies on a banker plant are critical while building

any banker plant system. We incorporated *D. pallidus* successfully in the PBS in this study and calibrated the densities of *D. pallidus* and *T. variabilis* required in the PBS to deliver a maximum number of *D. pallidus* to target high and low both densities of pest whiteflies in a cash crop successfully. The commercial production of the PBS would need this calibration to ensure high-quality and standardized products. This talk will demonstrate the incorporation and calibration of *D. pallidus* in the papaya banker plant system.

**Keywords:** banker plants, predatory beetle, whitefly

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## OC401. Control of *Bemisia tabaci* MEAM1 on poinsettia using pallidus beetle, *Delphastus pallidus* LeConte applied either directly or by a papaya banker plant system

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*Bemisia tabaci* (Gennadius) feeds on 900 different host plants, vectors over 111 plant viruses and is a major invasive pest species worldwide. Insecticides are used extensively for whitefly control consequently development of widespread resistance is always a major concern. We have shown how papaya, corn and ornamental peppers can serve as “banker plants” for a range of insect parasitoids and predators. This approach is considered an environmentally friendly alternative because it reduces insecticide use and offers growers a low-cost, self-perpetuating alternative. Our whitefly banker plant system uses papaya to rear the alternate prey (papaya whitefly, *Trialeurodes variabilis* (Quaintance)) which is host specific for papaya (*Carica papaya*) and used to feed the predatory beetles. Beetles leave the papaya banker plant to feed on the crop pest, *Bemisia tabaci* MEAM1 (Middle Eastern Asia Minor 1) in the cash crop, poinsettia in this case. We conducted two greenhouse trials in randomized complete block designs (RCBD). Our research determined the effectiveness of pallidus beetle to control *Bemisia tabaci* MEAM1 populations applied either directly to the poinsettia or by a papaya banker plant system and compared that efficacy to an insecticide grower standard and the untreated control. Outstanding results will be presented and discussed.

**Keywords:** biological control, predatory beetle, whitefly, banker plant

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## OC402. Investigating factors affecting the *Eretmocerus eremicus* parasitism level by comparative studies on two *Bemisia tabaci* populations from Greece

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Hymenoptera parasitoids are a very important tool for managing pests with the frame of IPM. However, unsatisfactory parasitism levels by *Eretmocerus eremicus* have been reported from commercial greenhouses in Northern Greece, which was confirmed by preliminary laboratory observations. Since the factors affecting parasitism levels are of key importance in agricultural practice, the percentage of parasitism by *E. eremicus* on two *Bemisia tabaci* populations (Bt 552 Southern Crete, Bt 22-616 Central Macedonia) was studied in relation to *B. tabaci* populations' origin (Northern vs Southern Greece) and parasitoids' experience (exposed to host nymphs: inexperienced 24h vs experienced 72h). Briefly*: B. tabaci* adults from each population were allowed to oviposit on cotton plants using clip cages. The number of nymphs was fixed and adjusted to 30 nymphs per replication. Once the nymphs reached the $L_2$ stage two pairs of parasitoids (experienced or inexperienced) were introduced for 24h to the nymphs using a clip cage. All experiments were replicated 4 times. Based on the results, differences in the parasitism levels between the two populations were detected. More specifically a higher parasitism rate was detected on Bt 552 than on Bt 22-616, when both experienced and inexperienced parotoids were used. In addition, a significantly higher parasitism rate was detected in experienced parasitoids compared to inexperienced ones on population Bt 22-616. However, these differences were not detected on population Bt 552. The underlying mechanisms responsible for the observed differences in parasitism rates between whitefly populations remain to be investigated.

Keyword *Bemisia tabaci* populations, *Eretmocerus eremicus*, parasitism

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## OC403. Differential effects of fungal biocontrol agents on spider mites under different application methods and environmental conditions

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*Tetranychus urticae* (Koch) is one of the most notorious pests of strawberries, damaging yield and quality. Entomopathogenic fungi (EPF) can infect and kill arthropods and thus be used as biopesticides. Moreover, several EPF live as root colonizers and endophytes. Root inoculations with EPF can affect aboveground herbivores, but mechanisms remain unclear. Here, we applied the EPF *Metarhizium brunneum* and another biocontrol fungus *Clonostachys rosea* as either foliar sprays or root inoculants in different strawberry cultivars, which were grown in coir substrate or field-collected soil. We evaluated the effects on *T. urticae* individual development using *in vitro* bioassays and population growth on whole plants, respectively. Surprisingly, these biocontrol fungi showed completely different effects depending on application methods, plant growth environments and cultivars. Specifically, foliar spray of a *M. brunneum* strain significantly decreased *T. urticae* survival and hatch rates on strawberry leaves compared to the other treatments. However, when the fungus was applied as root inoculant, it promoted *T. urticae* population growth in "Rumba" cultivar grown in soils, while it suppressed the growth in same cultivar when grown in coir substrate. *C. rosea* also inhibited *T. urticae* population growth in two cultivars. Fungal colonization of *Metarhizium* was found in roots not in leaves, which led us to hypothesize that the EPF may have affected the plant secondary metabolites and/or rhizosphere microbial communities, leading to differential changes in the *T. urticae* populations. Fungal biocontrol agents may therefore have different effects on the same pest depending on application methods, plant cultivars, and growing environments.

Keywords: plant-microbe-arthropod interactions, root inoculation, entomopathogenic fungi, biological control, *Tetranychus urticae*

## OC404. Assaying semiochemicals and entomopathogenic fungi for an auto-dissemination biocontrol strategy of *Frankliniella occidentalis*

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For creating an auto-inoculation trap for thrips with entomopathogenic fungi, three thrips populations collected from environmental plant samples, were reared in microcosms in climatic chambers and genetically identified as belonging to the species *Frankliniella occidentalis*. Simultaneously, a fungal bioprospecting campaign on the cadavers collected in thrips rearings allowed the isolation of a strain (Bb1), further identified as *Lecanicillium lecanii*, a species of interest for this study. Subsequently, the entomopathogenicity of a local proprietary collection of 11 *Beauveria* and *Metarhizium* spp. strains was screened on an egg-laying sample of *F. occidentalis*. The *Lecanicillium lecanii* strain Bb1 caused the greatest population decrease at 75%, and was the only one to be statistically significant from the negative control. In comparison, a commercial strain of *Beauveria bassiana* caused only 12% mortality. In parallel, the fungistatic capacities of 7 attractive molecules of *F. occidentalis* (attraction pheromone and kairomones) were screened on the same fungal isolates at two different concentrations, namely 1% and 10%. All treatments showed a fungistatic action, when compared to the negative control. However, linalool, nerol and geraniol at a concentration of 10% especially impacted the growth of the fungal isolates and were statistically significant compared to the other treatments. This work therefore allowed the selection of thrips semiochemical molecules with a weak fungistatic effect against the fungal strains of interest for the control of *F. occidentalis*, as well as the isolation, identification, demonstration of the virulence potential and sensitivity of a fungal strain potentially useful for controlling this thrips species.

Keywords: auto-dissemination system, entomopathogenic fungi, *Frankliniella occidentalis*, bioprospecting, biopesticides

## OC405. Evaluation of the parasitoid *Cotesia typhae* for augmentative biological control of the Mediterranean corn borer *Sesamia nonagrioides*

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A new hymenoptera parasitoid species, *Cotesia typhae* has been described in Kenya as strictly specialised on *Sesamia nonagrioides* (Lepidoptera, Noctuidae). This African moth is a corn pest in the Mediterranean basin. In France, the control of this pest faces challenges due to its expansion towards the north and the lack of efficient control methods against the second generation of the insect larvae. The possibility of using *C. typhae* in France as biocontrol agent against *S. nonagrioides* is being investigated. Four greenhouse experiments have been conducted to test the parasitoid efficiency. The first two trials aimed at estimating immediate post-release efficiency depending on the temperature and the parasitoid density. Results showed that the parasitoid was active between 15 and 25°C and the parasitism rate increased with the density of parasitoids released. A third and forth trials were conducted to evaluate the efficiency of one parasitoid release on two generations of the pest larvae, a pre-requisite for an affordable cost of the biocontrol solution. The third trial showed that one release of the parasitoid at mixed developmental stages allowed a stable parasitism rate of 58% in average along 3,5 months, during which one generation of *S. nonagrioides* had developed. An improved protocole used for the fourth trial resulted in an almost full suppression of the pest by *C. typhae* over the same duration, covering the first and the beginning of the second pest generation. Long lasting activity of *C. typhae* following a single release is very encouraging for future field trials.

**Keywords:** biocontrol, parasitoid, stemborer larvae, maize, greenhouse

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## OC406.Assessing risk for non-target species before introducing an exotic parasitoid for biocontrol of the corn stemborer *Sesamia nonagrioides* in France

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Biocontrol of non-native insect pests can be achieved by introducing their natural enemies. Although it has been successful against many pests worldwide, negative environmental impacts have sometime occurred. EU regulation obliges an environmental risk assessment before introducing any exotic macro organism useful for crop protection. This study aims at evaluating the non-target risks of the African larval parasitoid *Cotesia typhae* (Hymenoptera, Braconidae), a potential biocontrol agent of the corn stemborer *Sesamia nonagrioides* (Lepidoptera, Noctuidae) in France. Lepidopteran species, phylogenetically close to *S. nonagrioides* (stemborers of Poales) and sharing the same ecological niche than *S. nonagrioides-C. typhae* in their native range, were listed as non-target. The impact of *C. typhae* on 8 non-target species was determined by sequential analysis, including no-choice and choice tests (olfactometer and *in planta).* The attractiveness and acceptance of these species for parasitism varied greatly. The risk of development of *C. typhae* and the risk that non-target larvae die after parasitism were on average low (2% and 5%, respectively) compared to those of *S. nonagrioides* (49%). Only the cattail stemborer, *Nonagria typhae*, presented a higher mortality risk *in planta* (10%). Nonetheless, this risk is still smaller compared to the target species (33%), which supports the high specificity of *C. typhae* towards *S. nonagrioides* on cattail in their native region (East Africa). The risk of *C. typhae* to the nontarget species is therefore very low, as well as its survival capacity under winter temperatures, which predicts even a lower environmental risk of *C. typhae* on the long term.

**Keywords:** biocontrol, parasitoid, insect pest, maize, risk assessment

# Session 15:

## Integrated Pest Management

## OC407. Plant defenses in Integrated Pest Management

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Integrated Pest Management (IPM) is an effective and environmentally-friendly approach of pest control that relies on a combination of practices. These include human-recruited chemical, biological, behavioral, cultural, mechanical and biotechnological control methods to suppress pest populations below economic injury thresholds and protect crops from yield losses. On the other hand, plant defense includes all functions that plants- being unable to run away from their attackers- recruit to defend themselves against biotic stressors under natural and agricultural settings. Here, I present the mechanisms underlying plant defense responses to arthropod pests, and based on current knowledge on plant defenses, I explore opportunities for controlling pests in the context of IPM. Specifically, I focus on plant defense elicitors which could become important components of IPM in the future. Finally, I list research gaps and raise considerations relevant to the compatibility of plant defense with IPM in sustainable pest control.

**Keywords:** induced plant defense, IPM, elicitors, plant resistance

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## OC408. Conspecific and non-conspecific airborne cues prime *Brassica napus* defence response

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Plants frequently use warning cues from their environment that provide information about the risk of encountering herbivores or pathogens. Plant responses to such cues often entail defence priming, which enables faster deployment of induced defences following subsequent attacks in a wide range of systems. Priming can be used within the framework of IPM towards alternative solutions to the chemical control of pests. We have recently shown that the annual brassicaceous plant, *Brassica nigra*, responds to warning cues associated with oviposition-induced plant volatiles (OIPVs) emitted by undamaged neighbouring plants that harbour *Pieris brassicae* eggs. Focal *Brassica nigra* plants can detect volatiles produced by egg-infested plants in their vicinity, and these volatiles not only prime defences in the focal-receiver plant but also elicit changes in life history strategies, namely a rapid shift from growth to reproduction that enhances fitness via reproductive escape. Here we tested whether *Brassica napus*, one of the utmost importance economic crop plants, respond with priming of plant defences and increased reproduction to oviposition-induced plant volatiles (OIPVs) emitted by (1) neighbouring plants of the same species (intraspecific interaction) or by (2) neighbouring plants of different species (interspecific interaction). This was done by measuring *P. brassicae* larvae performance on OIPV-exposed *B. napus* as a proxy for defence. While for conspecific priming, we found pronounced effects on herbivores' performance, interspecific priming yielded inconclusive results. To further explain the observed results, we conducted OIPVs collection and identification experiments for each conspecific and heterospecific treatment group.

**Keyword:** IPM, priming, induced plant defences, egg deposition, *Pieris brassicae,* Brassicacea.

## OC409. Entomopathogenic fungus-related priming defense mechanisms in cucurbits impacts on *Spodoptera littoralis* (Boisduval) fitness

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Entomopathogenic fungi exhibit direct and indirect mechanisms to increase plant resistance against biotic and abiotic stresses. Plant responses to these stresses are interconnected by common regulators such as ethylene, which is involved in both iron (Fe) deficiency and Induced Systemic Resistance responses. In this work, the roots of cucurbits seedlings were primed with *Metarhizium brunneum* (EAMa 01/58-Su strain), relative expression of 19 genes related to Ethylene (ET), Jasmonic Acid (JA) and Salicylic Acid (SA) synthesis, as well as Pathogen Related (PR) protein genes were studied by qRT-PCR. Effects of priming on *Spodoptera littoralis* was studied by feeding larvae for 16 days with primed and control plants. Genes showed up-regulation in studied species, however, highest relative expression were observed in roots and shoots of plants with Fe deficiency, demonstrating the complexity and the overlapping degree of the regulatory network. *EIN2* and *EIN3* should be highlighted, both key genes of the ET transduction pathway that enhanced their expression levels up to eight and four times respectively in shoots of primed cucumber. Also, JA, SA synthesis and PR genes showed significant up-regulation during observation period (e.g. the JA gen *LOX1*, increased 506 times). Survivorship and fitness of *S. littoralis* were affected with significant effects on mortality of larvae fed on primed plants *vs.* controls, length of the larval stage, pupal weight and the percentage of abnormal pupae. These results highlight the role of EAMa 01/58-Su strain in the induction of resistance, which would be translated into direct benefits for plant development.

Keywords: *Metarhizium brunneum*, EAMa 01/58-Su strain, melon, ISR, Fe deficiency, multipurpose microorganism

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## OC410. Plants with benefits: enhancing direct and indirect plant defenses against insect herbivores by plant-beneficial fungi

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Plant-beneficial fungi represent a diverse group of fungi that have the potential to substitute or complement the widespread use of chemical pesticides to control pests and plant diseases. These fungi can have profound impacts on plant fitness, not only by increasing nutrient uptake or enhancing growth, but also by protecting plants against abiotic and biotic stress. Plant-beneficial fungi may protect plants against pests by enhancing direct and/or indirect plant defense responses. However, the precise outcome of these plant-microbe-insect interactions may depend on the specific biotic and abiotic context, leading to inconsistent or unpredictable results that hamper the widespread adoption of plant-beneficial fungi in practice, but only little is known in this regard. In this study, we investigated the effects of plant-associated fungi on both direct and indirect plant defenses against the southern green stink bug (*Nezara viridula*) in sweet pepper. Specifically, we evaluated how plant-beneficial fungi (*Beauveria bassiana* and *Trichoderma harzianum*) affected plant growth and resistance to *N. viridula*, and how these responses are mediated by fertilizer levels. Moreover, we examined if these fungi can affect the behavior and performance of *Trissolcus basalis*, the main egg parasitoid of *N. viridula*. Our results revealed that plant-beneficial fungi have the potential to enhance plant growth, reduce herbivore damage, modulate defense signaling pathways and enhance parasitoid attraction. Nevertheless, some of these effects were found to differ between fungal strains and were also dependent on the abiotic context, warranting against generalization of research results.

Keywords: *Nezara viridula*, plant-beneficial fungi, plant defense*, Trissolcus basalis, Beauveria bassiana, Trichoderma harzianum*

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## OC411. Unraveling direct and indirect control mechanisms of *P. ubiquitus* against the tomato russet mite and powdery mildew

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Tomato cultivation faces major challenges such as the tomato russet mite (TRM), *Aculops lycopersici,* and powdery mildew (PM). TRM is a member of the Eriophyidae family and can cause significant yield losses by feeding on the epidermal cell layer. On the other hand, PM is a group of devastating fungal pathogens that infect over 10 000 host species, including tomato. This disease is prevalent in glasshouse tomatoes worldwide and is increasingly becoming significant in field tomatoes. In European greenhouse tomato crops, *Oidium neolycopersici* has been identified as the causal agent of PM since the 1980s, causing powdery white lesions on all plant parts except the fruit. Recent research has discovered that *Pronematus ubiquitus* can be established on tomato plants as a “standing army”, providing protection against both TRM infestation as PM infection. *P. ubiquitus* is a small, omnivorous, leaf-feeding iolinid mite that is known to be partly mycophagous. However, the significant suppression of PM at all levels of the tomato plants suggest that plant defenses may also be involved. Induced plant defenses could potentially contribute to the control of TRM by *P. ubiquitus* as well. Therefore, our research investigates the mechanisms behind these effects with a combination of bio-assays and comparative transcriptome analysis.

Keywords: tomato russet mite, powdery mildew, predatory mites, Iolinidae, biological control, plant defense mechanisms

## OC412. Plant damage caused by the predatory bug *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) can be reduced by eliciting plant defenses

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Although widely used as a biological control agent for tomatoes, *Nesidiocoris tenuis* can also cause damage to host plants. Recent studies have shown that tomato plants attacked by sucking insects can trigger responses that make them more resistant to herbivores, more attractive to natural enemies, and less susceptible to pathogens. In this study, we hypothesized that eliciting plant defenses might reduce the damage caused by *N. tenuis*. We exposed tomato plants to (Z)-3-hexenyl propanoate (Z3HP), a green leaf volatile, applied through polymeric dispensers to induce a defensive response. Individuals of *N. tenuis* were released onto these plants, while control plants were not exposed to the volatile. In both cases, *N. tenuis* established on the plants. However, surprisingly, the number of necrotic rings, the characteristic damage caused by *N. tenuis*, was significantly lower in the Z3HP-exposed plants despite the higher number of individuals. These results may be explained by the activated plants having a higher callose deposition. Interestingly, the production of these compounds and others related to antioxidant activity in the activated plants made it possible to regenerate plant tissue in areas attacked by *N. tenuis*, as confirmed using fluorescent brightener microphotography. Also, the gene expression of markers related to the metabolic pathways of abscisic, jasmonic, and salicylic acids was evaluated and the results showed differences in gene expression, particularly for genes related to salicylic acid. These findings pave the way for improved management of this important natural enemy through enhancing plant defenses.

**Keywords:** necrotic rings, (Z)-3-hexenyl propanoate, gene expression, enhanced resistance, plant volatile

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## OC413. Exposure of sweet pepper plants to the volatile Z-3-HP enhances the plant's resilience to herbivore attacks and water deficits

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When plants are injured by herbivores, they may trigger direct defensive responses, such as the release f herbivore-induced plant volatiles (HIPVs). These chemical cues are involved in plant-plant communication and are used as warning signals by undamaged plants to trigger their own defenses and minimize in advantage herbivores damage. This work highlights the potential efficacy of the external application of green leaf volatile Z-3-hexenyl propanoate (Z-3-HP) as a tool to increase plant resilience against pests. Under semi-field conditions, the exposure of sweet pepper plants to Z-3-HP reduced the infestation of three sweet pepper key pests *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) in comparison with unexposed plants. In turn, the population development of the polyphagous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was greater in exposed plants in comparison with unexposed sweet pepper plants. On the other hand, the study of gas exchange activity revealed that the exposure of sweet pepper plants to Z-3-HP did not affect the photosynthesis rate ($A_N$) in exposed plants compared with unexposed plants. whereas, the water use efficiency ($A_N$/E) increased more than 40% in plants exposed to Z-3-HP, due to stomata closure associated to decrease of transpiration rate. The improvement of water use efficiency is an excellent measure to mitigate the effects of climate change on water resources. Overall, this work reveals insights into using plant volatiles as defense elicitors to increase plant resistance to pests and as a tool for adaptation to water stress.

**Keywords:** biological control, plant defense, HIPVs, transcriptome, gas exchange, sweet pepper

## OC414. Impact of visual and olfactory cues on *Psylliodes chrysocephala* (Coleoptera, Chrysomelidae) host plant location and selection in the field

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The stem flea beetle (SFB- *Psylliodes chrysocephala*) is among the main pests in winter oil seed rape (OSR- *Brassica napus*) across Europe. Intercropping OSR with faba beans (FB- *Vicia faba*) showed promising results to reduce SFB larval density. However, the underpinning mechanisms remain unclear. We therefore aimed at disentangling the visual and olfactory impact of FB on SFB host location and selection in intercropped OSR. In a field trial, OSR was planted with either natural FB, imitation FB made of polyethylene or alone. Plots (9 m$^2$) were sown spaced by 10 m in a barley field in a latin square design repeated twice. SFB adults were monitored with yellow sticky traps and larval population in the plants was assessed. The number of bites was recorded to assess insect damage to OSR. First results showed no significant difference in SFB migration between the OSR alone and crop associated with imitation FB, while OSR intercropped with FB was significantly different from them. Those results suggest a negligible effect of visual and physical disruption by FB on host plant location by SFB. The analyses of the bites and larva density will help us to understand the impact of the companion plant on the host selection.This study associated with olfactometer tests will bring a better understanding of SFB behavior contributing to integrated pest management design as an alternative to pesticide use.

**Keywords:** Berlese trap, canola, host searching behavior, mix cropping, plant odour, vision

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## OC415. host plant preference of citrus species and ornamental plants to *Trioza erytreae*, vector of HLB

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*Trioza erytreae* is one of the two known vectors of Huanglongbing, the most devastating disease of citrus. Recent studies show that *T. erytreae* transmits the most aggressive bacterial species (*Candidatus* Liberibacter asiaticus; *C*Las) with similar efficiency as *Diaphorina citri*. Thus, it is mandatory to identify which is the host plant preference of *T. erytreae* for the most common citrus species grown in Europe as well as those non-citrus species that may act as reservoirs of *T. erytreae*. Citrus and citrus-related species screened were the following: *Citrus limon* (cv. “Verna”), *Citrus limon* (cv. ”Fino”), *Citrus latifolia* (Tahiti lime), *Citrus hystrix* (Kaffir lime), *Citrus paradisi* (Grapefruit; cv. “Star Ruby”), Fortunella spp (Kumquat; cv. “Nagami”), *Microcitrus australasica*, *Citrus sinensis* (cv. “Navelina”), *Citrus sinensis* (cv. “Lane Late”), *Citrus sinensis* (cv. “Valencia Delta”) and *Citrus clementina* (cv. “Clemenules”). Non-citrus species screened were: *Hedera canariensis, Bergera koenigii, Ficus benjamina, Ruta* spp.*, Choisya ternata, Buxus sempervirens, Ficus repens, Hedera helix, Laurus nobilis and Nerium oleander*. First, a free-choice completely randomized block design test was conducted using 900 adults released in a greenhouse containing either citrus or non-citrus species. The number of adults, the number of eggs and the presence of nymphs on plants were counted 1, 3, 7, 15 days after release. Non-choice tests were also conducted with the most promising species. Preliminary results obtained so far show that *Choisya ternata, Hedera canariensis, Ruta* spp. and *Ficus benjamina* were non-hosts, while lemon (cv. "Verna") and Kaffir lime were the least preferred *Citrus* hosts for *T. erytreae*.

Keywords: *C*Las, HLB, *Citrus*, ornamentals, resistance, host plant preference

## OC416. Pheromone-based control of the strawberry tortrix, *Acleris comariana*, a major pest in Danish and Swedish strawberry production

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The strawberry tortrix, *Acleris comariana* (Lepidoptera: Tortricidae), is a destructive pest on strawberries in Denmark and southern Sweden. Larval feeding on flower buds results in small and malformed berries, which are rejected by consumers. Traditional insecticides are not sufficiently efficient for population control of *A. comariana*, and alternative management strategies thus need to be explored. We recently identified the female-produced sex pheromone of this pest as the single compound (*E*)-11,13-tetradecadienal (*E*11,13-14: Ald). In this study we evaluated the efficacy of pheromone-based mating disruption of *A. comariana*. In 2020-2021, pilot studies were performed treating 1 ha plots in large (>10 ha) commercial strawberry fields in southern Sweden. The total dose of *E*11,13-14: Ald applied per treatment plot was 7 g (2020) or 0.7 g (2021) with a second application after 3 weeks. In spite of the low doses of pheromone applied in these experiments compared to similar studies on other tortricid pests, catches of males in pheromone-baited traps were reduced by 98-100% in treatment plots versus control plots. In contrast, a corresponding reduction in larval numbers in the subsequent generation was not achieved, most probably due to immigration of mated females into the relatively small treatment plots. In 2023 whole crop fields will be pheromone-treated, and the preliminary results from these experiments will be presented. Efficient mating disruption of *A. comariana* could be an important part of integrated pest management to increase yields in Danish and Swedish strawberry production.

Keywords: *Acleris comariana*, strawberry, sex pheromone, pest control, mating disruption

## OC417. Exploring yeast-based formulations for environmentally friendly management of *Drosophila suzukii*: efficacy, chemical characterization and potential for attract-and-kill approach

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*Drosophila suzukii* is a global invasive pest of soft-skinned fruits. Its control is heavily relying on insecticide use but environmentally friendly and affordable management is being investigated. The flies and their fruit hosts are often associated with yeast communities which can be exploited in management systems. Among these, *Hanseniaspora uvarum* and *Saccharomycopsis vini* were isolated from *D. suzukii*-infested grapes and identified as the most attractive yeasts to the ovipositing females. Within the Dromytal project and following activities, we evaluated the efficacy of these two yeasts to attract gravid females in field assays and assessed how they could be exploited. Yeast-based formulations combined with insecticides were used in attract-and-kill approach in sweet cherry experimental fields for two years. Both formulations performed similarly in the first year. However, *S. vini* was significantly less attractive in the second year. Behavioural responses to yeasts are mediated via the detection of yeast volatiles with a highly specialized olfactory system. These semiochemicals can serve as a signature for each yeast species and contribute to their attractiveness in the field. We thus characterized the chemical composition of the volatile organic compounds released by the two yeasts to identify which compounds are detected by the flies and may induce the differences in attraction and oviposition. The main difference between the two yeasts was the release of several terpenoids by *S. vini*. We further identified which of these were detected by the flies with electro-antennography. These results imply the possibility of using both yeast-based formulations in an attract-and-kill approach.

**Keywords:** spotted wing drosophila, sustainable management, yeast formulation, sustainable agriculture

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## OC418. Host plant volatile lures attract the poplar bark beetle *Trypophloeus* (Coleoptera: Scolytidae)

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The poplar bark beetle *Trypophloeus* spp. (Coleoptera: Scolytidae), is a serious pest of poplar trees (*Salicaceae*, Genus *Populus*) across North Spain. Among the more than two hundred poplar clones available on the market, clone USA 184/411 has the highest susceptibility to *Thrypophloeus* attacks. In this study, we set out to identify and evaluate the potential attractiveness of host plant volatiles released by two poplar clones with very high different susceptibility to *Trypophloeus* spp. to lay the groundwork for the development of field attractants for their populations. The chemical volatile profiles of those *Populus* USA 184/411 and I-214 clones were determined by GC-MS. Coupled gas chromatographic-electroantenographic detection (CG-EAD), was carried out to identify compounds that elicit antennal response of *Trypophloeus* spp.GC-EAD analysis of the Tenax TA-captured volatiles from the bark and leaves of both clones revealed two compounds that consistently elicited antennal responses by adult male and female beetles. One of these, SA, significantly increased the number of catches of *Trypophloeus* adults in traps baited with ethanol in poplar plots located in Villasabariego, Villoria and Turcia (León province, NorthSpain).In addition, the most favorable temperature ranges for its capture have been observed in León province poplar plots. Regarding the flight of the insect, no notable peaks have been observed, but rather a staggered emergence. It will be necessary to continue with new research to continue providing new control tools for this pest.

Keywords: *Populus* spp., *Thrypohloeus*, bark beetle, attractants, monitoring

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## OC419. “Catch me if you can” – improving monitoring and control of vine weevil (Coleoptera: Curculionidae) in soft fruit and ornamental crops

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Vine weevil, *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae), is one of the most economically important insect pests of soft-fruit and ornamental crops globally. Growers currently lack effective and reliable monitoring systems to determine the presence of adult vine weevil within crops, meaning that controls are often applied too late to prevent economically significant losses. To identify an effective and reliable monitoring system for adult vine weevil, this study used paper cups as monitoring tools to investigate the influence of visual (colour, height, diameter as well as monitoring tool and plant density) and olfactory (conspecifics, host plant) cues on monitoring tool efficacy. Results indicate that vine weevil adults showed a preference for dark, tall and large-diameter monitoring tools. Increasing monitoring tool density had the effect of increasing the numbers of weevils recorded in monitoring tools. By contrast increasing plant density, within the range tested, did not affect the numbers of weevils in monitoring tools. Volatile chemical stimuli also influenced the number of weevils recorded in monitoring tools. More vine weevils were recorded in monitoring tools baited with yew or yew plus conspecifics compared to those left unbaited. The presence of only conspecifics did not increase the numbers of vine weevils recorded in monitoring tools.These results provide the first steps toward developing an effective and reliable vine weevil monitoring tool based on an improved understanding of vine weevil visual and olfactory ecology.

Keywords: Curculionidae, IPM, traps, visual cues, olfactory cues, semiochemicals

## OC420. A novel insect food-based attractant: the case of olive fruit fly *Bactrocera oleae*

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The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most important pest of olive culture in the Mediterranean basin as well as in other regions across the globe, affecting the quality and quantity of both oil and table olives. Food-based attractants are crucial for the monitoring as well as the control of *B. oleae* adults. However, commonly used food-based attractants have shown low attractiveness to olive fruit fly adults and are dependent on the prevailing climatic conditions such as temperature and humidity, resulting in underestimated *B. oleae* populations in the field and ineffective control. The aim of this study was to assess the efficacy of a novel attractant 'Na' compared to two commonly used food-based attractants, namely ammonium sulfate 'As' and hydrolyzed protein 'Hp', in attracting *B. oleae* adults in McPhail traps placed in olive orchards. We also evaluated the selectivity of these attractants towards natural enemies. Additionally, we investigated the potential of 'Na' for controlling *B. oleae* populations through a mass trapping system with plastic McPhail traps. The results of the study revealed that 'Na' captured significantly more olive fly adults throughout the cultivating season compared to 'As' and 'Hp' and did not capture high number of natural enemies. Moreover, the application of the novel attractant in mass trapping systems, resulted in significantly lower levels of both olive fly populations and fruit infestations compared to the control. These findings support that the novel attractant can be effectively used for monitoring and controlling the olive fruit fly.

**Keywords:** novel attractant, monitoring, control, olive fruit fly, *Bactrocera oleae*

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## OC421. Implementing the 'female removal' technique for apple orchard protection: recent results in the Trentino-Alto Adige region

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The key pest of pome fruit crops *Cydia pomonella* (Lepidoptera: Tortricidae) can cause even total economic loss in apple production. Fruits damaged by the larvae are susceptible to rotting and therefore completely unmarketable. While using synthetic insecticides allows keeping *C. pomonella* populations under control, few strategies are available in organic farming. Recent characterization of volatile compounds with kairomonal activity reopens the possibility to develop mass trapping techniques for *C. pomonella* by primarily targeting females, providing a new tool in organic farming. This study aimed to evaluate the female removal technique by using non-saturating bucket traps lured with the attractive 4K, based on pear ester, acetic acid, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and linalool oxide in apple orchards of Trentino-Alto Adige (Italy) during summer 2022 and 2023. Treatments based on paired plots of ca. 1 ha each whit and without (as control) buckets traps were performed. To evaluate the technique's effectiveness, a fruit damage assessment was conducted in pre-harvest by visually inspecting about 2,500 fruits per plot. At the end of the first year trials, the mean number of *C. pomonella* captured was

1.123,8/ha in organic orchards and to 540/ha in integrated orchards. Insects captured during the trials were sexed and the reproductive status of females was determined, allowing to find a high percentage (mean 59%) of females. Second-year trials are still ongoing and will be also shown. Preliminary analysis allows female removal to be considered a promising technique to significantly reduce the pressure of *C. pomonella* within organic apple orchards in northern Italy.

**Keywords:** codling moth, integrated pest management, organic agriculture, chemical ecology, codlemone, pear ester

## OC422. Integrated pest management and field efficacy of insecticides on potato tuber moth *Phthorimea opercutella* (Lepidoptera: Gelechidae)

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The potato tuber moth is considered one of the most serious potato pests, attacking potato leaves and tubers in the soil and stores. Also, it attacks tomatoes, eggplants, tobacco, and some weeds mostly *Solanum nigrum* and *Datura stramonium,* which are abundant in Greece.Type delta sex pheromones traps were used for monitoring the flights and the generations per year. Three generations were estimated in northern Greece. Capturing starts by the end of May and the maximum number of moths were caught in the middle of July, Augustand September. The main attacks on the tubers start by the end of the season when the leaves start to decay. Field experiments were conducted for estimating field efficacy of insecticides and combinations of them. The complete randomized block design was used with four replications, applying four foliar spays in intervals of 15 days, from the start of July till the end of August (harvesting period). Results showed that the average tubers' damage caused by the insecticides was about 16% and in the control was 67%. The best treatment was given by the chlorantraniliprole, with 15% damage. The insecticides lambda- cyhalothrin (zeon), metaflumizone, spinosad, clothianidin, a botanical oil mixture and a mix of beauveria basiana plus lambda- cyhalothrin gave equal satisfactory control with 20,7% damage. The Economic threshold to start spraying is 40adult captures per trap in a three-day period. For best results the sprays must start after the nightfall. Cultural practices must be applied also to avoid resistance problems.

**Keywords:** potato tuber moth , *Phthorimea opercutella ,* insecticides, potato, Gelechidae

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## OC423. First steps of developing a novel push-pull-kill strategy: Primary and secondary plant metabolites involved in the attraction and feeding behavior of BMSB

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The brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive pest native from Eastern Asia and has already gained pest status in most of the USA, as well as in southern and western Europe. It has a wide host range of about 170 different plants and causes severe damage to agricultural crops. The significantly increased application of broad-spectrum insecticides has severely disrupted previous integrated pest management (IPM) programs, causing negative economic and environmental impacts. Exploration of chemicals that exhibit attraction or repellent activities toward major stink bug species would be beneficial in developing push-pull strategies. Field monitoring of BMSB showed a high abundance of *H. halys* nymphs and adults on specific shrubs. These plants appear highly attractive despite being utterly distinct from typical host plants. These plants may have arrestant**,** attractant, or phagostimulant properties against stink bugs. Aiming to develop a biotechnical plant protection strategy against the BMSB in organic horticulture, we intend to develop a push-pull-kill strategy by encapsulating attractant volatiles (push) and feeding stimulants combined with biological insecticide or microbial antagonist (kill component). Screening compounds of the typical scent of those attractive shrubs and metabolome from their fruits, together with the behavioral reaction of the BMSB, is the first step in developing a potential attractant that can be used in a capsule-based attract-and-kill strategy. The first results of these plants' chemical analysis, both volatile and fruit metabolome components, and biotests with nymphs and adults of BMSB will be presented.

Keywords: attract-and-kill, BMSB, arrestant, IPM, volatiles, metabolome

## OC424. Study of the winter ecology of *Palomena prasina* (L.) (Hemiptera: Pentatomidae) in a perspective of Integrated Pest Management

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*P. prasina*, the green shield bug (GSB), is widely distributed in the Palearctic region. In the Southwest of France it is considered a serious pest of hazelnuts, its feeding punctures leading to blank nuts and kernel necrosis, causing heavy losses in commercial orchards. To date, there is no Integrated Pest Management strategy available to control *P. prasina*. Pest control strategies often focus on the Spring - Summer ecology of pests, when these are in-field or in the vicinity of crops. However, the abundance of pest populations in crops is also related to the Autumn-Winter ecology of pests. Therefore, to find new opportunities for pest control, there is a need to better account for the overall temporal and landscape ecology of pests. In the present work, we focused on the Autumn-Winter ecology of *P. prasina* to investigate i) where *P. prasina* overwinters, ii) if it aggregates in the overwintering sites, and iii) if it mates while overwintering. Samples were collected in different habitats (forests, hedges, orchards, houses) and microhabitats (litter, bushes/trees, dead trees, human made structures), in two years. The reproductive status of GBS individuals was monitored in Winter and when they emerged from overwintering sites, in Spring. Our results show that *P. prasina* adults overwinter individually in the litter of crop and natural habitats, and only mate after leaving the overwintering sites. The abundance of GSB in those sites is negatively correlated to litter temperature and positively to humidity levels. We discuss these results in a perspective of IPM.

Keywords: green shield bug, hazelnuts, overwintering, reproductive status

## OC425. Exploring refuge sizes to delay resistance by western corn rootworm to Bt maize

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Transgenic crops engineered to produce insecticidal toxins from the bacterium *Bacillus thuringiensis* (Bt) have been used in pest management for nearly 30 years. When planted with Bt crops, refuges of non-Bt host plants delay evolution of Bt resistance by reducing the frequency of homozygous resistant individuals. The higher the dose produced by the Bt crop, the lower the dominance of resistance, and the smaller the refuge size required to delay resistance evolution. Bt crops producing two toxins targeting the same pest are also paired with refuges to delay resistance. Additionally, fitness costs of resistance select against resistance within the refuge. Western corn rootworm is a major pest of maize in the US and an invasive species in Europe. Bt maize has been used for management of western corn rootworm in the US since 2003, and to date has evolved resistance to all four commercially available Bt toxins used to manage this pest. In the primary maize growing region of the United States, minimum integrated refuge sizes for Bt maize targeting western corn rootworm are 10% for single-toxin hybrids and 5% for two-toxin hybrids, while minimum structured refuge sizes are 20% for single-toxin hybrids and 5% for structured refuges. We applied a computer model to data from laboratory experiments measuring inheritance and fitness costs of resistance to estimate appropriate refuge sizes to delay resistance to Bt maize by western corn rootworm. The results of this study can help to improve strategies to manage resistance to Bt maize by western corn rootworm.

**Keywords**: *Bacillus thuringiensis*, western corn rootworm, resistance management

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## OC426. Inheritance and Fitness Costs of Field-Evolved Resistance to Gpp34/Tpp35Ab1 Maize by Western Corn Rootworm (Coleoptera: Chrysomelidae)

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is a pest of maize in the United States and Europe. Since 2003, this pest has been managed in the United States with maize hybrids that produce insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Bt). Bt maize reduces root-feeding injury by WCR larvae. The widespread use of the Bt protein Gpp34/Tpp35Ab1, and other factors, may have contributed to the field-evolved resistance documented to Gpp34/Tpp35Ab1 in some WCR populations in the United States. Insect resistance management (IRM) plans include the refuge strategy to delay evolution of resistance to Bt crops, where refuges of non-Bt host plants serve as a source of Bt-susceptible individuals. Mating between resistant individuals and susceptible individuals from refuges produces progeny that are heterozygous for resistance. As such, the dominance of resistance will influence the rate of resistance evolution, with more dominant resistance contributing to a higher rate of resistance evolution. The effectiveness of a refuge in sustaining Bt susceptible individuals can be compromised when resistance alleles accumulate in the refuge. However, fitness costs act to reduce resistance allele frequency within refuges and preserve the dynamic of refuges producing Bt-susceptible genotypes. Our objectives were to measure the inheritance and fitness costs of resistance to Gpp34/Tpp35Ab1 maize for WCR strains with field-evolved

resistance. This research will aid in assessing the risk of continued resistance evolution and developing more effective IRM approaches to manage WCR and other agricultural pests.

**Keywords:** Bt crops, insect resistance management, maize, population genetics, refuge strategy, rootworm

## OC427. CRISPR-Cas12a-based biosensing of pests

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Early detection of pests is critical for efficient control and elimination. Current identification approaches suffer from limitations such as time-consuming procedures, requirements for transport to centralized facilities, or highly trained personnel and sophisticated equipment. Here, we present a CRISPR/Cas12a-based approach for the identification and differentiation of two fruit fly pests, Ceratitis capitata and Bactrocera zonata, which are difficult to distinguish in early developmental stages. CRISPR/Cas12a detection is based on DNA sequence identification via a guide RNA, complementary to the target sequence to be identified. Upon recognition, Cas12a adopts a non-specific, single-stranded DNAse activity, releasing a fluorophore and resulting in a quantifiable fluorescent signal. Our protocol offers on-site detection within less than two hours, high specificity and sensitivity, and supports sample pooling to save both reagents and time. Additionally, the system's activity was tested in microgravity on the International Space Station, demonstrating its potential for developing self- and pest-diagnostic kits for long space missions. This study offers a fast, affordable, and easy-to-handle approach for early fruit fly pest detection, which could be expanded to many other pests of interest.

**Keywords:** CRISPR-Cas12a, biosensors, pest detection, *Ceratitis capitata, Bactrocera zonata*

## OC428. The oak pinhole borer, *Platypus cylindrus* F. (Coleoptera; Curculionidae): population dynamics and association with *Diplodia corticola* in productive stands of cork oak

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Changes in climate regimes together with the irruption of exotic and emergent pathologies are challenging the sustainable management of cork oak stands (*Quercus suber*). *Platypus cylindrus* is a xylomycetophagous beetle able to kill mature cork oaks in short periods of time (months). Despite the gaining economic impact of this species, its flight phenology during cork harvesting season is still unclear. Additionally, it is known that *P. cylindrus* can act as carrier of fungal pathogens, nevertheless, its role as vector of the oak pathogen *Diplodia corticola* (Botryosphaeriaceae) requires more research. We developed two complementary studies in the core cork-producing area of N-E Spain in 2021 (April-October). The first one aimed to model flight period of *P. cylindrus* during cork harvesting season (pheromone-baited trapping); the second one investigated the presence of *D. corticola´*s propagules associated with this insect. We fitted generalized additive models (GAMs) to investigate flight curve and we found early emergence pattern in May, followed by two-three capture peaks in June and July. In addition, GAMs highlighted the microclimatic window associated to high rate of captures. Otherwise, we confirmed *P. cylindrus* as carrier of *D. corticola* through a new molecular approach. We developed a nested quantitative PCR protocol based in a mitochondrial marker that allow us to detect DNA of *D. corticola* in the insects and estimate spore loads. The number of propagules carried significantly varied throughout the season and reached, as average, 33 spores/insect. The complementary use of modelling and molecular tools provided new insights of this understudied pathosystem.

**Keywords:** emergent forest pest, GAM, Insect-microorganism interaction, qPCR, spore load

## OC429. Facultative endosymbionts; new biological control agents of grain crop aphids and their horizontal transmission

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Aphids are major crop pests in southeastern regions of Australia. Some aphid species harbor heritable facultative endosymbionts that may induce either beneficial or detrimental impacts on aphids under certain ecological conditions. Aphid-parasitoid interactions can be greatly affected by facultative endosymbionts but there is limited research on species of economic significance. Here we assessed the effects of a facultative endosymbiont, *Ricketsiella viridis,* on parasitism of the major crop pests *Myzus persicae* and *Rhopalosiphum padi* by *Diaeretiella rapae* and *Aphidius colemani*, respectively. We found that *R. viridis* doesn't provide either *M. persicae* or *R. padi* with significant protection against *D. rapae* and *A. colemani*. However, fecundity of *M. persicae* is reduced due to infection with *R. viridis* regardless of the presence of parasitoids. Moreover, our results suggest that different parasitoids facilitate horizontal and subsequent vertical transmission of facultative endosymbionts such as *R. viridis*, *Regiella insecticola* and *Wolbachia* spp. in aphid populations. According to our data, simultaneous release of *D. rapae* and *M. persicae* infected with *R. viridis* in the early cropping season (lower population densities and cooler conditions) may be an effective strategy to control this pest. These results will help inform the costs and benefits associated with the newly generated infection which will dictate its potential as a biocontrol agent.

**Keywords:** grain crop aphids, facultative endosymbionts, biological control agents, horizontal transmission

## OC430. IPM strategies for managing leatherjackets in pasture

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Leatherjackets are the larvae of crane flies. In Northern Ireland the predominant pest species is *Tipula paludosa* (Tipulidae: Diptera). Having laid their eggs in late summer / early autumn, the larvae (leatherjackets) then develop over the winter months, reaching maturity in the spring, before pupating during the summer. Leatherjackets feed on the roots and stems of plants just below the surface and can cause significant damage (loss of yield and large bare patches) to pasture. In particular, grass reseeds and new leys can be devastated if leatherjackets are not controlled. In the past leatherjackets were controlled by agrochemicals containing chlorpyrifos (e.g. Dursban®); however, these products were banned in April 2016. New mitigation strategies are therefore required. Following discussions with farmers' groups and feedback from webinar sessions, a draft IPM framework has been constructed. The strategy consists of combined long-term, medium-term and short-term approaches to leatherjacket management. Recognising that there will be no silver-bullet replacement to chlorpyrifos, the framework uses a combination of standard agronomic approaches (drainage, reseeding, liming) coupled with tight autumn grazing and regular monitoring of leatherjacket counts. A number of factors (e.g. slurry application, silage cuts, rush control, natural enemies, multi-species swards) that have an influence on leatherjacket populations are factored in for further study. The intention is that this framework will be refined by discussions with farmers. In particular, the practicalities of implementing some of the approaches needs to be clarified.

Keywords: IPM, *Tipula*, insecticide, grassland, farmers

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## OC431. Field assessment of an IPM scheme for *Bemisia tabaci* based on laboratory scientific evidence

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The cotton whitefly *Bemisia tabaci* is the most destructive pest of vegetable crops worldwide. Due to the development of extreme resistance phenotypes, *B. tabaci* control with conventional chemistries is problematic. Recently, novel products based on green chemicals were evaluated under laboratory conditions exhibiting high efficacy levels. Based on the aforementioned scientific evidence an Integrated Pest Management (IPM) scheme was designed aiming to evaluate the efficacy of the tested green-chemicals on *B. tabaci* under controlled greenhouse conditions, in comparison to the standard conventional control scheme. An eight-compartment hydroponic tomato greenhouse was used, where each pest control scheme was implemented in four of the compartments ($12m^2$ with 12 plants) artificially infested with a resistant *B. tabaci* population. The IPM scheme included the use of the parasitoid *Eretmocerus eremicus* and mass trapping. Pest status was estimated weekly, throughout the six-month cropping season, based on the number of adults per leaf and the number of nymphs on each plant top. In the conventional control scheme a total of six applications were required alternating insecticides spiromesifen, thiacloprid, flupyradifurone and sulfoxaflor. In the IPM scheme 11 spray applications were conducted alternating maltodextrin, terpenoids, plant extracts, fatty acids and silica. Pest status was comparable between the two control schemes, however towards the end of the cropping season both adults and nymphs counts were significantly higher in the conventional scheme. Tomato quality parameters were evaluated and some exhibited significant differences. Residues for all four compounds were below the established EU Maximum Residue Levels (MRLs).

Keywords: *Bemisia tabaci,* IPM, novel insecticides, greenhouse, tomato

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## OC432. Integrated Pest Management strategies for cabbage stem flea beetle in the UK

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The cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala* L.) is the most abundant and widely distributed autumn pest of winter oilseed rape (OSR, *Brassica napus* L.). Traditionally, this pest has been controlled with the use of synthetic insecticides, mainly neonicotinoid seed treatments and pyrethroid sprays. However, in 2013 the EU restricted the use of neonicotinoid seed treatments for OSR, reducing the number of available insecticides to control CSFB to just one group: pyrethroids. Despite the EU efforts to reduce insecticide use, current control methods still rely upon the sustained and often prophylactic use of pyrethroids, which has caused widespread resistance in CSFB to pyrethroids and associated problems of environmental harm. Due largely to difficulties in controlling CSFB, many farmers are giving up growing the crop and the area sown in EU has fallen sharply. To maintain sustainable production of this important crop, there is a crucial need for alternative and environmentally benign management strategies for this pest. In this presentation we will cover our research on different aspects of integrated pest management of CSFB. We will present some recent research on management and weather factors affecting CSFB populations and how we are exploring the possibility of creating resistant cultivars. Lastly, we will highlight our research into CSFB biocontrol, looking at the distribution and parasitism rates of both CSFB adult and larval parasitoids in the UK.

**Keywords:** biocontrol, *Brassica napus*, *Psylliodes chrysocephala,* insect pest control, sustainable agriculture

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## OC433. Developing IPM techniques for the spherical mealybug, *Nipaecoccus viridis* for citrus orchards in a region of endemic Huanglongbing

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The spherical mealybug, *Nipaecoccus viridis*, is a recently established invasive pest in Florida with most damage to date seen in citrus orchards, which are actively managed for *Diaphorina citri*, the primary vector for *Candidatus* Liberibacter asiaticus that leads to Huanglongbing disease. Despite management activities, *N. viridis* has successfully established and led to new pest management challenges necessitating a rethinking of citrus arthropod management and a need to rebuild these practices based on proper implementation of integrated pest management (IPM) strategies.

As a new pest in Florida citrus orchards, nothing was known about the seasonal phenology of *N. viridis* and how that could be incorporated into developing a management program. Due to the temperate Florida climate and multivoltine nature of *N. viridis*, we anticipated multiple generations throughout the year. To determine the seasonal phenology of *N. viridis* populations, we performed biweekly monitoring in six commercial citrus orchards in central Florida.

Results showed that *N. viridis* completes 3 generations per year in central Florida, two of them being clearly defined and resulting in high populations that are largely driven by the juvenile life stages. The first population peak occurs from March to May immediately after flowering as fruit are setting and beginning the early stages of development. Later peaks are not clearly tied to tree phenology, but driven by the reproductive cycle of *N. viridis*. These data are the basis for current insecticide-based management while we seek for alternative strategies for the control of this pest.

**Keywords:** citrus, mealybug, phenology, integrated pest management

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## OC434. Integrated management of *Dysmicoccus grassii* Leonardi (Hemiptera: Pseudococcidae) based on *Acerophagus artelles* releases in commercial banana crops in the Canary Islands

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Banana mealybug *Dysmicoccus grassii* is one of the main pests in banana crops in the Canary Islands. Despite none of its natural enemies had been successfully used in an Integrated Management program, our laboratory and semi-field studies with the endemic parasitoid *Acerophagus artelles* showed a promising potential. Thus, a field trial was carried out in four commercial banana crops in 2018 with three treatments considered: 1. Chemical Control, based on the use of authorized pesticides; 2. Integrated Pest Management (IPM) based on inoculative releases of *A. artelles* and focus releases of *Cryptolaemus montrouzieri*, and 3. Integrated Pest Management + Ant Control (IPM+AC), combining IPM with the application of entomological glue (PEGAFIT®, Koppert) in plants pseudostem. On average, the pest density in plants was 2.5 times higher in Chemical Control plots. In clusters, Chemical Control plots registered 2 and 3 times more pest than IPM and IPM+AC respectively. The percentage of clean leaves increased throughout the trial in IPM and IPM+AC plots and decreased in Chemical Control plots. The percentage of clean clusters in collected season in Chemical Control plots was 20 and 25% lower than IPM and IPM+AC respectively. Ant density was also much higher in Chemical Control plots both in plants and clusters, being absent in clusters in IPM+AC treatment. The percentage of parasitism did not differ significantly between IPM and IPM+AC, although it was more than 3 times higher than the registered in Chemical Control plots.

**Keywords:** banana, Integrated Pest Management, *Acerophagus artelles*, *Dysmicoccus grassii*

## OC435. Advancing current IPM schemes based on scientific evidence and farmer engagement: the ZeroTuta project

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Pest management in the European Region is entering a period of major changes and challenges due to the implementation of the Green Deal EU regulations. Therefore, the limited range of registered pest control tools have to be used with high precision and in conjunctions with alternative control strategies. At the same time, targeted agricultural practices have to be enhanced at farmer level while novel plant protection products have to be evaluated for potential incorporation in future IPM schemes. The aforementioned bundle of actions are currently implemented in commercial tomato greenhouse in Southern Crete (Tympaki Cooperative) under the frame of the ZeroTuta project. Resistance monitoring and management, expected control likelihood and estimation of mutation frequency in local pest populations is one axis of interventions already successfully implement in experimental level in prior projects. The second and novel axis of interventions includes, farmer training, strict pest population monitoring, infestation level estimation and technical advisory services for optimum real time adjustment of the IPM scheme. In this presentation, each of the aforementioned interventions will be analyzed and the initial results will be presented. The scope of the project will be to highlight critical parameters that require attention for advancing current IPM schemes thus establishing a sustainable and viable crop production in the era of the Green Deal.

**Keywords:** *Tuta absoluta*, Bemisia tabaci, insecticide resistance

## OC436. The role of the landscape in supporting hoverfly populations and natural pest control of aphids

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Pest-regulating insects are broadly considered to benefit from habitats other than agricultural fields. These other habitats can be important in providing hibernation sites, alternative prey, or floral resources. The relative importance of these different habitats to the performance of pest-regulating insects is difficult to study empirically. This is where habitat-structured population-dynamical modelling can play an essential role. In this study, we modelled the dynamics of aphidophagous hoverflies (Diptera: Syrphidae) and investigated their effectiveness as control agents of aphids. Whilst larvae of these hoverflies feed on aphids, and enforce natural pest control, adult hoverflies require floral resources. Both types of resources are often spatially and temporally segregated in agricultural landscapes. Therefore, also in our model, hoverflies move between different habitats in the arable landscape. The model is designed and parameterised based on field observations of temporal and habitat-related availability of resources. The model indicates optimal hoverfly performance and aphid suppression when different habitats are present that can provide aphid and/or floral resources at different times of the year. Woody habitat can provide aphid and floral resources early and late in the year, and flower margins during the growing seasons of the crops. Arable crops, such as winter wheat and potato, can enhance each other's pest control when their aphid populations peak at different moments in time. The model can also be used to optimise agricultural management techniques, such as mowing and planting of flower margins, in such a way that resources are spatially and temporally complementary in the landscape.

**Keywords:** natural pest control, conservation biological control, aphidophagous hoverflies, population dynamical modelling, complementary habitats, landscape complementation

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## OC437. The impact of reduced tillage and distance to field margin on predator functional diversity

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Agricultural intensification results in biodiversity loss through land conversion and management practices. The abundance and diversity of ground-dwelling predators, e.g. ground beetles (Coleoptera: Carabidae) and spiders (Araneae), are negatively affected by soil disturbances such as tillage. Reducing soil disturbances can potentially conserve arthropod populations in the field and reduce the use of chemical pest control. The present study investigated the ground-dwelling predatory community using pitfall traps in cereal fields with three different levels of soil disturbance: conventional tillage (CT), reduced tillage (RT) and no tillage under Conservation Agriculture management (CA), in a two year field trial. Pitfall traps were placed in transects from the field margins. Overall, the activity-density of ground-dwelling predators was higher in fields with minimum soil disturbance and generally declined with increased distance to semi-natural habitats. Functional diversity, expressed by the body size of ground beetles, was also affected by soil disturbances; large ground beetles more consistently occurred in CA, while few or none of the largest ground beetles were found in RT and CT. In the second year only, species diversity was higher along field edges bordering semi-natural habitats when compared to the field area. Our results show that conservation of epigeic natural enemies in the agricultural landscape is improved by reducing soil-disturbing events such as tillage.

Keywords: soil disturbances, conservation agriculture, biodiversity, Carabidae, Araneae, semi-natural habitats

## OC438. Region wide assessment of the distribution of *Cacopsylla pruni* — main vector of European stone fruit yellows — and an agroecological approach for its management

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Phytoplasma diseases are a major problem in fruit production and cause significant economic losses. However, they remain very difficult to control and are poorly documented. *Cacopsylla pruni* (Psyllidae) is known to be the main vector of *Ca.* P. prunorum, the causing agent of European stone fruit yellows (ESFY). This psyllid produces one generation per year, is strictly oligophagous on Prunus spp. and overwinters on conifers and then migrates in spring to prunus trees where it breeds. During the breeding season, the psyllid passes through several hosts including apricot (*Prunus armeniaca*) and wild plum (*Prunus spinosa*). Here we describe its phenology in relation to weather and local conditions, as well as the incidence of *Ca.* P. prunorum with regard to host species (*Prunus spinosa* vs. *Prunus armeniaca*) and local conditions and finally correlate phytoplasma incidence in *Cacopsylla pruni* with incidence on apricot. In a region wide assessment of the vector in 90 study sites the psyllid were sampled on its principal host species and tested for the presence of the phytoplasma. The gained insights in the distribution of vector and disease allow pathogen flow modelling at the landscape scale. This will in turn enable the identification of management and control strategies against the vector and pathogen at the local scale.

Keywords: ESFY, secondary hosts, agroecology, *Cacopsylla*

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## OC439. Probing on tillage-alternatives juvenile Aphrophoridae vectors of *Xylella fastidiosa* (JAveX) control

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Vector management is law-required to restrict the spread of *Xylella fastidiosa* (*Xf*): juvenile management, established by winter/spring tillage, aims to disrupt and interrupt the postembryonic development of juvenile spittlebugs. Tillage to control juvenile Aphrophoridae vectors of *Xf* (JAveX) is only viable in some agricultural settings and is not sustainable in the long term. Improper soil management burdens the farm balance, alters the carbon balance, and results in organic matter loss.

JAveX control acts on pre-viruliferous stages and, contrary to adult control, does not allow the spread of the bacterium until adulticide treatment. Thus, we might propose tillage alternatives for JAveX control in this scenario.We present a three-year (ending in fall 2023) laboratory/field investigation of JAveX control. We selected tillage-substitute means of action (MoA) for their low impact on non-target entomofauna. In the laboratory, we tested the control efficacy of various substances by recording post-treatment field-collected JAveX survivorship. We repeat exposure to MoAs three times consecutively each year, allowing us to divide efficacy results into three instars intervals (intervals: II-III instars; III-IV

instars; IV-V instars). We quantify post-treatment JAveX (n./sqm) in field trials using AquaSampling (ASP). To conduct ASP, we systematically collected JAveX host plants within transects to rinse them and collect juveniles.
Statistical comparison of efficacy will allow the identification of effective substances for managing *Xylella fastidiosa* Aphrophoridae vectors.

**Keywords**: OQDS, insect-borne, *Xylella fastidiosa*, vector management

## OC440. Convergence in red palm weevil and *Xylella*-vector invasion events and consequent IPM options

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Experience in both *Rhynchophorus ferrugineus* (Olivier, 1790; RPW) and *Xylella fastidiosa pauca* Wells, Raiu et al., 1986 ST53 (Xf) invasive events suggest comparing the available management options, both the invasives organisms' interplay with their insect vector. The RPW interacts with a guild of bacteria and yeasts thriving in female Weevil genital lumina. Adult *Philaenus* or *Neophilaenus* spread Xf multiplying in spittlebugs' foregut. The vector/borne species interplay results in more than proportional damage to each vector's host/food plant. Bacterial transmission and the subsequent infective process are key points in RPW and Xf invasions. RPW-borne bacteria can damage to kill the infected palm, leaving the Weevil as a secondary actor in the scene, while Xf desperately needs spittlebugs to propagate over the land. Direct control actions versus insects, either the Weevil or the Spittlebug, will fail. Each vector can infect much more than one single plant propagating the insect-borne plant pathogenic microorganism to plants within the range of insect dispersal ability. A way to mitigate the pathogen spread may consist in killing vectors at their first feeding on concerned plants. This way pushes back to proportional or less than proportional the damage for that vector/pathogen interplay. Once a direct proportionality exists, the damage will correspond to the number of the key events, restituting a sense to a threshold evaluation process. Simple life table or more complex lattice modelling approaches support the analysis and the opportunities for effective IPM strategy building in RPW or Xf invasions and expected similar events.

**Keywords:** alien, invasive, quarantine pest, vector management

## OC441. Evaluation of *Mentha pulegium*, *Ocimum basilicum* and *Origanum majorana* essential oils encapsulated in nanosystems against *Tetranychus urticae, Aphis gossypii* and *Tuta absoluta*

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In this study the potential of *Mentha pulegium*, *Origanum majorana* and *Ocimum basilicum* essential oils (EOs) after their encapsulation in yeast cells or β-cyclodextrin was investigated against nymphs and adults of *Tetranychus urticae* (1‰ (v/v), on nymphs of *Aphis gossypii* (0.5‰) and eggs of *Tuta absoluta* (0.5‰) applied by spraying. Mortality was recorded daily for 7 days after treatment. The main components were pulegone, isomenthone for *M. pulegium* EO, terpinen-4-ol, γ-terpinene for *O. majorana* EO, and linalool, eucalyptol, for *O. basilicum* EO, respectively. In the case of *T. urticae* the emulsion of free *M. pulegium* EO caused 66% mortality on adults which increased to 84% after encapsulation in β-cyclodextrin. Similarly, the emulsion caused 58% on *T. urticae* nymphs but when encapsulated in β-cyclodextrin or yeast cells it was increased to 88% and 90%, respectively. After sprayed with the free *O. majorana* EO the nymphal mortality rate was 66%, while after treated with oil encapsulated in β-cyclodextrin or yeast cells the mortality increased to 80% and 88%, respectively. Generally, the encapsulated oils showed a prolonged bio-activity than the emulsion. The emulsion of *O. basilicum* free EO was the only that caused a relatively high mortality rate on *A. gossypii* and *T. absoluta* (42% and 32%, respectively). In conclusion, the EOs of *M. pulegium* and *O. majorana* showed a high efficacy against *T. urticae* which was further improved by encapsulation in both nanosystems. Further studies should be conducted to evaluate these encapsulation systems against these major pests under laboratory and field conditions.The work was implemented in the framework of the RESEARCH-CREATE-INNOVATE Action co-financed by the European Regional Development Fund (ERDF) of the European Union and national resources through the OP. Competitiveness, Entrepreneurship & Innovation (EPANEK) (project code: T2EDK-03105).

Keywords: mortality, β-cyclodextrin, yeast cell, spider mite, tomato pinworm, pest

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## OC442. Plant essential oils: a multifaceted tool for crop protection

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Plant essential oils (EOs) are becoming a key component in the sustainable control of agricultural pests and more knowledge on their impact on protected crops and associated food chains is needed. We studied in the laboratory whether different nanoformulated EOs belonging to four botanical families (i.e., Apiaceae, Asteraceae, Liliaceae and Lamiaceae) can control greenhouse key pests having different feeding strategies (i.e., a chewer, *Tuta absoluta*, and a sap sucker, *Aphis gossypii*). The non-target effects of EOs were also investigated on plants and on two entomophagous insect species used in biological control applications (i.e. *Nesidiocoris tenuis* and *Aphidius colemani*). Finally, we assessed the potential activation of plant defenses mediated by EOs and the cascading effects on the related food networks. Most of the assayed EOs caused significant mortality on insect pests and the estimated lethal concentrations varied significantly. Fennel and anise EOs caused significant mortality on *T. absoluta*, while garlic was the most effective against *A. gossypii*. Although most EOs had negligible toxicity on tomato and sweet pepper plants, they showed a changing selectivity towards tested predators and/or parasitoids. Foliar applications of garlic and peppermint EOs activated defense signalling pathways and triggered different behavioral responses on pests and natural enemies in olfactory trials. Our findings disclose new advances in the current understanding of EOs for plant protection strategies in the Integrated Pest Management framework of modern cropping systems.

**Keywords:** botanicals, glasshouse pest, horticultural crops, IPM, nanomaterial, plant defense

## OC443. The long and challenging road to capitalize on plant-based extracts from the lab to the field

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Negative impacts of chemical pesticide, social demand (healthier food), organic farming organization, rising costs of inputs linked to oil market and their importation (in Africa), and the circular economy approach are a few arguments/opportunities that underlie the roll-out of alternative solutions, including botanicals. Technology readiness levels (TRLs) offer a means to rate relevance of these solutions in the process from plant identification and local knowledge to large-scale use of plant extracts. Options range from do-it-yourself (homemade preparations)- often based on smallholders' traditional know-how- to application of commercial input formulations. The constraints to be overcome vary in scope: technical (biomass availability/sustainability, extraction, chemical analysis, standardization, formulation), societal (grower and consumer perception/acceptability, labour demand, gender issue), and regulatory (adaptation, environmental/ecotoxicological aspects). The following are a few examples of TRL ratings in terrestrial insect management: aqueous extracts of *Tephrosia vogelii* in Malawi and Tanzania (TRL 4-5), organic extracts in Brazil (Annonaceae, TRL 3-4), essential oils of *Piper borbodense* in Réunion (TRL 3-4) and of *Piper aduncum* in the western Amazon (TRL 5-6). In Brazil, the use of commercial formulations (Orobor, Orange Power, etc.) derived from agroindustrial residue processing (orange oil) is an example of a TRL 9 rating. Potential solutions are presented to foster individual or collective (e.g. in African villages) usage (blends, mixtures with chemical pesticides, quality control) and the pathway towards certification. In particular, the creation of specific regulatory categories (e.g. basic substance) within the framework of the European regulation EC n°1107/2009 could help facilitate the adoption/use of plant-based extracts.

**Keywords**: botanicals, agroecology, protection, pests

## OC444. Effect of conventional acaricides and mineral oils on *Anagyrus vladimiri*, a potential biological control agent of citrus mealybug

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Chemical pesticides should be applied in a way that minimizes harm to natural enemies. In the Mediterranean region, the parasitoid *Anagyrus vladimiri* Triapitsyn (Hymenoptera: Encyrtidae) is an important biological control agent of mealybug pests on citrus trees. The study aimed to compare the effects of mineral oils (Levanola, EOS, JMS, and Ultrapaz) and commonly used acaricides (Spirodiclofen, Spirotetramat, Sulphur, Fenpyroximate, and Abamectin) on the acute mortality of *A. vladimiri*. Three toxicity scenarios were examined: (i) direct spray application on adults, (ii) consumption of food contaminated with pesticide, and (iii) contact with pesticide residue. In all bioassays, Abamectin was extremely toxic to the parasitoids when used alone or in combination with Levanola oil. Moreover, Fenpyroximate was also toxic when applied directly, while sulfur was only marginally toxic. Mineral oils did not significantly increase adult mortality unless consumed with food. The study results indicate that all pesticides examined, except Abamectin and Fenpyroximate, are safe for *A. vladimiri* survival. However, consuming oils directly can result in high mortality. The indiscriminate use of certain pesticides can impact the population ecology of *A. vladimiri*; therefore, caution should be exercised when using them.

**Keywords**: biocontrol, citrus, pest management, pesticide, toxicity

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## OC445. Larval cannibalism in *Spodoptera frugiperda* rearings and experimental designs – which factors are amplifying the problem?

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*Spodoptera frugiperda* (Lepidoptera, Noctuidae) is economically one of the most important pest species of the world, its importance is increasing, due to becoming a nearly global pest in recent years. A lot of research was conducted during the last decades, resulting in hundreds of publications. Nevertheless, there is one major problem occurring during its rearings and experiments: the larvae of *S. frugiperda* are cannibalistic. But the extent of cannibalism between experimental designs, and among the different rearing methods. Stabilized conditions regarding low mortality rates are crucial for any scientific purpose, as otherwise the interpretation of the data becomes more difficult. Therefore, we tested several different conditions to see if they had a positive or negative impact on cannibalism among *S. frugiperda* larvae. Some of these factors are abiotic like light and temperature and others are biotic like density or the host plant species. Our data show that nutrition source and plant choice are at least two of the key factors and are therefore crucial for any experimental or rearing set up.

Keywords: *Spodoptera frugiperda*, cannibalism, experimental set up, insect rearing

## OC446. The Animal Landscape and Man Simulation System (ALMaSS), a simulation framework for developing IPM

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ALMaSS is a mature simulation framework developed over 25 years to represent the effects of human activities on populations of natural organisms. The system has been used extensively for risk assessment and regulation of pesticides. The core of ALMaSS is landscape simulation, which provides the environment for simulated species models. This highly-detailed environment describes farms, their fields, their growing crops, and their day-to-day management. All activities, such as soil cultivation, pesticide and fertilizer applications, are explicitly simulated in crop management simulations for over 300 crops grown in Europe. Operational in seven EU countries, with a further five under development, the simulated landscape structure is highly detailed with a 1m$^2$ resolution and area coverage of typically 100 to 2500 km$^2$. The animal species models represent pollinators, beneficial arthropods, aphid pests, birds and mammals and are highly detailed. Combining these elements as an integrated mechanistic model enables a detailed evaluation of the impacts of management such as IPM, including spatial and temporal interactions and feedback loops. By using scenario analysis, strategies can be compared and optimal approaches identified. Although not yet used to evaluate IPM approaches, ALMaSS' use in pesticide risk assessment and detailed representation of mechanistic interactions provide the basis to develop IPM strategies and quantify their effects across a broad range of agricultural environments. Key results from simulation studies will be used to demonstrate the potential gains and pitfalls of using this approach in future IPM evaluation.

**Keywords:** agent-based modelling, landscape, integration, evaluation

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## OC447. The application of Smart Technologies in agriculture for insect pest monitoring and control

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Smart farming and Agriculture 4.0 are new concepts emerged in recent years concerning the way agricultural goods are produced. They give greater attention to innovative approaches such as precision agriculture, Internet of Things (IoT) and big data processing. These tools are used for tracking, monitoring, automating and analysing operations by means of sensors in the field to collect information and in-silico approaches to elaborate them, as the case of decision support systems (DSS), which provide suggestions to end users for optimising cultivation processes.

In the present contribution, examples of smart applications are given for monitoring and pest control of two fruit flies, *Bactrocera oleae* Rossi and *Ceratitis capitata* Wiedemann. IoT were applied to the monitoring of fruit flies by using automatic traps, equipped with a camera that collects images of the insects glued on adhesive panel and sends them to a remote server. Flies can be counted by visual observation of images or an algorithm that correctly recognize them. Software allowing precision targeting application of pest control is also presented, which includes farm digitization; specific DSS for each fruit fly, which create risk maps to make precision insecticidal treatments; a module to guide the operator in the field during treatments; web application that returns inputs and outputs to be visualized and managed.The incorporation of such an innovative technology into integrated pest management (IPM) practices will provide to end users' broad benefits including a better control of agricultural processes to reduce production inputs, such as chemicals, and labor costs.

**Keywords:** IPM, agriculture 4.0, automated trap, DSS, tephritids

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## OC448. Do drones affect the behaviour of *Halyomorpha halys*? Implications for pest monitoring in pear orchards

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Drones have been recently applied in the agronomical field for spraying pesticides and fertilisers. The HALY.ID project (https://www.halyid.eu) is developing a drone application to detect and monitor the invasive pest *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) on pear trees. The effects of a drone flying at different heights on the behaviour of *H. halys* were evaluated, quantifying the walking, flight, and freezing responses on adults and immature stages. Insect specimens were placed on pear trees at a height of 1m while the drone flew at heights of 1, 4, 8 m. Results for adults show that the drone has limited impact on walking and flight at any height, whereas at 4m height it induced freezing behaviour. On immature stages the responses were different: for the older nymphs the results were similar to those of the adults, while the younger nymphs increased the walking or let themselves fall from the leaf or the branch. To understand the reason for the freezing response during drone flight, the two components of noise and wind were tested separately and replicated by using Bluetooth speakers and noiseless computer vents. Noise did not induce significant behavioural alterations, while wind induced the freezing response with results comparable to those obtained with the drone. These results enabled the development of an autonomous flight protocol to monitor the presence of *H. halys* in a pear orchard. Associated with machine learning algorithms, the whole system allows for reliable pest monitoring with a substantial reduction of time and energies.

**Keywords:** invasive plant pest, behaviour, pest monitoring, drones

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## OC449. Early detection of wireworm (Coleoptera: Elateridae) infestation and drought stress in maize using hyperspectral imaging

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The aim of our research was to test hyperspectral imaging as a method for early detection of wireworm herbivory and drought stress in maize. Individual and combined effects of these two stresses were assessed on several morphological and physiological traits of two maize hybrids, one drought-tolerant and one drought-sensitive. Hyperspectral imaging and physiological parameters were determined at three time points (days 14, 21, and 28 after adding wireworms and inducing drought). Most differences in maize physiology between treatments were observed on day 21, but our results indicated that the combined effect of both stressors did not always have a synergistic effect. Plants exposed to drought had lower relative leaf water content (RWC), leaf stomatal conductivity (gs), net photosynthesis (Pn), transpiration rate (E), and effective quantum yield of PSII (Fv'/Fm'). Conversely, the presence of wireworms had a greater effect on maize morphological traits than on physiological traits. Hyperspectral data were collected in a combined range of 400 – 2500 nm. There were no significant differences between treatments in the visible region of the spectrum. However, we were able to detect abiotic and biotic stresses and distinguish between them in the near-infrared and short-wave infrared regions, with the highest overall accuracy on day 14 (86.7 %) and the lowest on day 28 (67 %). Hyperspectral imaging detected pest infestations and water stress before they were detected by classical methods (or the naked eye), and therefore can be used as a method for early detection of wireworm attack and/or drought in maize.

**Keywords:** wireworms, hyperspectral imaging, drought stress, maize, early detection, precision agriculture

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## OC450. Search for peptides acting on *Ceratitis capitata* larvae

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Oostatic peptides are molecules that act over insect reproduction due to a regulation on egg development. It is known that a specific oostatic decapeptide isolated from the mosquito *Aedes aegypti* inhibits trypsin activity in the midgut of the mosquito. This peptide, denominated TMOF (Trypsin Modulating Oostatic Factor), was isolated from *Aedes aegypti* ovaries and has proven to inhibit the expression of serine proteases and trypsin in larvae's gut, disrupting digestion and leading to larvae starvation and death. This mechanism was demonstrated to have effect also on herbivore pests such as *Plutella xylostella*, *Diaprepes abbreviatus* or *Heliothis virescens*. Preliminary laboratory data indicated that this mechanism of action, involving modulators of the expression of enzymes, essential for digestion in insects, is transversal to insect species, namely agricultural pests. This knowledge led us to hypothesize that such a mechanism of digestion inhibition could also exist in some Mediterranean insect pests such as for the species *Ceratitis capitata* (considered to be one of the most destructive fruit pests in the Mediterranean region). A search for *C. capitata* peptide TMOF-like was initiated. A *Ceratitis capitata* rearing unit was established where eggs and larvae were obtained. TMOF and TMOF-like peptides were tested on larvae. Ovarian peptide extracts were analysed. *In silico* homology search for TMOF receptors was also performed. Results showed some detrimental effects on larvae mobility and development. The use of these peptides as biocontrol for larvae development is further discussed.

Keywords: TMOF, biocontrol, insect, pest, Mediterranean

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## OC451. Identification of the predators of oilseed rape pests using camera traps

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Cameras are becoming common tools for ecologists studying species abundance, richness, and interactions. They are mainly used to study large animals, but with their increasing availability they are increasingly used to study invertebrates: e.g., to record insect-flower and predator-prey interactions. Cameras can be powerful tools to identify and quantify the predators of specific agricultural pests. This knowledge can then be applied to adapt farming practices to favour the species of the predators identified. We used cameras to monitor the predation of two important pests of oilseed rape, the pollen beetle (*Brassicogethes aeneus*) and the brassica pod midge (*Dasineura brassicae*). Both species are vulnerable to predation by ground dwelling invertebrates in spring when larval stages drop from the plant canopy to the ground before pupation. Over 2 years of sampling and 240 days of cumulated observation, we identified the main ground predators of these species in the UK. Our observations showed that *Carabidae* and *Staphylinidae* larvae are key predators of pollen beetles and brassica pod midge larvae. Only adults of the Genus *Bembidions* or *Trechus* were frequently observed feeding on these preys. Other predators such as Acari and ants were also observed in predatory behaviours. These results show that neglected species and development stages can be important contributors to the biocontrol services provided in agroecosystems. This knowledge can be used to guide farmer's management practices, with the aim to support important predator populations in their fields.

Keywords: pollen beetle, brassica pod midge, carabid, predatory larvae, biocontrol

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## OC452. Connecting global expertise in plant virology and entomology to develop sustainable pest management solutions: The community network for vector-borne plant viruses

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The Community Network for Vector-Borne Plant Viruses (CONNECTED) builds multidisciplinary research capacity to tackle crop diseases spread by insects to ensure fair, nutritious and sustainable food production for all. Integrated pest management is an environmentally sensitive approach to sustainable management of vector-borne crop diseases. CONNECTED brings together expertise in plant pathology and entomology to tackle the complex problems of vector-borne crop diseases. CONNECTED comprises 1624 members across 92 countries, mostly in Sub-Saharan Africa, representing a valuable resource of technical expertise, knowledge and innovation. CONNECTED has funded a portfolio of 20 innovative projects (including pest/disease surveillance, diagnostics and biopesticides) involving 14 countries, 11 different crops, and collaborations involving 55 researchers across 34 institutions, representing new collaborations between UK and African institutions. CONNECTED has made over 100 training awards to delegates from 18 countries for multidisciplinary courses or educational visits. Social network analysis reveals that CONNECTED has been successful in developing a core of related technical expertise and knowledge capacity: the phased programme of research, capacity building and networking activities has been central to this and has provided a framework for measurable collaborative outcomes. CONNECTED is currently working with stakeholders to evaluate climate-smart Integrated Pest Management approaches for vector-borne plant viruses, and to map international provision and need for weather and climate information, and tools to support sustainable vector-borne plant virus management.

**Keywords:** network, vector-borne plant virus, capacity building, climate-smart, IPM

# Session 16:

## Stored Product Protection

## OC453. Lessons from the study of stored-product insect interactions with pheromones and their application to monitoring and management programs for the food industry

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A diverse community of stored product insect species can be found exploiting commercial food facilities such as mills, processing plants, warehouses and retail stores. These species can have significant impacts due to their contamination of the food supply and the cost of management programs to reduce food infestation. Food facilities tend to be complex landscapes which make monitoring and targeted pest management tactics difficult. In this presentation a series of experiments evaluating insect responses to pheromones and the lessons they provide for improving the implementation of pest management programs for the food industry will be presented. Focus will be on improving the design of trapping devices, implementation of monitoring devices, interpretation of monitoring results, and use of pheromone for the manipulation of insect behavior. How smaller scale experiments can be effectively scaled up to the commercial scale by considering the landscape structure of food facilities will be emphasized. Considerable progress has been made on improving monitoring and managing programs, but remaining knowledge gaps will be highlighted.

**Keywords:** behavior, stored products, pest management, pheromones, ecology

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## OC454. The microbial ecology of stored products insect pests in Greece

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There has been no work assessing the ability of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), to vector microbes after harvest. While there has been some work assessing the microbial ecology of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Cucurlionidae), none has directly assessed whether they vector microbes. However, this has important ramifications for the safety of the post-harvest agricultural supply chain. Thus, we evaluated the ability of both *P. truncatus* and *S. zeamais* (from colony strains) to vector microbes when given the opportunity to forage on potato dextrose agar dishes for 3 and 5 days. We also used field caught *S. zeamais* and *Rhyzopertha dominica*, the lesser grain borer, since *P. truncatus* were in low abundance in Greece. We subsequently photographed the dishes and quantified the microbial growth using ImageJ. In addition, we isolated unique morphotypes of fungi, and extracted DNA using a Zymo kit, amplified the DNA using PCR, and sent samples for sequencing to determine fungal identity. We found that both species readily vectored several plant pathogenic microbes, including *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium spp*. Microbial growth was greater at 5 days compared to 3 days. We also found important differences between colony and field caught individuals, as well as species, with more microbes vectored by *S. zeamais* than *P. truncatus*. This has important implications for food safety in the postharvest environment, especially for maize production.

**Keywords:** mobility, microbial growth, food safety, behavior, trapping, PCR

### OC455. Dispersal ability and prey preference of the predatory mites *Blattisocius* sp. and *Cheyletus* sp. in stored rice

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Predatory mites occur spontaneously in storage facilities, and they can decrease stored product pests. However, their mobility potential in grain piles and structures is not well assessed. The aim of this study was to evaluate these predatory mite species as biological control agents through their dispersal ability and prey preference in stored rice. Dispersal was tested in three structurally different designs resembling warehouse conditions. First, dispersal ability was tested vertically in polyvinyl chloride tubes of three different depths 40, 100, and 150 cm filled with paddy rice. The objective was to see if predatory mites were able to reach the bottom where a trap with its target pests' eggs was located. Eggs were checked for mite damage and larval emergence of the target pest. Secondly, horizontal dispersion was tested, in 2-meter cable channels filled with different thicknesses of rice flour residues. The objective was to see if predatory mites can reach a group of pest insects' eggs at different distances, time periods, and thicknesses of residues. Finally, prey preference was tested in 2 L plastic containers where the two pest species were exposed to both mite species. Predation and survival rates were evaluated. Mites were effectively reaching their target pests' eggs at 40 cm depth but barely reached 1m. Horizontally, mites could reach their target species at 2 meters in 24 hours, but predation decreased when mites were given less time.

**Keywords:** predators, biological control, dispersal, prey preference, grain, storage pests

### OC456. Monitoring stored-product insect pests to build better predictive models for migration and seasonal variation

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For insects that infest post-harvest grain, movement is often attributed to human activities such as transportation from bulk storage or shipping of warehoused goods. However, these insects also navigate more natural habitats and may migrate outside of human influences. Here a system of collaborators through the central United States trap flying insects near stored grain. Samples were collected weekly during the warm season (April through October). Known stored product insect species were identified and counted and other non-stored product insects were identified to family. We find that in more northern states, stored product insect pests emerge just as early as more southern states but there is only a single peak of large numbers of insects. In southern states, there can be two peaks during these warm months. We find significant variation in insect species found at our different locations, which could be attributed to the type of facility or structure where traps were placed. For example, traps near a location that shipped and received large amounts of corn captured high numbers of Indian meal moths, *Plodia interpunctella* Hübner, while traps near bin storage of wheat captured low numbers of *P. interpunctella,* but higher numbers of lesser grain borers, *Rhyzopertha dominica* Fabricius. These data are the beginning of a long-term dataset that can be used for predictive modeling and tracking of these insects throughout the Great Plains states. Knowing when and where to expect these insects can help pest managers best prepare for remediation to reduce the damage of these pests.

**Keywords:** stored products, funnel traps, grain, seasonal variation, field trapping

## OC457. Mating disruption technique for the European grain moth infestations

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*Nemapogon granellus* (L.) (Lepidoptera: Tineidae) is a stored-product moth, found to infest stored animal products such as meat, sausages and cheese. Damages are caused by larvae digging tunnels for feeding on cured meat and cheese. Control method based on the use of sexual pheromone to prevent or limit mating between *N. granellus* males and females was evaluated.The trials were conducted in two ham factories where the European grain moth was infesting cured meat during storage in warehouse. A treated area and a control area were selected in each ham factory: in the treated ones, dispensers loaded with 10 mg of *N. granellus* pheromone were positioned at a density equal to 1 unit/22.5 m$^3$ (factory A) and 1 unit/25 m$^3$ (factory B). The efficacy of mating disruption was evaluated on the reduction in the number of mated females, captured in a plastic container filled with water and placed in the control and test warehouses. The results showed a significant reduction of mated females in the test warehouses compared to the control warehouses, in both ham factories. The total number of mated females was greater than 90% in untreated warehouses, less than 50% in warehouses with mating disruption. These results indicate that the use of pheromones to reduce the couplings is a technique that can be successfully applied in the integrated management of *N. granellus* in ham factories.

**Keywords:** Integrated Pest Management, water trap, *Nemapogon granellus*, stored-product pests, ham factory

## OC458. Lessons learned for phosphine distribution in “real world” fumigations

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Recent advancements in wireless phosphine sensors and computational modeling enable extensive and continuous monitoring of fumigation applications. We conducted an evaluation of wireless phosphine sensors to illustrate gas concentrations in various storage facilities, including silos, warehouses, and shipping containers. Commercial applications were carried out to assess the impact of multiple parameters, such as the type of commodity, the temperature, the relative humidity level, and the phosphine concentration. The results indicated that in horizontal warehouses, phosphine distribution was often insufficient to control insect mortality, as gas concentrations varied significantly over time and space. However, commercial treatments in shipping containers were effective in controlling stored product insects with varying susceptibilities to phosphine. Our work demonstrated that forced recirculation systems are necessary in silos to improve phosphine distribution and increase insect mortality. Moreover, our data suggested that wireless sensors are effective in measuring phosphine concentrations and evaluating the success of fumigations. Computational models can predict phosphine concentrations and insect mortality levels, enabling corrective actions, such as the addition of extra fumigant. In conclusion, real-time monitoring is an available technology that can be used in Integrated Pest Management (IPM) programs during the post-harvest stages of agricultural commodities.

**Keywords:** wireless sensors, distribution, mortality, phosphine

This research was carried out as part of the project «PrecisionFEEDProtect: Precision protection of stored feed from entomological infestations using innovative technologies» (Project code: KMP6-0077613) under the framework of the Action «Investment Plans of Innovation» of the Operational Program «Central Macedonia 2021-2027», that is co-funded by the European Regional Development Fund and Greece.

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## OC459. Feasibility of grain aeration for safe storage and insect infestation prevention in moderate climates and the alternative use of artificially cooled air using a grain chiller

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FAO estimates that annually up to 40 percent of global crop production is lost to pests, resulting in a total $220 billion of losses. Grain storage facilities usually provide the ideal environment for insects to flourish, and insect infestation hence poses one of the biggest threats for stored grain. The ban of highly efficient fumigants, resistances to available fumigants as well as the spread of invasive insect species further aggravate the situation. Finding alternatives for infestation protection of stored grain thus is of utmost importance. It is well-known that the development of insects slows down significantly with decreasing temperatures. Lowering the temperature of the grain bulk sufficiently hence can contribute to keeping the grain insect free. Grain aeration is an old and established method to bring down the grain temperature in moderate climates, in order to keep the grain safe its use however is restricted to certain temperature and relative humidity ranges. This study evaluates the use of grain aeration in central Europe in view of climate change and based on historical weather data for the previous five years, as well as the real-life experiences of grain storage facilities in the region. The results show that aeration with ambient air is not apt to bring down the grain temperature fast enough to efficiently avoid insect infestation. The use of a grain chiller on the other hand can make a positive contribution to infestation prevention and proofs to be an economic solution, requiring only one initial chilling cycle in moderate climates.

**Keywords:** grain chilling, grain cooling, safe storage

## OC460. It´s just a point of view – destructions and creations of cultural objects by artifact pests

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Woodworms, carpet beetles, clothes moths and termites are well known pests on artifacts of cultural importance. Lately also silverfish have gained importance. Protection of our cultural heritage for future generations is not a new task but the methods have changed. Conventional use of pesticides in the past has preserved precious objects up to the presence, which otherwise probably would have been lost over time due to the destruction by insects and microbes. However, several of the objects were contaminated with poisons in such a manner that they became unsafe to be handled and thus are useless for exhibitions or scientific studies. Today, the concept of Integrated Museum Pest Management (IMPM) provides guidance for non-residue treatments of infested objects, followed by save storage or display under pest free (or maximum pest reduced) environments. Inert fumigants, physical and biological control measure, precise monitoring, threshold evaluations and detailed knowledge of the pest`s biology are key elements within IMPM.
Woodworms, clothes moths and termites, however, are not always considered as only pests. Some professionals have also used them to produce new pieces of art or for the installation of ephemeral displays. Under more or less controlled conditions, the insects´ destructive nature on wood or textiles has been turned around to a process of creation with aesthetic or philosophical messages. Destructive or creative - it just depends on the point of view!

**Keywords:** museum pests, Integrated Museum Pest Management (IMPM), cultural Entomology

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## OC462. Developing a molecular insect-free test for the detection of infestations in stored paddy rice

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Arthropod pests cause significant production losses in stored products. More than 120 species are considered cosmopolitan storage pests of raw materials and final food products that become contaminated in the facilities where food is stored and processed. To detect adult insect infestations in stored grain, routine analyses are carried out, usually by visual inspection of the grain trough sieving, being a tool that is not very sensitive at low population densities or when infestations are of pre-imaginal stages. Even if the use of molecular diagnostic methods is increasing in the food industry due to their high sensitivity, reliability, and accuracy, they have almost never been used to detect the presence of insects in stored grain. Even if few years ago we developed a multiplex PCR test to detect and identify the main five primary insect pest species in infested grain, this technique was not suitable to detect any other arthropod species present in the stored grain. Here we present the development of an insect-free qPCR test to detect infestations of any arthropod species commonly found in stored grain of the Mediterranean area. The main handicap of this test, which is the impossibility of identifying which species are present in the grain sample analyzed is discussed. This lack of identification could be solved either by using a conventional PCR analysis with a pair of specific primers for each target pest species, or even by a metagenomic analysis using High-Throughput Sequencing (HTS). Pros and cons of this "insect-free" test are discussed.

**Keywords**: insect infestations, insect-free test, pest detection, stored rice pests, qPCR, HTS

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## OC464. How does pirimiphos-methyl affect the progeny of *Tribolium castaneum* (Herbst)?

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This study investigates the effect of exposure time to pirimiphos-methyl on progeny fitness of *Tribolium castaneum* (Coleoptera: Tenebrionidae) parental individuals. Thus, *T. castaneum* parental females were exposed for 1, 3, 8, 16, 24 and 72 h to pirimiphos-methyl and progeny birth or death rates were calculated. The parental exposure to the insecticide affected both the fertility and the survival of their progeny. The net reproductive rates of progeny were 4.1 and 4.3 females/female when females were exposed for 1 and 3 h to pirimiphos-methyl, respectively. These values did not differ with the progeny in the control. The increase of exposure time of parental females to pirimiphos-methyl resulted in a detrimental effect to progeny fitness on the basis of the intrinsic and finite rates of increase. The lowest values of these parameters were noted at the 72-h exposure interval (0.001 females/female/day and 1.000, respectively). Similar trend was recorded in the values of the doubling time. However, the mean generation time did not differ between the control and all insecticidal treatments. The exposure of parental *T. castaneum* females to pirimiphos-methyl affected negatively the demographic parameters of the progeny production. This finding could be the basis of the moderation of the application of the pirimiphos-methyl against the commonly found *T. castaneum* in storages.

**Keywords:** progeny, Tenebrionidae, red flour beetle, pirimiphos-methyl

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## OC465. Deltamethrin applied on concrete against *Alphitobius diaperinus*: six-week residual trials

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This study reveals the immediate and delayed efficacy of deltamethrin (half label and label dose) applied on concrete, against adults and larvae of *Alphitobius diaperinus*. Bioassays were conducted for six weeks on concrete surfaces in the presence or absence of food. The first week, deltamethrin (both doses) killed the highest number of individuals, for both food scenarios, developmental stages in both immediate and delayed assays. The following weeks its efficacy gradually decreased. Regarding adult mortality, the first week and the sixth week, it ranged from 6.7% to 31.1% and from 1.1 to 13.3%, 7 days post-exposure respectively for both doses. Both doses of deltamethrin exhibited elevated efficacy against *A. diaperinus* larvae. The first week, label dose without and with food killed 100% of larvae after 3 days and 7 days of exposure respectively. At the end of second week, label dose (both food/no food

scenarios) killed all larvae. The 7th day of the third week, only label dose without food killed all *A. diaperinus* larvae. At the end of sixth week, mortality ranged from 71.1% to 92.2%, at all tested scenarios. Regarding delayed adult mortality, it ranged from 63.6% (first week) to 7.9% (sixth week). For the delayed larval mortality assays, all individuals died the fifth week (label dose without food), the fourth week (label dose with food, the second (half label dose without food) and the first week (with food). Deltamethrin has elevated efficacy against *A. diaperinus* larvae regardless dose and presence or absence of food.

**Keywords:** adult, larva, food, pyrethroid insecticide, stored product pest, long term protection, storage

## OC466. Extracts of *Acmella oleracea* are effective green pesticides for the management of key stored-product arthropods

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The hexane and methanol extracts of *Acmella oleracea* aerial parts were applied on stored wheat against the major stored-product pests *Cryptolestes ferrugineus, Tenebrio molitor, Oryzaephilus surinamesis, Trogoderma granarium, Tribolium castaneum, Tribolium confusum, Alphitobius diaperinus*, and *Acarus siro*. Two concentrations of both extracts (500 and 1000 ppm) were evaluated after 4, 8, and 16 h and 1 to 7 days of exposure. The extracts caused 100.0% mortality to *C. ferrugineus* adults vs. *A. diaperinus* adults that exhibited the lowest mortalities. Hexane extract was more effective than methanol extract. The susceptibility order, from least to most susceptible species/stage, was *C. ferrugineus* adults < *A. diaperinus* larvae < *C. ferrugineus* larvae < *T. granarium* adults < *T. molitor* larvae < *O. surinamesis* adults < *O. surinamesis* larvae < *T. confusum* larvae < *T. castaneum* larvae < *A. siro* adults < *T. molitor* adults < *A. siro* nymphs < *T. granarium* larvae < *T. castaneum* adults < *T. confusum* adults < *A. diaperinus* adults.

**Keywords:** natural pesticides, stored wheat protectants, noxious species, developmental stages

## OC467. Insects for feed: sustainability and circular economy in practice

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Since about a decade, there is a growing interest for the use of insect-derived products in animal feeds. Indeed, insects have an interesting nutritional profile in terms of protein and essential amino acids, lipids, vitamins and minerals. They also contain a series of bioactive compounds (chitin, specific fatty acids, and anti-microbial peptides), which have been shown to possess nutraceutical properties and exert an animal health- and welfare-promoting effect. Moreover, insects can be efficiently reared on organic wastes and side-streams, fully applying the circular economy concept. Closing the loop, insect frass, i.e., the residues of the insect rearing process, can be further exploited as an organic soil fertilizer with valuable nutrients and plant immunostimulating properties. In Europe, insects grown for the production of food, feed or other purposes are classified as “farmed animals” [Regulation (EC) No 1069/2009], facing specific limitations on the substrate type that can be used for their rearing. According to EU legislation, eight insect species can be used to produce insect-derived proteins for aquafeeds [EU Regulation 2017/893], as well as for poultry and swine feeds [EU Regulation 2021/1372], while for ruminants the use of insect proteins is still forbidden. No limitations apply though to insect-derived oils. Among the species used for feed production, the black soldier fly (*Hermetia illucens*), the common housefly (*Musca domestica*), and the yellow mealworm (*Tenebrio molitor*) are considered as the most promising. This talk aims to present recent research advances on insects as feed.

**Keywords:** insects for feed, circular economy, feed ingredients, insect frass

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## OC468. Wet feed effect on mealworms’ growth and performance: an important but rather underrated factor

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Mealworms, particularly the yellow mealworm, *Tenebrio molitor* L., and the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), have gained during the last decade a lot of scientific and commercial interest. Research efforts aim to define the optimum conditions for their rearing and optimize their production. However, when it comes to mealworm nutrition, most studies focus on the dry feed that is used as insect feedstock. Apart from that, mealworms are heavily dependent on the presence of a moisture source, commonly referred to as wet feed. Information though on the effect of wet feed on the performance of mealworms is rather limited. To close this gap, a series of trials was conducted to quantify the effect of wet feed on the growth of larvae of *T. molitor* and *A. diaperinus*. Based on the results, larval growth and development of the two mealworm species tested, as well as other larval fitness-related traits (e.g., survival, feed conversion ratio, growth rate etc.) can be considerably impacted by the wet feed type and characteristics. These findings highlight the importance of wet feed for the lab- and mass-rearing of *T. molitor* and *A. diaperinus* larvae.

This research is part of the project « EntoFeed» (Project code: MIS 513644) that is co-funded by Greece and European Union by the Action «Investment Plans of Innovation» in Central Macedonia under the framework of the Operational Program «Central Macedonia 2014 2020».

**Keywords:** *Alphitobius diaperinus*, *Tenebrio molitor*, insect rearing, larval growth, mealworms, wet feed

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## OC469. Evaluation of isoproteinic diets composed of agricultural by-products as feed for *Tenebrio molitor* larvae

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Insect feed represents an essential operating cost of insect production. Thus, researchers and industries aim at its improvement while, at the same time, balancing practical considerations. Insects can upcycle agricultural side-streams and wastes and bio-convert them to high-value nutrient products. In this way, insect rearing gets aligned with circular economy practices while, at the same time, the production cost can be reduced. In this context, the objective of the present study was to evaluate the suitability of five agro-industrial by-products. More specifically, spent grains, rice bran, and by-products of oats, maize, and sunflower, utilized as feeding substrates for larvae of the yellow mealworm, *Tenebrio molitor*. At first, isoproteinic diets at three different protein levels (i.e., 17,4, 20,3, and 22,6%) were formulated and evaluated in a lab bioassay [50 early-instar larvae per vial] in terms of larval growth and performance. Based on these results, the best-performing diets were also evaluated at a pilot scale in crates (60x40 cm) with approximately 10,000 larvae. The results were promising for most isoproteinic diets showing the potential of the tested agricultural by-products as feed ingredients for *T. molitor* larvae. Interestingly, the high-protein diets did not necessarily lead to higher larval growth, indicating the need to consider other factors besides protein content when designing optimal diets. This research is part of the project «EntoFeed» that is co-funded by Greece and European Union by the Action «Investment Plans of Innovation» in Central Macedonia under the framework of the Operational Program «Central Macedonia 2014-2020».

**Keywords:** larval growth, yellow mealworm, agricultural by-products, isoproteinic diets

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## OC470. Exposing edible insects to livestock viruses, a step towards sustainability

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The mass production of insects offers the potential for a sustainable source of nutrients for livestock feed. In order to ensure this sustainability, there is a need to explore new substrates for the insects, including those of animal origin, such as slaughter byproducts, manure and communal waste. However, such substrates could pose risks that need to be assessed. Experimental exposure studies with animal viruses can provide knowledge about such hazards for risk assessments. In a series of experimental studies, we exposed two important insect species, *Tenebrio molitor* and *Hermetia illucens*, to Porcine respiratory coronavirus (PRCV), African swine fever virus (ASFV), and Porcine parvovirus (PPV). These studies had the aims of establishing virus exposure bioassays for larvae of the two insect species and of providing knowledge of the survival of the different types of viruses within these insect larvae. For virus exposure of *T. molitor* larvae, a newly developed virus exposure bioassay, using direct oral inoculation, was established. For virus exposure of *H. illucens* larvae, a method for inoculation via spiked feed was tested. Both species of larvae were sampled immediately following virus exposure and until 8-9 days post inoculation. The presence of viral genomic material was determined using (RT)-qPCR. The results underline the importance of such exposure studies if the sustainability goals of insects as feed are to be achieved. Future work will focus on other species of insects, other livestock viruses as well as assessments of the risks of pathogen transmission via oral consumption in livestock species.

**Keywords:** feed safety, sustainability, insect rearing, virus exposure bioassays, virus survival

## OC471. Valorization of agricultural side-streams for the rearing of larvae of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae): Effect on larval growth and body composition

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The lesser mealworm has attracted attention as an alternative source of nutrients, with its addition to the list of insect species that can be used as feed ingredients for fish and poultry. Yet, information on the suitability of various feeding substrates for this species is still scarce, setting limitations to its industrial mass rearing. The present study aims to shed light on the potential of agricultural side-streams as nourishment for *A. diaperinus* larvae. The suitability of certain byproducts originating from the production of rice, oats, barley, lucerne and maize as larval feeding substrates was investigated at lab-and pilot-scale trials. All byproducts were tested either individually or by designing isonitrogenous diets. Larval development and survival, as well as the time required until pupation and the quantity of ingested feed were monitored at weekly intervals. According to our findings, numerous byproducts of the seed cleaning process might be utilized as feeding substrates for *A. diaperinus* larvae. Moreover, when the larvae were fed with diets containing varying percentages of these byproducts, the cost of larval production was significantly altered, either positively or negatively. These data contribute to the strengthening of the sustainability profile of insect farming and link this profile with circular economy attributes. This research is part of the project « EntoFeed» (Project code: MIS 513644) that is co-funded by Greece and European Union by the Action «Investment Plans of Innovation» in Central Macedonia under the framework of the Operational Program «Central Macedonia 2014 2020».

**Keywords:** circular economy, edible insects, feed conversion efficiency, insect farming, insects as food and feed, organic side-streams

## OC472. Inbreeding depression impacts mating and reproductive performances in *Hermetia illucens*

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The black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) is a species commonly used as feed, which has been also promising as bioconvertion tool for organic wastes. Despite several recent studies about this insect, research about the biology of this species is needed to optimize mass rearing and bioconversion efficiency. This study aimed to investigate the impact of inbreeding in *H. illucens*, exploring both effects on behavior and progeny outcomes. Concerning mating, females could not distinguish sibling (acceptance rate: 58%; copula: 36.2 ± 2.8 min) from non-related males (acceptance rate: 52%; copula: 34 ± 3.1 mi), but females generated from a sibling mate (F1-sib) were generally less inclined to accept courtship, showing longer copula durations when mating with inbreeded males (copula: 52.2 ± 6.5 min). Similar to mating, F1-sib mated females lays less fertile eggs compared to parental females. Considering fecundity and progeny outcomes, every female produced an average of 262.4 ± 39.8 larvae with a weight of 0.193 ± 0.007 g/larva, while larvae produced from inbreeded mates were in average 277.6 ± 61.3, with a lower median weight (0.168 ± 0.011 g/larva). The progeny was consistently reduced in F1-sib females (100 ± 25.6 and 194.9 ± 46 larvae from sibling and un-related mates, respectively). Lasty, inbreeding affected both the emergence rates and the progeny sex ratio.

**Keywords:** black soldier fly, feed, mass rearing, inbreeding, behavior, fecundity

## OC473. Effects of insecticide residues in organic residual streams on insects reared for food and feed

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Certain insect species, such as black soldier fly (*Hermetia illucens*) and lesser mealworm (*Alphitobius diaperinus*), are increasingly considered as a suitable alternative protein source for both human food and animal feed purposes in the European Union (EU). The substrate on which these species are reared may contain a variety of chemical contaminants, e.g. residues of pesticides. This entails two potential risks: bioaccumulation of these residues in the insect biomass may present a potential safety concern for the consumer; and insect exposure to insecticidal substances may affect survival and insect biomass yield, presenting economic concerns. Maximum residue limits (MRLs) for pesticides have been laid down in EU legislation. However, findings of our recent studies suggest that exposure to concentrations at or below those limits (MRL) can nonetheless result in significant reductions in insect survival and yield. We have tested the effects of several substances with different modes of action (MoA), differences between species, effects of pesticide cocktails with the same or different MoA, bioaccumulation factors, concentration/response curves for selected insecticides, and sub-lethal effects resulting from chronic exposure. Results suggest an urgent need for commercial insect farms to monitor pesticide concentrations in feed materials, and for policymakers to consider adjustment of legal limits tailored to reared insects.

Keywords: pesticides, *Hermetia*, *Alphitobius*, reared

## OC474. New insights in khapra beetle research: results from a five-year transnational research in USA and Europe

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The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is an important quarantine pest with a large variety of food preferences that threats global food security. In this work, we summarize a series of experiments that have been carried out in the USA and Greece between 2018 and 2022, that mostly focus on detection, trapping and sampling of this pest, along with its population growth on different grains. Trials with different types of traps and attractants have indicated that this species does not respond vigorously in multiple commercial combinations of lures and traps tested compared with other major stored product beetle species. In addition, its population growth on different grains was comparable with that of the warehouse beetle, *Trogoderma variabile* Ballion, a relative, nonquarantined species that is the most commonly found dermestid in the USA. Nevertheless, at elevated temperatures, *T. granarium* outcompeted *T. variabile*. We developed a molecular technique for identifying larvae when measuring the comparable development of these two species on the same commodities, which can also be applied to field detection programs. All the above data highlight the importance of further developments of early detection tools for this species and the salience of estimating its presence in international trade.

**Keywords:** khapra beetle; invasive species; trapping; sampling; population growth

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## OC475. Biological control of some arthropod pests present in stored rice using the generalist predatory mites *Blattisocius sp.* and *Cheyletus sp.*

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Significant losses in quantity and quality of stored products are caused by numerous arthropod pest species. Currently, the main control strategy is the use of a limited number of authorized synthetic insecticides whose overuse has led to an increase in pesticide resistance, resulting in a lack of effectiveness. As an alternative, biological control using natural enemies (predators and parasitoids) is an effective method to prevent insect populations from reaching pest status, especially in storage

facilities due to the stable environmental conditions. Generalist predatory mites are noted for their potential as biocontrol agents due to their ability to prey on a large variety of pest species and their ease of management and application.
We evaluated the suitability of two predatory mites commonly found in grain warehouses, *Blattisocius sp.* and *Cheyletus sp.*, against arthropod pests usually present in stored rice. Host acceptance and predation rates were tested in laboratory experiments, in which a known number of preys was offered to a single predatory female in small arenas. A subsequent experiment in which we increased the dimensions of the arenas and triplicated the number of prey and female mites introduced was also performed. High predation rates were observed for both mites, reducing the prey by up to 90% in some cases. The combinations of one predatory mite with a specific parasitoid, and both predators together were also tested in larger arenas. Obtained results suggest that periodical releases of these mites could maintain some of the pest populations under control.

**Keywords:** predatory mites, biological control, stored pests, natural enemies

### OC476. Evaluation of the suitability of by-products of the cotton industry for the rearing of four insect species: Promoting circular economy through insect farming

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Cotton is one of the main crops cultivated in the Region of Thessaly, Greece, whilst during its production and processing a number of by-products and side-streams are generated. Among the produced cotton by-products, cotton cake, cotton gin trash, cotton seeds with lint hairs, and delinted cotton seeds are included. As insects can be easily reared in a controlled environment and fed on a variety of agricultural by-products, the objective of the present study was to investigate the suitability of the aforementioned four by-products of cotton production as insect feed for the larvae of the yellow mealworm, *Tenebrio molitor*, the lesser mealworm, *Alphitobius diaperinus*, the superworm, *Zophobas morio*, and the black soldier fly, *Hermetia illucens*. Trials were conducted at laboratory scale and early-instar larvae of the various insect species were fed on each one of the aforementioned by-products, while larval growth and performance were recorded. Based on the results, the suitability of the tested by-products as insect feedstocks varied among the different insect species and by-products tested. Indicatively, cotton cake was able to support the larval growth and development of all species tested, indicating its potential to be included as ingredient in compound insect diets. This was not the case though for cotton gin trash. The results of the present study aim to exploit locally available resources for insect rearing and subsequently decrease the dependency of Mediterranean countries on imported resources.

This research is supported by the EU-PRIMA program project ADVAGROMED (Prima 2021 – Section 2).

**Keywords**: cotton production, insects as food and feed, larval growth, local by-products

## OC478. Insect infestations of insect meals under storage: a real-case scenario?

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As the insect sector grows, the quantities of insect meals and related insect-based food and feed products that will be kept in storage will inevitably increase. As is the case for most organic commodities of vegetal or animal origin, while at storage, insect meals may be infested by stored-product insects. To test this hypothesis, insect meals of two tenebrionid species that are commercially produced for food and feed, namely the yellow mealworm, *Tenebrio molitor* L., and the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), as well as substrates based on these two insect meals, were artificially infested with some of the major storage insects in a series of laboratory trials. Based on the results, several storage insect species, e.g., the confused flour beetle, *Tribolium confusum* Jacquelin du Val, and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), or the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), were able to infest the *A. diaperinus* meal building high populations in a short time. Similarly, *T. confusum* infested pure *T. molitor* meal and produced progeny on this substrate. The implications of these findings for the storage of insect meals and related insect-based products in real-world conditions are yet to be estimated. However, it becomes evident that effective post-harvest strategies must be designed and implemented for the protection of insect meals during storage, in order to ensure their safety and high quality.

**Keywords:** alternative proteins, insect infestation, insect meal, population growth, stored-product insects

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## OC479. Evaluation of cold and heat treatment on different life stages of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

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As insect resistance to various contact and fumigant insecticides increases, the necessity of finding alternative control methods should be considered a priority. Among the most promising alternatives is the use of extreme temperatures which is considered a non-chemical option. The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) is one of the most common stored product insect species of grains. Laboratory bioassays were carried out to evaluate the cold tolerance of different life stages (adults, larvae and eggs) of *O. surinamensis* at five different temperatures i.e. 5, 0, -5, -10, -15 and -18$^{o}$C for 1, 3, 6, 9, 24 and 168 hours. In addition, the evaluation of the efficacy of heat was assessed by exposing all individuals at 30, 45, 50 and 55$^{o}$C for 60 minutes, 3, 12 and 24 hours. The results of our study demonstrate different susceptibility among life stages, highlighting the exposure interval as an important factor. Our results indicate that extreme temperatures could be used in control strategies of *O. surinamensis*.

Keywords: heat treatment, cold treatment, saw-toothed grain beetle, life stages, non-chemical method

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### OC480. Evaluation of graphene for the control of stored product insects

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In a series of bioassays, the efficacy of different formulations of graphene powders were evaluated for the control of stored product beetle species. In a first bioassay, three formulations with the same nanoparticle size (XER, XEF and ML) were tested, while in a second bioassay graphene formulations with different microparticle sizes (5, 10 and 25 mm) were evaluated. Insect species tested for both bioassays were *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) on wheat and *Prostephanus truncatus* (Horn) on maize at different concentrations, i.e. 0, 100, 500 and 1000 ppm. Adult mortality was recorded after 3, 7, 14 and 21 days of exposure. In the first bioassay, *R. dominica* and *S. oryzae* were found to be the most susceptible species with high mortality rates after 21 days of exposure to 1000 ppm, while *P. truncantus* was the most tolerant species for all graphene formulations tested. Furthermore, XEF was more effective for *S. oryzae* and *R. dominica* at 500 ppm, while ML was more effective for *P. truncatus* in all concentrations examined. In the second bioassay, mortality ranged at low levels for all insect species tested. The results of the present work illustrate that some graphene formulations are effective in controlling the stored product insect species tested and can contribute to the inclusion of graphene in stored product pest management programs, as an alternative method over the use of traditional insecticides, encouraging the production of graphene-based insecticides that can be commercialized for this purpose in the near future.

Keywords: graphene, stored product insects, nanoparticles, alternative control methods

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### OC481. Insecticidal effect of phosphine against eggs of different stored product insect species in laboratory bioassays

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Phosphine is the key fumigant for the disinfestation of durable agricultural commodities. Sub-lethal exposures have resulted in the selection of less susceptible insect populations in many stored product pests worldwide. Eggs have been shown to be the least susceptible and therefore the most critical stage concerning the efficacy of phosphine treatments. In the present study, we evaluated the effectiveness of phosphine against eggs of key stored product insect species, i.e. the red flour beetle, *Tribolium castaneum* (Herbst), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), the lesser grain borer, *Rhyzopertha dominica* (F.), the cigarette beetle, *Lasioderma serricorne* (F.), and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). For each species, two different strains were tested, one susceptible and one resistant to phosphine, and two different egg ages, 1 and 2-day old. The tested concentrations ranged between 50 and 2000 ppm for three different exposure intervals (2.5, 5, and 7 days). The data of the present study indicated that phosphine affected egg hatching in all species tested, given that no hatching was observed for all susceptible populations after exposure to 50 ppm for 2.5 days. A significant reduction in hatching was noted for the resistant populations when the concentration was increased. This study confirms different susceptibility levels among species and populations, and underlines the critical role of the evaluation of the efficacy of this gas against eggs, in order to define effective phosphine-based control strategies.

**Keywords:** concentration, phosphine, eggs, beetles, susceptibility, protocols

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## OC482. Bio-insecticide effectiveness of plant extracts powder against insects of stored products

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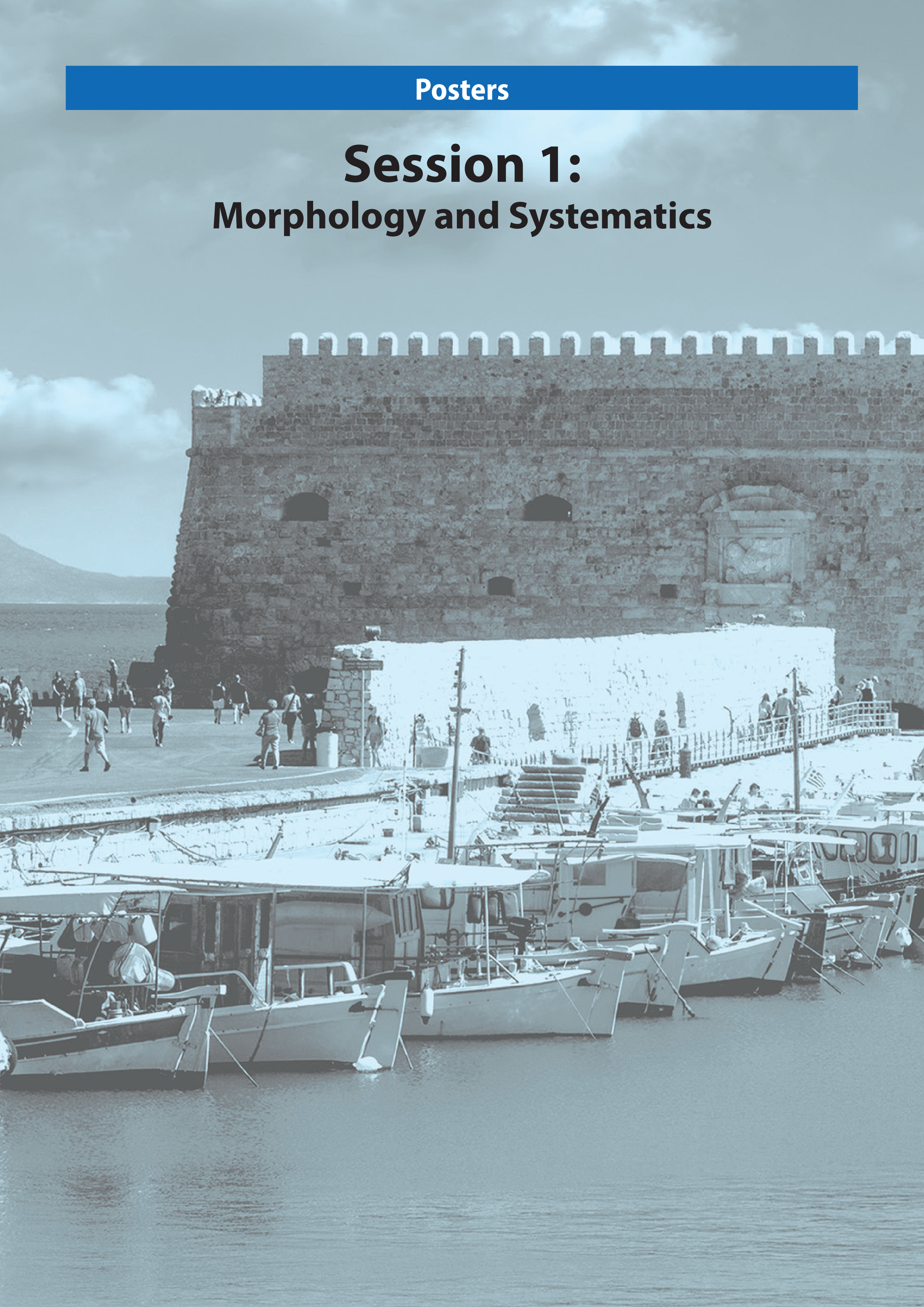
The current study was conducted to evaluate the repellency of 11 plant powders on a major stored-product insect, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The plant species studied were, *Laurus nobilis*, *Salvia rosmarinus*, *Citrus aurantifolia*, *Peganum harmala*, *Cuminum cyminum*, *Cinnamomum cassia*, *Zingiber officinale*, *Lavandula angustifolia*, *Myristica fragrans*, *Trigonella foenum-graecum* and *Syzygium aromaticum*. The repellency was observed at three concentrations for each plant powder (5%W/W,10%W/W and 20%W/W). The obtained results show that all plant powders showed repellency against *T. castaneum*. Although, significant difference between plant powders repellency was observed. The most repellent powder at the immediate observation was *Cinnamomum cassia*, after 1 hour *Cuminum cyminum* had the highest repellency 100% and after 3 hours *Cinnamomum cassia*, *Cuminum cyminum* and *Lavandula angustifolia* were of the same high repellency with no significant difference detected between concentrations for *Cinnamomum cassia* and *Lavandula angustifolia*.

**Keywords:** *Tribolium castaneum*, plant powders, repellency

# Session 1:

## Morphology and Systematics



## P001. Characterization of a new island radiation on endemic *Convolvulus floridus* (Convolvulaceae) in the Canary Islands

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The Canary Islands are considered a biodiversity hotspot and have been the subject of numerous evolutionary studies of endemic arthropod taxa. Here, we investigate the newly discovered island radiation of the endemic genus *Percyella* (Psylloidea: Triozidae) in the Canary Islands, using haplotype analyses and Bayesian dating of mitochondrial cytochrome oxidase I. The genus *Percyella* includes four species, all of which are single-island endemics feeding on the same host species *Convolvulus floridus*: *Percyella canari* on Gran Canaria, *P. guanche* on Tenerife, *P. benahorita* on La Palma and *P. gomerita* on La Gomera. Although modest in species diversity, this radiation is atypical in diversification on a single host plant species, but typical in the primary influence of allopatry in driving the diversification process. Our analyses support monophyly of *Percyella* and hence a single colonization event in the archipelago. The age calibrated Bayesian analysis show that the two taxa on Tenerife and La Palma are older than the taxa on La Gomera and Gran Canaria, and the colonizer of La Gomera most likely originated from La Palma and that of Gran Canaria from Tenerife. Our data also suggests that the structure and extent of diversification within each species is less a product of maximum island age, but more likely influenced by recent periods of volcanism and the topology of individual islands.

**Keywords:** Canary Islands, molecular dating, *Percyella*, *Convolvulus floridus*, radiation

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## P002. Experimentally induced host-shift changes morphology and fluctuating asymmetry in sex-specific manner in a seed beetle: an experimental evolution approach

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Host-shift induced stress in phytophagous insects could have effect on developmental destabilization. Indicator of developmental instability is fluctuating asymmetry (FA)- small random deviations between right and left side of bilateral symmetrical traits. In theory, symmetry is subject to sexual selection in insects, since FA is considered as an indicator of male quality. Response to environmental variation is commonly sex-specific and can affect patterns of sexual dimorphism. We performed reciprocal transplant experiment in eight replicated seed beetle (*Acanthoscelides obtectus*) populations adapted to bean and chickpea. Within the framework of experimental evolution and using the method of geometric morphometrics we: 1. observed the effects of long and short term host-shift on patterns of shape variation of beetles of both sexes and 2. tested hypothesis that both sexes will have a higher level of FA after long or short term change from optimal to suboptimal host. We found that long term host-shift process affected shape in a way that bean adapted beetles had wider and shorter abdomen compared to chickpea adapted ones. Short-term effects are more notable in bean selected beetles, especially in the abdominal region. Bean adapted males had the most canalized development, i.e. the lowest level of FA, compared to chickpea beetles. In chickpea adapted females, difference in level of FA significantly decreased after the short term host-shift. Our work indicates that host-shift process can change morphological aspects of beetles in a sex-specific manner and consequently influence their developmental trajectories.

**Keywords:** host-shift, *Acanthoscelides obtectus*, geometric morphometrics, fluctuating asymmetry, developmental instability

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## P003. A new species, a new invader, or an old neighbour? The curious tale of the aphid *Longicaudinus corydalisicola* (Tao, 1963)

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Determining the geographical origin of species is usually a difficult matter. Talking about aphids, there are many cases in which species have been described when found outside their original distribution areas. At present, citizen science helps finding and monitoring them, while molecular analysis aid with their identification. However, this kind of studies still has a long way to go. In this work, we present a clear example of these difficulties. Thanks to citizen science, an aphid species, which initially could not be identified based on its morphological characteristics, has been located on species of *Fumaria* in the Iberian Peninsula. Subsequently, it has been found in native spontaneous cover crops in citrus orchards in Valencia and in other natural areas of Spain. The analysis of the molecular sequence of the cytochrome oxidase I gene (COI) highlighted a high coincidence with a species native to Southeast Asia, *Longicaudinus corydalisicola* (Tao, 1962), but sometimes the COI is not enough. We try to confirm the molecular identification with morphological analyses. At the moment we have found this species in several localities of the Iberian Peninsula (León in the Northwest, Valencia in the East, and Cadiz in the South) on species of *Fumaria* (*F. agraria*, *F. capreolata*, *F. officinalis*). We made a complete description of the species and illustrated it conveniently. Its status, its possible origin and its presence in Europe, an area apparently very far from its known distribution area to date (South Korea, Taiwan, China and Japan), are discussed.

**Keywords**: aphids, citizen science, alien species, Spain, Fumaria, Iberian Peninsula, cover crops, COI

## P004. Use of Infrared Spectroscopy (ATR-FTIR) to identify aphid species

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Aphids are sap-sucking insects known as major pests for trees, herbaceous and cultivated plants. Due to the large number of known species and their polymorphism, which means several kinds of morphs of adults in the same species, their recognition and identification is very difficult and is based mainly on morphological features. The aims of our study were to suggest the possibility of identifying aphids through the use of Fourier-transform infrared (FTIR) spectroscopy. Our results showed differences in the chemical composition of the aphid body. On the basis of the chemical composition of the body, we were able to distinguish 12 species of aphid. Our research has shown that the main factors enabling the differentiation of the species of aphids are lipid-specific vibrations (peaks around 2958, 2913, 2849, 1737 and 1408 $cm^{-1}$), amides I and II molecular vibrations (peaks around 1626 and 1545 $cm^{-1}$) and molecular vibrations in carbohydrates (peaks around 1248 and 1173 $cm^{-1}$). It has been shown that by using a FTIR spectroscopy it is possible to accurately identify aphid species with an efficiency of 98%.

**Keywords:** aphid, chemotaxonomy; rapid species identification; mid-infrared spectroscopy

## P005. Scaling of the antennal sensory system of the smallest insects

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Insects receive most types of sensory information with antennal receptors, sensitive to temperature, humidity, smell, taste, sound, and other stimuli. Antennal sensory system consists of up to 100,000 receptors and several nervous centers. It is involved in such vital processes as the search for a breeding partner, host, food resources, communication and many others. At the same time, it is fully functional even in miniature insects smaller than unicellular organisms. The effect of miniaturization on antennae was not previously studied in detail. We investigate how size affects antennal sensory system in insects, including effects of extreme miniaturization. Using TEM, SEM, FIB-SEM imaging and 3D reconstruction we studied the antennae of the smallest insect, male *Dicopomorpha echmepterygis* (Hymenoptera: Mymaridae), the miniature parasitic wasp *Megaphragma viggianii* (Hymenoptera: Trichogrammatidae), and the smallest free-living insect *Scydosella musawasensis* (Coleoptera: Ptiliidae). A thorough allometric analysis including data on 483 species from 21 orders (including previously published data) made it possible to formulate general principles of size-related adaptations. We found that the number of structural units (sensilla and scolopidia) strongly depends on the body size, while size, structure and diversity of structural units demonstrates lesser correlation with the body size or does not correlate with at all. This work was supported by Non-commercial Foundation for the Advancement of Science and Education «INTELLECT».

**Keywords:** antenna, sensilla, miniaturization, Johnston's organ, 3D reconstruction, FIB-SEM

## P006. Evolution of pesticide resistance in seed beetles: transgenerational effects of sub-lethal pyrethroid concentration on morphology and development

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Insecticide resistance is an example of rapid evolution under strong selection pressure and, is therefore of great importance for human and environmental health and economy. *Acanthoscelides obtectus* is a globally distributed Coleoptera pest that attacks bean seeds and degrades their quality. One method of protecting against seed beetles is the application of a widely used group of pesticides- pyrethroids. Insecticides, as xenobiotics, can induce stress in pests, leading to destabilization of their development and changes in their morphology. Fluctuating asymmetry (FA), small random deviations between right and left sides of bilateral symmetrical traits, is considered as a result of developmental instability and is thus a measurable indicator of phenotypic response to stress during morphogenesis in insects. In this work, we exposed four populations of *A. obtectus* to sub-lethal doses ($LD_{20}$) of pyrethroid insecticide for five generations in a laboratory evolution experiment. To estimate the level of stress, we monitored the effects of the pesticide on shape changes and the level of FA using a geometric morphometric approach. Our results showed that beetles treated with $LD_{20}$ significantly changed their morphology in the thorax region, while the effect on the level of FA was minor. This work uses experimental evolution as a powerful tool to study pesticide resistance and paves the way for further studies on morphological adaptations, modularity and developmental stability under stress conditions.

**Keywords:** experimental evolution, *Acanthoscelides obtectus*, fluctuating asymmetry, developmental instability, pyrethroids

## P007. The species of the carabid *Platynus* Bonelli, 1801 from the Italian W Alps (Coleoptera, Carabidae)

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The genus *Platynus* – characterized by a worldwide distribution – includes at present about 190 species. The present research focused on the endemic species of the Italian W Alps, which share similar ecological requirements, showing a marked preference for humid and cold environments, sometimes associated with altitude. We studied more than two thousand specimens which were collected to the last 60 years, and are now housed in institutional and private collections. The materials were initially assigned to the five known species from the Italian Alpine area, but since these carabids share quite similar external features, sometimes the species identification was uncertain. The identification, at first carried out with the new detailed dichotomous keys, was then coupled with the geometric morphometrics approach (semilandmarks method), to evaluate and quantify the amount of overall shape variation within and among these taxa. The internal and external anatomical structures usually employed in coleopteran studies were separately examined in both sexes. Together with the georeferenced distribution data, the qualitative and quantitative results confirmed the presence of cryptic groups. The Italian Western Alps *Platynus* are characterized by an increased level of biodiversity, which is commensurated to the detected morphological complexity.

**Keywords:** Italian W Alps, Carabidae, Platynini, ground beetles, Palearctic, cryptic taxa

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## P008. Taxo-Fly project- a EU funded service contract to generate Taxonomic Resources for European hoverflies

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The overall aim of the Taxo-Fly project is to generate Taxonomic Resources for European hoverflies by identifying and capturing taxonomic and other information (ecology, distribution) for the hoverflies of Europe, and making these data openly available on the web in a well-structured system. The taxonomic resources generated within this project will constitute an important basis for future digital (web) tools, e.g. available for use in both European and regionally adapted identification keys important for the EU Pollinator Monitoring Scheme. High-quality digital images of all hoverfly species generated within the project will constitute a new resource of standardized hoverfly images, with diagnostic characters clearly portrayed and indicated. The background and recent developments of the project are presented.

## P009. Identification of endoparasites and parasitoids in colonies of Asian giant hornet, *Vespa mandarinia* (Hymenoptera: Vespidae), using morphology and DNA barcodes in South Korea

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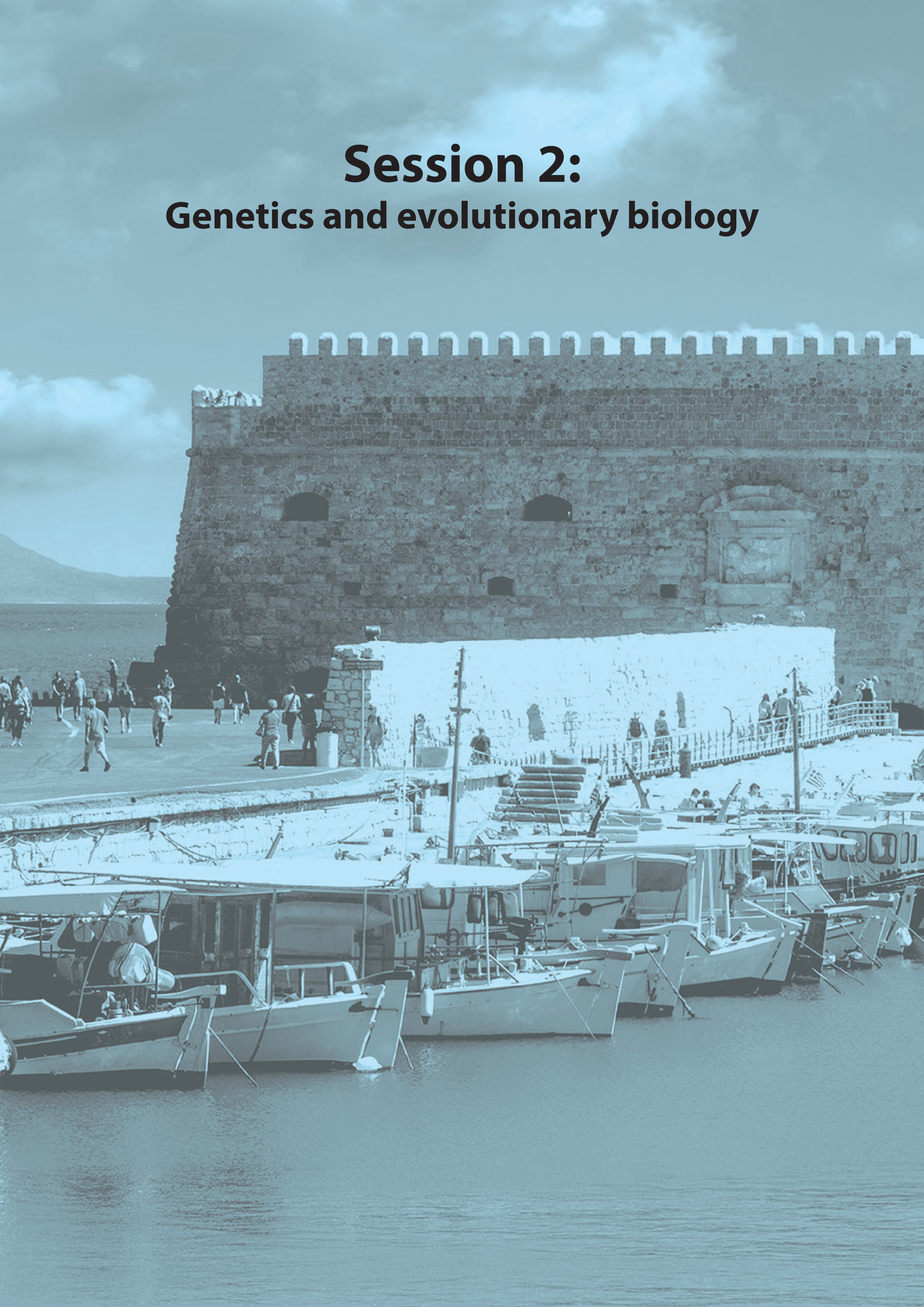
*Vespa mandarinia* is the largest hornet in the world with a size of 30-45 mm and mainly inhabits Northeast Asian countries. *V. mandarinia* has stung many people with their stings and has caused a significant negative economic impact regarding the beekeeping industry, especially as they attack honeybee hives in groups and annihilate the honeybees. Therefore, in order to reduce such attacks, various physicochemical control methods have been attempted, and natural enemies for biological control have been continually searched for and discovered. As *V. mandarinia* has recently been discovered in North America, various information on the control and management of *V. mandarinia* is required. Therefore, in this study, all endoparasites and parasitoids that appeared in the hornet colony in Korea, where *V. mandarinia* is an endemic species, were identified using morphology and DNA barcodes. A total of six species, five families and five orders were identified, and *Xenos moutoni* du Buysson (Stylopidae: Strepsiptera) and *Pheromermis vesparum* Kaiser (Mermithidae: Nematoda) were identified as endoparasites. In parasitoids, *Volucella suzukii* Matsumura and *V. coreana* Shiraki (Syrphidae: Diptera), *Pyralis regalis* Denis and Schiffermüller (Pyralidae: Lepidoptera), and *Quedius pectinatus* Sharp (Staphylinidae: Coleoptera) were found. Among them, *Volucella suzukii* and *Pyralis regalis* were found in almost all colonies, and nematodes were first recorded in Korea. Although they do not directly disrupt the *V. mandarinia* colony, they have been found to have a negative effect on colony development, such as preying on larvae and pupae within the nest or interfering with the activity and reproduction of adults.

**Keywords:** *Vespa mandarinia*, endoparasites, parasitoids, DNA barcode, Korea

# Session 2:

## Genetics and evolutionary biology



## P128. Long read assembly of the insect model species *Rhodnius prolixus* (Hemiptera: Reduviidae), a Chagas disease vector

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Sequencing efforts have provided many insect vector genome assemblies to tackle neglected tropical diseases' transmission; however, for the >150 species of Triatominae, vectors of the Chagas disease parasite *Trypanosoma cruzi*, only three whole genomes are available. Our goal was to generate a new draft assembly for *Rhodnius prolixus*, the main domiciliated vector in Colombia and Venezuela and a model species for insect physiology and development. The individual sequenced was obtained from a Colombian colony; DNA from its head, thorax and legs was extracted and later sequenced by Oxford Nanopore Technologies and Illumina to obtain long and short reads, respectively. The long reads were assembled; completeness and basic statistics were computed, comparing assemblies with and without scaffolding and after one or two rounds of short read polishing. As a result, we obtained a smaller-sized draft assembly (564.6 v/s 706.8 Mbp), which shows higher levels of completeness (98.3% v/s 96.6% Hemiptera database BUSCO genes) and higher average contig size (133,350.18 v/s 42,741.98), with reduced fragmentation compared to the current reference genome available (4,234 v/s 16,537 scaffolds). Our long read draft assembly provides 2467 complete Hemiptera BUSCO genes- 42 more than the reference- which portends that more genes and other relevant genomic sequences would be discriminated in the annotation process, unveiling more of the biology that characterizes this blood-feeding hemipteran, and hopefully providing possible targets for control. This work was supported by Minciencias Convenio 727 DIeI from UR Colombia; ANID- Programa Becas- Doctorado Becas Chile 2019 72200391; Wellcome [204820/Z/16/Z].

**Keywords:** Triatominae, kissing bug, genome, vector, Chagas disease, neglected tropical disease

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## P129. Study of Auchenorrhynchan species composition in vineyards in Greece and phylogenetic relationships of the dominant species of the genus *Empoasca*

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The suborder Auchenorrhyncha is one of the most abundant and diverse insect taxa, containing mostly phytophagous species that feed on a wide variety of plants, including crops of economic importance. They

damage the plants either directly through feeding, causing stippling, or indirectly, through phytopathogens' transmission.
The aims of this research are 1) to study the seasonal abundance of Auchenorrhyncha in a conventional, an organic, and an abandoned vineyard, and 2) to study the phylogenetic relationships of *Empoasca* species using the sequencing analysis of COI mitochondrial DNA (mtDNA) gene segment.
Samples for identification were collected fortnightly, using, yellow sticky traps, Malaise traps, and sweeping net. The samples for sequencing analysis were stored in absolute ethanol. Total DNA extraction was performed using DNeasy Blood & Tissue Kit (Qiagen), and Polymerase Chain Reaction (PCR) using BARCODE primers for the COI mt DNA gene segment. PCR products were purified using the Nucleospin Extract II kit (Macherey-Nagel). Individual sequences were determined via automated sequencing of the above mtDNA gene segment provided by CEMIA S.A. Company (Larissa, Greece). For data processing, the packages BioEdit 7.0.9.0, CLUSTALW2, DnaSP 5.10, MEGA 6.0.0, and GenAlEx 6.5 were applied.
The first results show 1) the most abundant species collected above grapevines belong to the subfamily Typhlocybinae and 2) genetic variability was detected.

**Keywords:** Auchenorrhyncha, sweeping net, Malaise trap, yellow sticky traps, Typhlocybinae, Phylogenetics

## P130. Molecular mapping of the neuroectoderm across phyla – conservation and divergence of brain regions between insects and vertebrates

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Gene expression has been employed for homologizing body regions across bilateria. The molecular comparison of vertebrate and fly brains has led to a number of disputed homology hypotheses. Data from the fly *Drosophila melanogaster* has recently been complemented by extensive data from the red flour beetle *Tribolium castaneum* with its more insect-typical development. In this review, we revisit the molecular mapping of the neuroectoderm of insects and vertebrates to reconsider homology hypotheses. We claim that the protocerebrum is non-segmental and homologous to the vertebrate fore- and midbrain. The boundary between antennal and ocular regions correspond to the vertebrate mid-hindbrain boundary while the deutocerebrum represents the anterior-most ganglion with serial homology to the trunk. The *insect head placode* shares common embryonic origin with the vertebrate adenohypophyseal placode. Intriguingly, vertebrate eyes develop from a different region compared to the insect compound eyes calling organ homology into question. Finally, we suggest a molecular re-definition of the classic concepts of archi- and prosocerebrum.

**Keywords:** brain evolution, insect, vertebrate, mid-hind-brain boundary, eye evolution, archicerebrum

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Alpine aquatic communities are characterized by specialist, poor-species assemblages adapted to low temperatures restricted to high elevations. Alpine species might present wider thermal niche and higher cold tolerance than lowlad species as a consequence of inhabiting regions subject to higher climatic variability, which may determine communities altitudinal segregation. In the present work, we explored the thermal niche of five alpine diving beetles from Sierra Nevada (Spain) and five related species from lowlands, belonging to the genera *Agabus, Hydroporus* and *Boreonectes*. Our aim was to study whether thermal tolerances prevent lowland generalists from colonizing alpine environments and if this could happen in a context of climate warming. To study tolerance to cold, we determined the supercooling point (SCP), lower lethal temperature (LLT) and ice enclosure tolerance, whilst heat coma temperature (HCT) was estimated to explore heat tolerance. All the endpoints were determined at two acclimation temperatures. Alpine species had wider thermal niches (lower SCP and higher HCT) than lowland ones, but the latter showed lower LLTs. In most species, both SCP and LLT were higher at lower acclimation temperature. Most of the species from both regions can be considered as freeze-tolerant, as their LLTs were few degrees lower than their SCPs. All the studied species survived ice enclosure. Overall, our results indicate that cold tolerance does not prevent lowland species from currently inhabiting alpine lakes, suggesting that other factors such as oxygen concentration or biological interactions may be determining species segregation between both areas.

**Keywords:** climate change, Dytiscidae, alpine lakes, lower thermal limit, upper thermal limit

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### P132. Allopatric and ecological diversification in *Cyaniris semiargus* (Lepidoptera, Lycaenidae)

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*Cyaniris semiargus*, the Mazarine blue, is a relatively common Lycaenidae in the western Palaearctic, typically inhabiting grasslands and feeding on various Fabaceae species. It is widely distributed across most of its range but has somewhat isolated populations at mountain ranges at the southern part of its range. Previous studies on mtDNA revealed three lineages in southern isolates, one of which also individually represents one of two evolutionary significant units and corresponds to a population from Doñana, southwestern Spain. This population represents an ecological contradiction: it occupies sandy areas at or slightly above sea level and the larvae feed exclusively on *Armeria velutina* (Plumbaginaceae). Another population with the same apparent ecological needs was later found in Aljezur, southwestern Portugal. Our aim was to explore these lineages and patterns of differentiation with ddRADseq data. Results suggest the existence of two different lineages in the southern isolated populations and very low levels of admixture between these and the widely distributed main lineage. Results also indicate mild to strong divergence of each southern isolate. Further analyses on gene flow, species delimitation, ecology and morphology need to be done, but we have solid indication that these populations present unique diversity important to consider in terms of conservation.

Keywords: ecological diversification, allopatry, isolation

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## P133. Comparative transcriptomics provides insights into insecticide resistance-related genes in phlebotomine *Leishmania* vectors

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Sand flies are important vectors of various human pathogens including *Leishmania* protozoans, bacteria and viruses. They have high adaptability to xenobiotic-imposed selection pressures, while climate change is expected to expand their current eco-geographical ranges. Progress in sand fly research, towards the mechanisms responsible for sand fly adaptations to challenges imposed by naturally-derived or chemically produced xenobiotics has been hampered by the paucity of genomic resources. To fill this gap, we sequenced and assembled the transcriptomes of 11 phlebotomine sand fly species that belong to the *Phlebotomus*, *Lutzomyia* and *Sergentomyia* genera. These species originate from a variety of eco-geographical regions of Europe, Africa, Asia and South America. They represent a wide range of phylogenetic distances among and within the three genera including major proven, potential as well as presumably non-vector species of human leishmaniases. We combine evolutionary and functional genomics approaches to examine patterns of gene content evolution pertaining to phlebotomine sand fly adaptations. More specifically, we delineated orthology relationships and studied the diversity and evolution of gene families related to xenobiotic detoxification, including Cytochrome P450s (CYPs), Glutathione-S-transferases (GSTs), Carboxyl/cholinesterases (CCEs), as well as all known insecticide targets.

The genomic resources generated within the frame of this study provide a basis for the support of effective control strategies against these Leishmania vectors.

**Keywords:** sand flies, RNAseq, comparative genomics, gene family evolution, cytochrome P450s

## P134. The identity of *Neoleucopis* spp. (Diptera: Chamaemyiidae) in Greece and their impact on *Marchalina hellenica* (Hemiptera: Marchalinidae)

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The Giant Pine Scale, *Marchalina hellenica* (Hemiptera: Marchalinidae), is endemic in Greece and Turkey, feeding mostly on pine trees (*Pinus* spp.). While *M. hellenica* is widely regarded as a beneficial insect in its native range and is known to significantly contribute to the production of pine honey, its introduction to other countries (e.g., Italy, Croatia, Australia) has had severe consequences. In these regions, *M. hellenica* has been identified as a destructive invasive species, causing extensive damage to host trees. A preliminary study in northern Greece highlighted the silver fly *Neoleucopis kartliana* (Diptera: Chamaemyiidae) as the most abundant predator of *M. hellenica* among its natural enemies. Studies on *Neoleucopis* spp. were initiated to unravel the identity of all *Neoleucopis* species in Greece and assess their potential as biological control agents against *M. hellenica* in the areas it has invaded. To disentangle the identities of *Neoleucopis* spp. that feed on *M. hellenica* in Greece, we molecularly screened 15 populations throughout Greece using mtDNA COI and assessed their distribution and relative abundance under different *M. hellenica* infestation levels. Our results suggest that a *Neoleucopis* spp. complex is responsible for the suppression of *M. hellenica* in Greece, exhibiting considerable potential as biological control agents against *M. hellenica.* Nevertheless, further studies on the biology and prey-specificity of *Neoleucopis* spp. are essential to distinguish the most suitable *Neoleucopis* species for the biocontrol of *M. hellenica* in the invaded areas.

**Keywords:** invasive species; classical biological control; predatory silver flies

## P135. piRNAs as a new level of regulation of insect oogenesis

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The piRNAs are small non-coding RNAs, with a length between 26 and 31 nt, and are poorly conserved even in closely related species. The first function associated with piRNAs was the repression of transposable elements, as part of an evolutionarily conserved mechanism that preserves genomic stability. At present, new regulatory functions for piRNAs have been described, and it is in this context we want to study the regulatory action of piRNAs in insect oogenesis.

Taking the cockroach *Blattella germanica* as an experimental model, we studied the expression and the function of Bg_piRNA_2367078, one of the most expressed piRNA in small RNA ovarian libraries of *B. germanica*. The expression of this piRNA was analyzed throughout the first gonadotrophic cycle, and by FISH we found that it is expressed in ovarian somatic cells. Treatments with antisense oligonucleotides to reduce this expression allowed us to unveil its function on the ovary. Some delays in the oviposition and defects in embryo development suggested that the action of Bg_piRNA_2367078 is essential to complete oocyte maturation. The results obtained, although preliminary, demonstrate that is possible to modify piRNA levels in an organism, facilitating the study of a new level of regulation of gene expression.

Keywords: *Blattella germanica*, cockroach, insect reproduction, sncRNA

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## P136. Phylogenetic analysis of the cytochrome P450 genes of Lepidoptera

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The species-rich order Lepidoptera includes many of the most important pest species of agriculture and forestry. We present a phylogenetic analysis of the cytochrome P450 (CYP) genes from 20 species of Lepidoptera, covering 12 superfamilies and 16 families and thus representative of the diversity of this order. The CYPomes were manually curated from the best available genome assemblies, and ranged from a low of 70 to a high of 173 P450 genes. The nearly 2000 sequences were distributed in four CYP clans, with the CYP3 and CYP4 clan sequences being the most numerous and the most variable in number.

Phylogenetic analysis revealed two major types of P450 genes, those under single-copy control and those under multi-copy license. Except for the non-ditrysian (outgroup) representative (*Neomicropteryx cornuta*), we found at least 20 genes under single copy control in all 19 species, including 5 involved in

ecdysteroidogenesis. Many such genes are specific to Lepidoptera and of unknown function. The genes under multi-copy licence are often organized in clusters of variable size and constitute lineage-specific expansions or blooms. There was no significant trend between the number of P450 genes and feeding habit (herbivore/detritivore, monophagous/polyphagous) or between the number of P450 genes and phylogenetic position. Many P450 genes have been implicated, often indirectly (constitutive overexpression or RNAi knock-down), in resistance to insecticides. However, functional evidence (heterologous expression or CRISPR/Cas knock-out) remains sparse. Furthermore, a comparative CYPome-wide analysis of P450 expression remains a challenge.

## P137. Genetic diversity, host range and virus transmission ability of the Mediterranean populations of *Bemisia tabaci* Sub-Saharan Africa 2 species

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Whiteflies of the *Bemisia tabaci* complex are among the most important pests of many vegetable, fiber, root and ornamental crops worldwide. The species Sub-Saharan Africa 2 (SSA2, formerly biotype S) of *B. tabaci* was initially described on a single plant species (*Ipomoea indica*) in a small area of the Spanish Mediterranean coast (Nerja, Málaga province). Later, SSA2 populations were also identified in eastern and western sub-Saharan Africa. In this work, we carried out an extensive survey of *B. tabaci* SSA2 populations in the Iberian Peninsula, genotyped them by sequencing the mitochondrial cytochrome oxidase I (mtCOI) gene and established a pure colony. Geographical distribution of this whitefly species was wider than previously known, including southern Portugal. Also, new natural hosts were identified. Sequence analysis of mtCOI gene showed that the Mediterranean populations of *B. tabaci* SSA2 are highly homogeneous but differentiated from the populations present in sub-Saharan Africa. Experimental biological studies showed that this whitefly species is able to survive (oviposite eggs, develop nymphal instars and eclose adults), with different efficiencies, on *Capsicum annuum*, *Dorycnium rectum*, *Ipomoea batatas*, *I. indica*, *Manihot esculenta*, *Mentha suaveolens*, *Nicotiana glauca*, *N. rustica*, *N. tabacum*, *Solanum muricatum* and *Solanum lycopersicum*. Finally, adults of the pure colony were used to confirm the ability of *B. tabaci* SSA2 to transmit begomoviruses (tomato leaf curl New Delhi virus-strain Spain, tomato yellow leaf curl virus-strain IL and tomato yellow leaf curl virus-recombinant variant IS76) and criniviruses (tomato chlorosis virus).
*Acknowledgements: This work was partially supported by grants from the Bill & Melinda Gates Foundation (African cassava whitefly project, OPP1058938) and the European Union's Horizon 2020 research and innovation programme (project VIRTIGATION, grant agreement No 101000570).*

Keywords: *Bemisia tabaci*, genetic diversity, mtCOI gene, Sub-Saharan Africa 2, virus transmission, whiteflies

## P138. Macroevolution and shifts in the feeding biology of the New World scarab beetle tribe Phanaeini (Coleoptera: Scarabaeidae: Scarabaeinae)

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The New World scarab beetle tribe Phanaeini contains coprophagous, necrophagous, mycetophagous and suspected myrmecophilous species. We analyse the largest tribal molecular dataset assembled, incorporating, for the first time, the enigmatic monobasic genus *Megatharsis*, the thalassinus group of the subgenus *Coprophanaeus* (*Metallophanaeus*), and the subgenus *Dendropaemon* (*Eurypodea*) (formerly *Tetramereia*), unveiling their macroevolutionary and biogeographical history in light of Cenozoic abiotic changes and inferring shifts in feeding biology through time. We recover the contentious genus *Gromphas* outside an otherwise monophyletic Phanaeini. We infer *Megatharsis* in a clade containing the apparent myrmecophilous genus *Dendropaemon*, within the *Coprophanaeus* clade, and demonstrate that the subgenus *Coprophanaeus* (*Metallophanaeus*) is polyphyletic, whilst species groups within the subgenus *Coprophanaeus* (*Coprophanaeus*) are monophyletic. Our divergence time analyses and ancestral range estimation indicate an eastern South American origin for Phanaeini in the early Eocene, with subsequent colonization of Central America and the Nearctic during the Oligocene, long before a Panamanian land bridge. A shift to necrophagy in *Coprophanaeus* is possibly linked to increasing Neotropical small vertebrate diversity since the Eocene and, astonishingly, myrmecophily evolved from necrophagy 35 Mya. These drastic shifts in lifestyle are not concordant with variations in diversification rates and appear unlinked to Quaternary extinction of large mammals.

Keywords: diet evolution, dung beetles, Eocene, necrophagy, Panamanian land bridge

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## P139. Assessment of Mediterranean fruit fly population genetics for effective sterile insect technique (SIT) application in Türkiye

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The Mediterranean fruit fly, *Ceratitis capitata*, (Wiedemann, 1824), is on the European and Mediterranean Plant Protection Organization A2 quarantine list. *C. capitata* has a high spreading capability, tolerates diverse environmental conditions, and feeds on a broad number of plant species. *C. capitata* is among the most significant pests affecting the production and trade of fresh fruits, especially citrus, in Türkiye and worldwide. Effective and collective management efforts have been studied because of the increasing Mediterranean fruit fly populations in agricultural areas of Türkiye, and the response to changes in the pest

populations associated with global warming registered in recent years. Hence, population genetic information is needed for the management of sustainable agricultural programs in Türkiye. This study aims to assess the efficacy of the sterile insect technique by molecular methods. The COI barcoding region will be sequenced to determine the different haplotypes within the region subject of the study. Sequencing will allow access to *C. capitata* individual genotypes by multiplexing microsatellite loci to estimate the genetic diversity which will improve the effectiveness of the sterile insect technique. The difference between laboratory-reared Mediterranean fruit flies and field-collected populations will be also studied in areas under SIT regime to determine the best sterile insect release methodology aiming to eradicate *C. capitata*.

Keywords: Mediterranean fruit fly, mitochondrial COI, microsatellites, allele diversity, SIT, IPM

## P140. Genomics of domestication in the arboviral vector *Aedes aegypti*

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*Aedes aegypti* is the primary vector of arboviruses worldwide and exists as two distinct phenotypic subspecies: the generalist *Aedes aegypti formosus* (*Aaf*) and the domesticated *Aedes aegypti aegypti* (*Aaa*). Domestication manifests in mosquitoes living in proximity with humans by laying eggs in artificial containers and blood-feeding on humans preferentially. *Aaf* has remained confined in Africa, whereas *Aaa* emerged from Africa, distributed globally through the Atlantic slave trade, enhancing its vectorial capacity. Following the distinct geographic distributions of both subspecies, we sequenced and used public data to compared genomes of 511 African and 123 out-of-Africa mosquitoes to identify genomic changes associated with the behavioral switch to domestication. From the identification of ≥300 million high-confidence SNPs, we applied genetic structure analyses to infer divergence across populations and to identify genomic regions with different evolutionary signals between *Aaa* and *Aaf*. After a large-scale comparative and functional analysis of the 14,677 *Ae. aegypti* genes across populations, we identified a clear signal of genetic differentiation between *Aaa* and *Aaf*, circumscribed to a catalogue of positively-selected genes. These "*Aaa molecular signature genes*" include chemosensory and immunity genes, which are known to impact host seeking behavior, vector competence and overall response to external stimuli, and extended to genes with neuronal and hormonal functions. Several of these genes have switched to positive selection across Aaa populations from a rich stock of ancestral and weakly evolving standing variants in *Aaf* mosquitoes. Besides providing effective markers to genotype *Aaa* mosquitoes, our findings generate foundations to understand *Ae. aegypti* domestication process.

Keywords: *Aedes aegypti*, genome, population differentiation, domestication, selection, local adaptation, vector-borne diseases.

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## P141. Eight new mitogenomes of *Bemisia* (Hemiptera: Aleyrodidae): Insights into the phylogeny of *B. afer* sensu lato and endemic Canary Islands *Bemisia* spp

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The whitefly (Hemiptera: Aleyrodidae) fauna of the Canary Islands (Spain) comprises more than 20 named species distributed among a dozen of genera, including a number of endemic species. Whitefly taxonomy is largely based on the morphology of the puparia, although it is also recognized that puparia can vary considerably depending on the plant hosts where whiteflies develop. A paradigmatic example of this phenomenon is the *Bemisia afer* complex (*B. afer* sensu lato), that as a whole is highly polyphagous. Thus, *B. afer* sensu lato appears to be an assemblage of taxa with a particularly broad range of puparial forms. Thus, in the Canary Islands, forms A to H have been described. In this work, we have applied molecular technologies to get a better understanding of the evolutionary history and phylogeny of the *B. afer* sensu lato populations present in the Canary Islands. For this, the complete sequence of mitochondrial genomes (mitogenomes) of single individuals of *B. afer* sensu lato forms B, C, F and G from Tenerife Island have been obtained. Also, mitogenomes have been characterized for a *B. afer* sensu lato population from Málaga (continental Spain) and for three species endemics to the Canary Islands, *B. medinae*, *B. reyesi* and *B. euphorbiarum*. The phylogenetic analysis carried out including available mitogenomes of *B. afer* sensu lato and other whitefly species has provided insights into the relationships between the populations analyzed in this work and with other Aleyrodidae taxa.
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**Keywords:** *Bemisia afer*, *Bemisia* spp., DNA sequence, mitogenomes, phylogeny, whiteflies

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## P142. Metagenomics for ecological inference: Unravelling the unknown biology of smoke-flies (Diptera: Platypezidae: Microsaniinae)

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Flat-footed flies (Diptera: Platypezidae) are a group of small (3-6 mm) flies classified into four subfamilies based on diagnostic morphological characteristics. There are approximately 277 species worldwide. One of the subfamilies, the cosmopolitan Microsaniinae or the smoke-flies, is of particular interest. Smoke-flies are fumotropic i.e. they are attracted to smoke from burning wood or forest debris where they swarm. The knowledge of the immature stages and host fungi remain completely unknown for the smoke-flies, but exist for most other Palaearctic flat-footed flies. Determining the characteristics of the larvae is critical for unambiguously resolving the phylogenetic affinities of the smoke-flies. We have identified two different approaches to resolve the enigma. We use DNA barcoding to identity the phoretic mites (which smoke-flies occasionally carry) to infer the ecology and habitat preferences of the mites, which can help identify the habitat of the smoke-fly larvae. Additionally, we apply short read shotgun metagenomics of entire smoke-flies samples to explore if we can identify a molecular signature of the fungi in the obtained genomic reads for ecological inference of habitat. Taxonomic classifiers are used for the identification and extraction of reads of fungal origin, followed by extraction and investigation of the ecological preferences of the identified fungi and a possible fungal host. Integrating the results obtained from these novel approaches will provide keyl information to help locate the specific environment / substrate of the unknown larvae and eventually the larvae.

**Keywords:** smoke-flies, Microsaniinae**,** fumotropic, phoresy, metagenomics

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## P143. Pollinator genomics as a tool to develop intraspecific biomonitoring applications

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The last century of anthropogenic activities drastically affected earth ecosystems causing a severe biodiversity crisis. Insect pollinators have been affected by these changes, with drastic declines observed in diversity, density and distribution of natural populations. These insects are responsible for up to 65% of pollination of flowering plants and three out of four crops producing fruits or seeds for human consumption are dependent on pollinators, making them essential for our food supply. Preserving intraspecific genetic diversity, which ultimately determines the adaptive capacity of species, is a major aspect of biodiversity conservation that has often been overlooked. Characterizing and monitoring this basal level of biodiversity is thus key to build adequate conservation strategies. Here, we will use three pollinator species as models to develop intraspecific monitoring tools: *Maniola jurtina* (butterfly), *Syritta pipiens* (hoverflies) and *Bombus terrestris* (bumblebees). We will sample populations spread across Europe and use whole genome resequencing data mapped onto high quality, chromosome-level, reference genomes of each species to capture in detail their intraspecific genetic composition. These population genomics analyses will dissect the history and demographic structure of the three pollinator species and scan for signals of adaptation to the different European biomes. The data will be used to build reduced genomic representation toolkits that recover intraspecific patterns of genetic diversity, and can be used as monitoring tools. This research project is integrated in the Biodiversity Genomics Europe project (https://biodiversitygenomics.eu), a pan-European initiative that aims at promoting the application of genomics to improve our understanding and protection of biodiversity.

**Keywords:** biodiversity, insects pollinators, genomics, bioindicator, conservation, biodiversity genomics Europe

## P144. Methoprene tolerant (Met) is required for the correct formation of the basal ovarian follicle in cockroach panoistic ovaries

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Methoprene tolerant (Met) is a transcription factor belonging to the basic helix–loop–helix/Per-ARNT-SIM (bHLH–PAS) family, whose most well-studied function is to act as a receptor for juvenile hormone. In the cockroach *Blattella germanica*, which have the ancestral panoistic type of ovaries, we had observed that Met is expressed in the ovaries of the sixth (last) nymphal instar (N6), although, paradoxically, the levels of circulating JH are very low or undetectable in N6. We established the Met expression profile in the N6 ovaries, which shows an expression peak towards the end of the instar, on days 6, 7 and 8. Then, we depleted Met transcript levels in N6 using RNAi, obtaining the maximum reduction on day 8 (N6D8), the last day of the instar. We observed that *Met* depletion affected oogenesis, in particular the number of oocytes in the ovariole, and the formation of the follicular epithelium of the basal ovarian follicle. In Met-depleted insects, we observed that there were fewer oocytes in the ovarioles (one less, on average) than in the controls. The number of mitoses in the follicular epithelial cells was lower, and the basal ovarian follicle was smaller in Met-depleted insects than in controls. We conclude that Met is important in oogenesis of the panoistic ovaries of *B. germanica*. Work is in progress to find out whether Met's action is associated with JH or has nothing to do with this hormone.

**Keyword**: *Blattella germanica*, Juvenile hormone, kr-h1, ecdysone, insect reproduction

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## P145. piRNA and hormones in the panoistic ovary of the cockroach *Blattella germanica.*

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The Piwi-interacting RNAs (piRNAs) are involved in the control of transposable elements (TEs) as part of an evolutionarily conserved mechanism that protects the genome from viral or parasitic threats, thus preserving genomic stability. Moreover, recent research suggests possible piRNA functions as regulators of gene expression. Unlike miRNAs, the sequences of piRNAs are poorly conserved, even in closely related species, and thousands of unique piRNA sequences have been described in different insect species. Thus, taking into consideration the high amount of piRNAs found in insect ovaries and their low conservation rate, the existence of some conserved piRNAs common to distantly-related insect species suggests that these piRNAs may have relevant functions beyond counteracting TEs.

Using ovarian small-RNA libraries from the cockroach *Blattella germanica*, we identified the piRNAs most abundantly expressed in each key stage of *B. germanica* oogenesis. We are studying their expression profile by real-time PCR and their localization in the ovarian cells by *in situ* hybridization. We will identify their putative mRNA targets describing possible piRNA co-expression modules.

**Keywords:** insect oogenesis, panoistic ovary, ecdysone, juvenile hormone, sncRNA

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## P146. Embryonic development of the spider *Tegenaria pagana* C. L. Koch, 1840 (Araneae: Agelenidae)

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The study of embryonic development is a basic step in evo-devo studies and is important for the understanding of phenotypic evolution. Not much is known about comparative embryonic development within spiders, as only three model species, *Cupiennius salei* Keyserling, 1877 (Araneae: Trechaleidae) (Wolff, Hilbrant, 2011), *Parasteatoda tepidariorum* (C. L. Koch, 1841) (Araneae: Theridiidae) (Mittmann, Wolff, 2012), and *Acanthoscurria geniculata* (C. L. Koch, 1841) (Pechmann 2020), were previously studied extensively. Although in many respects their development is similar, there are differences in the number of stages, the rate of development, and the details of embryogenesis. In our study, which is part of a larger project to investigate the evolution of eye development in cave spiders, we describe the embryogenesis of *Tegenaria pagana* C. L. Koch, 1840, a troglophilic species, using confocal microscopy. The development of *T. pagana* is slower than that of *P. tepidariorum*, with a similar number of stages. In this presentation, I discuss the characteristic features of *T. pagana* embryogenesis. In general, we can conclude that the embryogenesis of spiders is rather conservative in the three different araneomorph families that were studied thus far. Now we can more accurately describe the embryological development of spiders. Our next step will be to compare the embryonic development of an eye-reduced *Tegenaria* species and find the differences between these two sister species that have gone through ecological speciation.

**Keywords:** spiders, development, embryology

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## P147. Origin of the European grapevine moth in California

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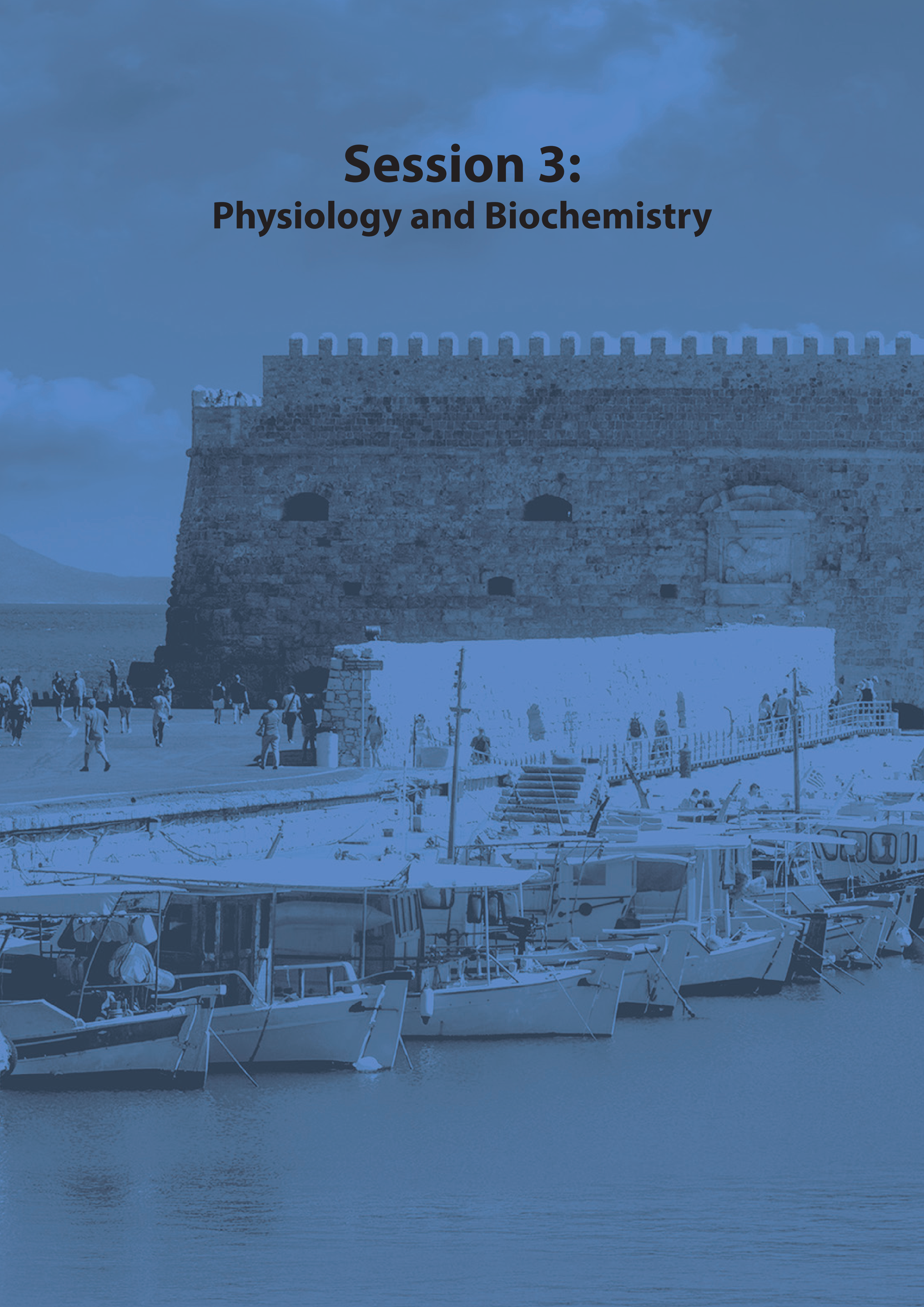
The European grapevine moth (EGVM), *Lobesia botrana*, is a pest of grapevines in the Palearctic ecozone. In 2008, the moth was reported in Chile and subsequently California in 2009. Determining geographical origins of insect pests can aid in developing local management methods and genetic approaches were used to determine the geographical source of the EGVM population in California. Thirteen countries originating from Europe, South America and the Middle East were sampled using field bated pheromone traps. Phylogenetic analysis was completed on DNA data from the mitochondrial CO1 and ITS2 loci, in addition to SNP information gathered from sequencing restriction-site associated DNA tags (RAD Seq). The CO1 and ITS2 gene regions show very similar genetics among and within sample populations. Applications of RAD Seq revealed detailed patterns of phylogeography indicating the EGVM in California and Chile was introduced from the Spanish and French regions of Europe.

Keywords: phylogenetics, grape berry moth, vine pest, biological control

# Session 3:

## Physiology and Biochemistry



### P188. Using insect surfaces to guide the design of engineered material surfaces with superhydrophobic and bactericidal activity

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Insect wing surfaces have micro- and nano- features that create a topography which results in multifunctional properties like hydrophobicity, antibacterial activity, and anti-reflectivity. For instance, cicadas (Hemiptera: Cicadidae) have nanopillars on the surfaces of the wings that make them superhydrophobic and bactericidal. Leafhoppers (Hemiptera: Cicadellidae) are unique in that they produce and excrete molecular granules (brochosomes) that are spread onto the integument by the insect. These adaptations have captured the interest of researchers looking to draw inspiration from the natural world to generate novel solutions and technologies. Here, we use a bioinspired design framework to compare these different approaches for achieving similar functionalities, prototype engineered surfaces inspired by insect surfaces, and discuss possible applications. Our work provides valuable insight into the guided design of novel functionalized materials that promote hydrophobic and self-cleaning activities

**Keywords:** bioinspiration, biomimicry, brochosome, hydrophobicity

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### P189. The heat shock response in *Polistes* spp. from differing climates following heat stress

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With climate change increasing the average global temperatures and the frequency of extreme thermal events, mechanisms to cope with heat stress are becoming progressively more important. One of these mechanisms is the heat shock response, consisting of several chaperone proteins whose expression is upregulated in response to different physiological stresses such as heat stress. The heat shock response is almost completely uncharacterized in the cosmopolitan paper wasps (*Polistes*). We aimed to investigate how the heat shock response differed between three closely related *Polistes* species from three different climates (alpine Austria: *P. biglumis*, temperate Austria: *P. dominula*, mediterranean Italy: *P. dominula* and *P. gallicus*). Experiments were conducted on *Polistes* brood (small larvae, large larvae, early pupae, late pupae). Nests were collected from wild populations and exposed for one hour to either 25°C (control), 35°C (moderately stressful), or 45°C (highly stressful). Gene expression of three heat shock proteins (Hsp70, Hsc70, Hsp83) was then assessed through a qPCR assay. Preliminary results for Austrian *P. dominula* showed

that expression of both Hsp70, Hsc70, and Hsp83 was upregulated in the highly stressful treatment compared to the control, while no upregulation was seen in the moderately stressful treatment. Furthermore, the scope of upregulation differed between developmental stages, with large larvae having the smallest response. Further results will show whether these patterns can be seen in the two other species. The results of this study will help expand the current understanding of how this genus will cope with future climate changes.

Keywords: heat shock proteins, *Polistes*, heat stress, temperature, climate

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## P190. Yeast glucan particles for macrophage-specific regulation of cellular metabolism in *Drosophila*

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Insect immune cells perform a large number of non-immune functions in the body. Our data from *Drosophila melanogaster* show that situations accompanied by severe metabolic stress, such as starvation, bacterial infection, metamorphosis, or experimentally induced lipolysis, are accompanied by infiltration of the fat body by macrophages. We have found that macrophages infiltrating the larval fat body during postmetamorphic maturation temporarily assume the role of histolysis-undergoing adipocytes, converting leaking lipids and cellular debris into further exploitable storage peptides and lipoproteins.

Although analogous non-canonical roles of macrophages can be assumed in other insect species, their investigation is limited by the lack of tools for macrophage isolation and experimental manipulation in vivo. Given the wide ecological variation in insect species, a wide range of previously undiscovered non-canonical roles of immune cells in many physiological processes can be anticipated.

To overcome these limitations, we introduced yeast-derived glucan particles (GP) as a highly specific macrophage delivery tool with negligible immunogenicity and toxicity in *Drosophila*. GPs can be used for efficient delivery of macrophage-specific transcription factors, small metabolic inhibitors, and siRNAs, and thus can be used for experimental manipulation of phagocytosing cells in vivo. GPs can be easily modified by the covalent binding of fluorescent tags and paramagnetic nanoparticles, allowing their tracking, localization, and magnetic separation for subsequent analyses.

Our preliminary data from *Pyrrhocoris apterus* and *Apis mellifera* indicate the broad potential of these tools for future studies of immune cells in a broad spectrum of insect species.

Keyword: plasmatocytes, macrophages, fat body infiltration, metabolic stress, macrophage-specific manipulations

## P191. Gene composition and expression regarding fatty acid absorption in three Hemiptera species with disparate fat content in their diet

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*Mahanarva fimbriolata*, *Dysdercus peruvianus*, and *Rhodnius prolixus* have remarkably different dietary fat content suggesting gene expression variations regarding proteins involved in fatty acid (FA) activation and binding. Both mechanisms allow retaining FAs intracellularly, although FA binding also reduces the amount of cytosolic free FAs, thus increasing their absorption. The expression of FA-Transport Protein (FATP), Acyl-CoA Synthetase Ligase (ACSL), FA-Binding Protein (FABP), and Acyl-CoA-Binding Protein (ACBP) genes was analyzed in all species by RNAseq of four midgut regions and the carcass. FA-binding proteins are the most expressed genes in all insects. However, *D. peruvianus* has the highest expression in all analyzed gene families, followed by *R. prolixus* and *M. fimbriolata*, which agrees with a higher fat content in cottonseed than in blood and especially the xylem. In contrast, *M. fimbriolata* has more *FABP* and *ACBP* genes than the heteropterans, a unique *ACSL* expressed along the midgut, and filtration chamber-specific genes. Results suggest a relationship between the gene expression of FA activation and binding proteins and the dietary fat content. High-fat content diets involve more FA activation, whereas low-fat content diets compensate with extensive intracellular FA binding. All insects absorb FA in the posterior midgut, the central region of nutrient absorption. However, *M. fimbriolata* also absorbs in the filtration chamber, which agrees with the scarce fat content in the xylem. *R. prolixus* FA absorption occurs mainly in the second half of the midgut, where blood cell digestion increases. Finally, *D. peruvianus* absorbs FAs along the midgut consistent with its high-fat content diet.

**Keywords:** fatty acid absorption, dietary fat content, Hemiptera

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## P192. Ontogeny of the oscillator controlling the sperm release rhythm from the testes in the yellow mealworm beetle - *T. molitor* (Coleoptera: Tenebrionidae)

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A spectacular example of a biological rhythm implemented by a peripheral system, is the moth's circadian, endogenous and temperature-compensated rhythm of sperm release (RoSR) from the testes to the vas deferens. This rhythm has so far been described only in lepidopterans. Here, our primary goal was to verify the hypothesis that this peripheral rhythm occurs in other insects. We commenced our search among beetles, the largest group of insects. We examined sperm release from the testes during ontogenesis in the

yellow mealworm beetle, *Tenebrio molitor*. We correlated RoSR with activity of the molecular oscillator in the reproductive organs, and verified the influence of the oscillator (and its malfunction) on males' reproductive fitness.
We show that RoSR in the beetle, *T. molitor,* operates according to a ~24 hour cycle. Further, it demonstrates characteristics of a typical circadian rhythm, controlled by an oscillator located in the male reproductive system. This oscillator was found to be fully brain-independent as it generates RoSR in organs cultured *in vitro*. We have demonstrated that this rhythm is established between the 8th and 9th days of pupal development. Maintaining the rhythm affects the fertility of males, expressed by the number of spermatozoa transferred to females during copulation and sperm quality. In conclusion, we have determined that RoSR is not a phenomenon limited to lepidopterans. Indeed, it seems to be common in other insect groups and is crucial for successful reproduction. Supported by the funding of the University of Warsaw IDUB grant #BOB-IDUB-622-322/2022 (PSP: 501-D114-20-1004310) awarded to P.B.

**Keywords:** biological oscillator, sperm release, male fertility, Tenebrionidae

## P193. Effect of *Geniotrigona thoracica* propolis on the growth inhibition of *Malassezia globosa*

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Stingless bee propolis has been widely reported for many bioactivities. The difference and potential in bioactivities of propolis depends mainly on bee species, food plants, and external factors. Here, *Geniotrigona thoracica* propolis was focused due to very rare reports on bioactivities. The growth inhibition of *Malassezia globosa*, pathogenic yeasts which cause skin diseases to many people living in tropical zones, was chosen. By agar well diffusion and broth microdilution assays, the potential in growth inhibition of *M. globosa* was determined by inhibitory concentration at 50% ($IC_{50}$). The $IC_{50}$ value of crude MeOH extract was 2.21 mg/mL while the $IC_{50}$ value of crude MeOH partitioned extract was 1.22 mg/mL. By silica gel 60 column chromatography, fraction 1 gave the better $IC_{50}$ value of 185 micrograms/mL. By NMR, a main active compound in fraction 1 was analysed to be methyl gallate (MG). Minimum fungicidal concentration of MG was found to be at 8 mg/mL. Since *M. globosa* cannot synthesize its own fatty acid, it produces lipase to digest lipid on host skin instead. Thus, inhibition of lipase by MG was assayed. It was found that MG at $IC_{50}$ value could inhibit the lipase activity at 30.25 ± 1.91%. However, the potential in lipase inhibition of MG did not depend on the dose of MG. In overall, it can be concluded that *G. thoracica* propolis is potential in inhibiting the growth of *M. globosa*. Also, this inhibition may be relating to the lipase inhibition if the low dose of MG is used.

**Keywords:** lipase, methyl gallate, pathogen, propolis, stingless bee

## P194. Fat body content: Indicator of what?

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Various aspects have been considered to study bee health, especially immune and detoxification responses. Research in these areas has been conducted at the cellular (e.g., haemocyte) and humoral (e.g., antimicrobial peptide (AMP), melanisation and detoxification pathway) levels. However, focusing on these metrics requires costly devices (e.g., quantitative PCR instruments) and highly trained experimenters. As an alternative, scientists have focussed on fat body content as a proxy for immunocompetence, since this tissue is known to be the main site for AMP and detoxification enzyme production, and since required equipment are cheap and easy to use. Yet, no research has ever properly demonstrated a link between fat body content and immune-related metrics. Motivated by incongruencies found in the literature and in previous research in our laboratories, we exposed bumblebee *Bombus terrestris* microcolonies to various stressors (i.e., nutrition, xenobiotic, infection and physical) and then assessed fat body content together with more direct immune-related parameters. In the haemolymph, we determined (i) phenoloxidase activity (i.e., an enzyme involved in the melanisation process), (ii) bacterial growth inhibition (i.e., due to AMP) and (iii) haemocyte populations (i.e., involved in several immune responses). Our preliminary results showed no significant differences among treatments, neither in phenoloxidase activity nor in fat body content, while haemocyte counts varied significantly. In addition to demonstrating that fat body content may fail to accurately reflect the immune status, it definitely showed that fat body content failed to capture discrepancies among treatments. We argue that fat body content should be interpreted carefully in future studies.

**Keywords:** bee, immunity, metrics, fat content, proxy

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## P195. Allatotropin affects immune system activity of mealworm beetle *Tenebrio molitor* L.

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The insect's immune system plays a major role in defending the body against pathogens. Its activity may be regulated by neuropeptides produced and secreted by the neuroendocrine system. One of the largest and most important families of neuropeptides are allatotropins (AT), mainly responsible for the synthesis and release of the juvenile hormone. Despite many studies on the activity of the insect immune system, the knowledge of its hormonal regulation is still scarce.

After application of Tenmo-AT (GIEHFKYHNMDLGTARGYa), the activity of cellular and humoral mechanisms of mealworm beetle *Tenebrio molitor*, was investigated. Obtained results clearly suggest that Tenmo-AT influences different immune parameters. The regulatory role of tested neuropeptides was especially visible in case of humoral response, including phenoloxidase activity and lysozyme-like activity. In addition, the effects of Tenmo-AT were time- and dose-dependent, which may suggest multifunctional role of AT during pathogen infection.
Learning about the effects of AT on insect immune system activity is next step in understanding the hormonal regulation of innate immune mechanisms as well as their evolution. In addition, the presented results may be also useful for searching new, selective insecticides sustainable for crop protection.

**Keywords**: neuropeptides, insect immunity, hormonal regulation

## P196. Critical thermal maxima ($CT_{max}$) of larvae and adults of three European paper wasp species from differing climatic areas (*Polistes dominula* CHRIST 1791*, P. gallicus* LINNÉ 1767*, P. biglumis* LINNÉ 1758)

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An insect's thermal niche and boundaries are utilized to determine physiological bases of adaptation and to model future dispersal under changing environmental conditions. Originally from the Mediterranean, European *Polistes* paper wasps spread across the continent over areas with varied climatic conditions. *P. dominula* is increasing its range northward, *P. biglumis* conquered montane terrain (above 1000 m MSL), *P. gallicus* remained in its original settlement area. The larvae's possibilities for temperature regulation are minuscule, they depend on the adult's actions. Adults strive to keep nest temperatures within a range favorable for brood development, below 42°C (behavioural thermoregulation by means of water droplets and fanning). We examined the $CT_{max}$ of larvae and adults via respirometry or behavioral observation. Single individuals were tested in a measurement chamber while driving a temperature ramp from 25°C to 55°C at a rate of 0.25°C per minute. The characteristic $CO_2$ curves were evaluated for cease of spiracular activity (onset of respiratory failure). In *P. biglumis* adults, behavioral observation was used to determine the cease of coordinated movement. Both parameters are routinely used as a metric for $CT_{max}$. In the adults, *P. biglumis* showed the highest $CT_{max}$ (48.5±0.6C°); it differed significantly from *P. dominula* (47.4±1.1°C), and *P. gallicus* (47.7±1.0°C; $p<0.05$). In the larvae, only *P. dominula* had a higher $CT_{max}$ (48.8±0.8°C) than the adults ($p<0.05$). Further experiments will clarify these preliminary findings.

**Keywords:** insects, *Polistes*, critical thermal maximum, temperature, climate

## P197. The respiratory metabolism of paper wasps' larvae and pupae from differing climates (*Polistes dominula, Polistes gallicus*)

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European paper wasps (*Polistes* sp.) originated in the Mediterranean area, but are now widely distributed and inhabit quite different climates. These primitively eusocial insects developed strategies of nest thermoregulation, mainly to prevent overheating of the brood. The development speed of the brood depends strongly on ambient temperature. The adults try to keep the nest temperature within the viable range. We investigated the respiratory metabolism of single larvae and pupae of *Polistes dominula* from the temperate climate (Austria) and *Polistes gallicus* from the Mediterranean climate (Italy), in order to reveal physiological adaptations to their habitat's microclimate. Respirometry experiments were conducted in a temperature range from 5-45°C. The mass specific metabolic rate of larvae and pupae increased exponentially with temperature in both species. The temperate species (*Polistes dominula*) overall had a higher mass-specific metabolic rate compared to the Mediterranean species (*Polistes gallicus*). In contrast to *Polistes dominula*, in *Polistes gallicus* the pupae had a significantly higher metabolic rate than the larvae. As the metabolic rate mainly depends on temperature, we presume the Mediterranean species exhibits a lower metabolism, especially at higher temperatures, to preserve energy for development. This way they compensate for increased metabolic costs due to their habitat's higher microclimate temperature.

**Keywords:** insects, *Polistes*, brood, metabolism, temperature, climate

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## P198. Macrophage-induced insulin resistance is an adaptive strategy for lipoprotein mobilization upon bacterial infection

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The immune response is an energy-demanding process that must be coordinated with systemic metabolic changes redirecting nutrients from stores to the immune system. Although this interplay is fundamental for the function of the immune system, the underlying mechanisms remain elusive. Our data show that the pro-inflammatory polarization of *Drosophila* macrophages is coupled to the production of the insulin antagonist *ImpL2* through the activity of the transcription factor HIF1α. *ImpL2* production, reflecting nutritional demands of activated macrophages, subsequently impairs insulin signaling in the fat body, thereby triggering FOXO-driven mobilization of lipoproteins. This metabolic adaptation is fundamental for the function of the immune system and an individual's resistance to infection. We demonstrated that analogically to *Drosophila*, mammalian immune-activated macrophages produce *ImpL2* homolog IGFBP7 in a HIF1α-dependent manner and that enhanced IGFBP7 production by these cells induces mobilization of lipoproteins from hepatocytes. Hence, the production of IMPL2/IGFBP7 by macrophages represents an evolutionarily conserved mechanism by which macrophages alleviate insulin signaling in the central metabolic organ to secure nutrients necessary for their function upon bacterial infection.

**Keyword:** hemocytes, *Drosophila*, ImpL2, plasmatocytes, fat body, infection

### P199. Seal lice survive in the sea breathing underwater

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Sucking lice are obligate and permanent ectoparasites, spending their whole life in the fur or among the hairs of their mammal hosts. Among them, seal-lice (Echinophthiriidae;Anoplura) infest amphibious hosts, such as pinnipeds and otters, who perform deep dives and remain in the open sea for long periods. During the evolutionary transition of pinnipeds from land to the ocean, these lice had to manage the amphibian biology of their hosts, who may spend more than 80% of the time submerged and performing extreme dives beyond 2000m profundity. These obligate and permanent ectoparasites have adapted to tolerate hypoxia, high salinity, low temperature, and, in particular, high hydrostatic pressure conditions. A major question, debated since decades ago, is how do seal lice survive without contact with atmospheric air, during the long excursion of their hosts in the open sea. Two possibilities can be proposed, either lice drastically reduce their metabolism to spare energy or they obtain the oxygen that they need from seawater. To shed some light on this question, we measured the consumption of oxygen by lice either exposed to air or submerged in water, at different temperatures. Additionally, we assembled, annotated, and examined the genomes of six species, looking for candidate genes potentially related to respiratory functions. We found that lice were able to obtain oxygen from seawater and that they reduce their metabolism when submerged. The presence of respiratory pigment genes (globins and hexamerins) also suggests that during deep-diving seal lice could store cellular oxygen.

**Keywords:** co-evolution, adaptation, metabolism, respiration

### P200. The role of insect neuropeptides in response to temperature stress in mealworm beetle, *Tenebrio molitor* L.

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Through the process called neuroendocrine integration, nervous and endocrine system interplay together to regulate a number of physiological processes and maintain system-wide homeostasis in regular as well as stressful situations such as temperature stress. One of the most important molecules in the nervous system of all living animals are neuropeptides, which regulate physiological processes. In our research we studied the expression level of five genes encoding insect neuropeptides, namely short neuropeptide F (SSRSPSLRLRFa), PISCF (QSRYXQCYFNPISCF-OH) and MIP (SKWDNFRGSWa) allatostatins, proctolin (RYLPT) and tachykinin-related peptide (RPKPQQFFGLMa) in brain and ventral nerve cord (VNC) and their effect on survival of *Tenebrio molitor* after cold (-5 °C) and heat (40 °C) stress.

The heat stress causes in the brain the up-regulation of the expression of only two genes: proctolin and MIP/AST, while in VNC a down-regulation of almost all tested genes can be observe, except for MIP/AST which is highly up-regulated. In case of cold stress, the changes in expression in the brain were observed only for the MIP/AST – high up-regulation. However, in VNC we noted a down-regulation of four tested genes, except for MIP/AST which is highly up-regulated. In turn, the injection of neuropeptides affected the survival of the insects, as the MIP/AST significantly decreased the longevity of *T. molitor* especially when combined with temperature stress. These results demonstrate that neuropeptides are key players in insect response to temperature stress.

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**Keywords:** insect neuropeptides, temperature stress, insect survival

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### P201. Ovarian transcriptomes of epoxidase null mutants reveal candidate genes related to JH signaling and reproduction fitness in *Aedes aegypti*.

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Juvenile hormones (JH) are a group of acyclic sesquiterpenoids that regulate metamorphosis and reproduction in insects. In mosquitoes, the last two steps of JH synthesis occur via methylation and epoxidation reactions. Our previous data have shown that a mutation in the P450 epoxidase (EPOX) gene affects *Aedes aegypti* reproduction fitness. In *epox*$^{-/-}$ females, previtellogenic ovarian development is impaired, and the number of eggs laid are significantly smaller. Therefore, we examined gene expression changes at four time points during the development of the ovaries of *epox*$^{-/-}$ and wild-type female

mosquitoes. Comparison of the four different transcriptomes revealed genes that are differentially expressed in mutants with modified JH signaling. We are functionally studying these genes to understand their role in ovarian development.

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## P203. Possible involvement of *SIFamide receptor* and *allatotropin* in the regulation of juvenile hormone biosynthesis during metamorphosis in a cricket

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In insects, metamorphosis is promoted by the suppression of juvenile hormone (JH) biosynthesis in the endocrine organ, corpora allata (CA). Myoglianin (Myo), a TGF-beta ligand, acts as the key molecule in the suppression of JH biosynthesis. However, how Myo regulates JH biosynthesis is still unknown. We found in a cricket *Svercacheta siamensis* (formerly known as *Modicogryllus siamensis*) that *myo* mRNA was upregulated in the brain, but not in the corpora cardiaca (CC)-CA complex, of the last instar nymphs when compared to the early instar nymphs. These results suggest that Myo in the brain regulates JH biosynthesis during metamorphosis. Next, we focused on the neuropeptides and their receptors under the control of Myo in the brain. We found that *allatotropin* (*at*) and *SIFamide receptor* (*SIFar*) mRNA levels were regulated by Myo; *myo* RNAi downregulated *at* expression in the brain, while it upregulated *SIFar* in the CC-CA complex. We detected immunoreactive signals of SIFamide (SIFa, the putative ligand for SIFar) in the CC-CA complex and in the pars intercerebralis (PI) of the brain. Immunoreactive signals of AT were observed in the CC, nerves surrounding the CA, and the brain region ventral to the central body. These results suggest that, in the last instar nymphs, Myo in the brain suppresses JH biosynthesis through upregulation of *at* in the brain and downregulation of *SIFar* in the CC-CA complex, to promote the metamorphosis.

**Keywords:** metamorphosis, Myoglianin, SIFamide receptor, allatotropin

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## P204. Genetic diversity and developmental characteristics of the two-spotted cricket *Gryllus bimaculatus* De Geer (Orthoptera: Gryllidae) in South Korea

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In this study, we investigated the developmental characteristics and genetic diversity of seven populations of two-spotted crickets (*Gryllus bimaculatus* De Geer, 1773 (Orthoptera: Gryllidae)) raised in South Korea. Regarding the developmental characteristics of the species, we observed no statistically significant

difference in the weight of the nymphs in the six populations we tested. After molting, although weight differences were observed between the populations in each stage of the developmental period, the average weight for each developmental stage was constant. We also analyzed mitochondrial *COI* gene sequences (DNA barcoding region) of the reared crickets collected from five insect farms and two national insect rearing facilities and the resultant sequences were analyzed together with the 12 sequences from foreign countries specimens obtained from public data. We detected six haplotypes from 111 specimens, indicating a low intraspecific genetic distance (~1.8%). The most dominant haplotype was overwhelmingly haplotype 1, which was found in all South Korean specimens and four specimens from China, Indonesia, and Germany. These findings indicate that the low genetic diversity of South Korean specimens can be explained by the fact that the *G. bimaculatus* population imported for feed from Japan in the early 2000s became a maternal group that spread throughout cricket farms in South Korea. In order to breed healthy cricket strains, it is necessary to increase genetic diversity by importing them from other countries through appropriate quarantine procedures.

Keywords: *Gryllus bimaculatus*, genetic diversity, *COI*, haplotype, developmental characteristics, Korea

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## P205. Selected humoral aspects of *Galleria mellonella* immune reaction upon secondary infection with *Pseudomonas entomophila*. Preliminary tests of antibacterial properties of newly identified Kazal peptide Pr13a

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In recent years, one of the leading topics in immunobiological studies is so-called immune priming - a phenomenon of increased survival of invertebrates during repeated contact with a given pathogen. We checked the survival rate of non-primed and primed *Galleria mellonella* larvae after infection with *Pseudomonas entomophila*. We analyzed the antibacterial activity of the hemolymph of infected primed and non-primed individuals and compared the low molecular protein content using Tris-Tricine SDS-PAGE. Furthermore, reversed-phase high-performance liquid chromatography (RP-HPLC) analysis was carried out and proteins whose amount was higher in the primed *versus* non-primed infected larvae were identified. The analysis allowed us to distinguish a protein encoded by the LOC113509813 gene, which has recently been described as Kazal peptide Pr13a. To elucidate the antibacterial properties of the peptide, we conducted an anti-*P. entomophila* plating assay and atomic force microscopy (AFM) analysis of *P. entomophila* peptide-treated cells. The increased survivability of primed larvae was correlated with the antimicrobial activity of their hemolymph and with the total low molecular protein content. The RP-HPLC analysis revealed differences in the protein pattern and indicated a few proteins that were more abundant in the primed infected individuals. The most significant one, identified as Kazal peptide Pr13a, showed high anti-*P. entomophila* potential in the plating assay and AFM imaging, which indicates its role in insect humoral response.

*Pseudomonas entomophila* is a kind gift from dr. Frideric Boccard (France), whose group isolated this strain in Guadeloupe. The work was financed by National Science Centre, Poland, project number 2020/37/B/NZ6/00167.

**Keywords:** *Galleria mellonella*, *Pseudomonas entomophila*, peptides, priming

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## P206. Can the solitary bee *Centris analis* recover from the effects of exposure to the fungicide azoxystrobin?

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Most fungicides are less toxic to adult bees than insecticides, but this does not indicate they are safe for pollinators. Aiming to evaluate the effect of oral exposure to the fungicide azoxystrobin on the midgut and the behavior of the solitary bee *Centris analis*, as well as its recovery capacity, newly-emerged males were submitted to continuous oral exposure to syrup with fungicide (10 ng i.a./µL) for 48 hours (T1). After exposure, bees were fed with syrup without fungicide for 48 hours (T2). Behavior alterations (N=40 bees) and midgut immunofluorescence labeling of heat shock protein (Hsp70) were assessed at T1 and T2 (N=12 bees). Histological analyzes (N=16 bees) were performed at T2. Behavior alterations were evaluated using 15-minute videos-tracking per bee in an arena with a light source. At T1 and T2, no behavioral alterations were observed in the locomotion (P=0.27) and light perception of bees (P=0.54). Midgut alterations occurred only at T2. Hsp70 labeling increased by 1.16 times in exposed bees (P<0.001). The number of regenerative nests was reduced by 1.46 times (P=0.004). These midgut alterations showed that bees were under stress and cellular mechanisms were activated to repair epithelium damages and prevent de novo cell death. Those mechanisms maintain individual homeostasis explaining the unaffected behaviors. Therefore, the azoxystrobin caused subtle effects on *C. analis* that may be reversible in a healthy bee. However, under natural conditions, bees can be exposed to other pesticides and pathogens and these subtle effects can cause high-load stress affecting bee' health and performance.

**Keywords:** Neotropical bees, sublethal effects, stress proteins, midgut

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## P207. Whether and how plant secondary metabolites affect metabolism of the storage pest tenebrio molitor?

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Many plant species produce glycoalkaloids (GAs) as protection against herbivores and pathogenes. These substances are sugar derivatives of alkaloids with a ring structure and have high biological activity. There are only a few studies concerning the mechanisms of GAs action in insects, thus, their activity remains largely unknown. To evaluate the effect of GAs on insect tissues, their ability to change the metabolism can be assessed. The aim of the study was to determine the effect of GAs on the level of following enzymes, phosphofructokinase, citrate synthase, and 3-hydroxyacyl-CoA dehydrogenase, in gut and fat body. During the experiments, three GAs (solanine, chaconine and tomatine) were tested at a concentration of 10-8 and 10-5 M. Tomato leaf GA extract was also used, which made it possible to compare its activity with the activity of pure GAs. Tested substances were administered to the larvae using the microinjection technique. The level of enzymes were determined in tissues of Tenebrio molitor mealworm larvae 2 and 24 h after the application of the compounds using immunodetection techniques. The analysis revealed the effect of the tested substances on expression levels of metabolic enzymes in insect tissues. The results of the research confirm the high biological activity of these compounds. The observed changes are time- and concentration dependent. Based on the conducted experiments, it can be concluded that GAs affect the metabolism pathways in beetle tissues. Taken together, this research provides important insights into GAs action in insects.

**Keywords:** metabolism, glycoalkaloids, solanine, chaconine, tomatine, *Tenebrio molitor*

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## P208. Revealing the binding specificity and interaction mode of bio-inspired compounds with Odorant Binding Protein 1 of the mosquito *Anopheles gambiae*

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Over the last decades, the search for new insect repellents has been directed towards products of plant origin as they are considered safer and more sustainable than synthetic ones. However, the main drawback

of plant-repellents is their limited protection time due to high volatility. To this end, synthetic bioinspired compounds exhibiting lower volatility could provide prolonged efficacy compared to their plant-derived parental compounds.

Odorant Binding Proteins (OBPs), constitute the first molecular filter in the insects' olfactory system for the selective capture of airborne chemical cues. A subset of 10 *Anopheles gambiae* OBPs have been found to exhibit strong female-specific expression and suggested to be involved in host-seeking behavior. Among them, AgamOBP1 has emerged as a molecular target for insect repellents.

Herein, utilizing a fluorescence competition assay, we determined the affinity of 25 bioinspired compounds discovered by AgamOBP1-structure-based approaches. Seven of them that were top-ranked in molecular docking studies were further validated by Isothermal Titration Calorimetry. Finally, regarding the most bioactive ligand we determined its interactions with the AgamOBP1 by $^{1}$H-$^{15}$N HSQC-NMR and X-ray crystallography at 1.3 Å resolution. The ligand was found to occupy a binding site located at the interface of the crystal dimer. This work revealed AgamOBP1-specificity for novel bioactive molecules, which belong to chemical classes divergent from those of known synthetic repellents, and the structural determinants that define ligand affinity at atomic level. Together, such information extends the available chemical space and can lead to the generation of improved QSAR and "olfactophore" models toward AgamOBP1-structure-based repellent discovery.

**Keywords:** mosquito repellents, bioinspired compounds, AgamOBP1, crystal structure

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# Session 4:

## Ecology and Behavior

## P087. Uncovering the olfactory system of the beet armyworm (*Spodoptera exigua*): Insights for ecology and control strategies

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The beet armyworm, *Spodoptera exigua*, is a common lepidopteran pest worldwide that feeds on various crops and weeds during its caterpillar stage. Caterpillars use their sense of smell to identify feeding spots and to detect natural enemies, while adults use it also for mating and egg-laying behaviour. Characterizing the olfactory system is crucial for the understanding of the ecology of insect species, and in designing control strategies based on manipulating olfactory-driven behaviours.

In the first part of our study, we annotate the full repertoire of odorant receptor (OR) genes in *S. exigua* genome and compared to related species. Gene birth and death analysis showed specific losses and one gains in occurred in *S. exigua* lineage. We also use RNA-seq data to identify genes with sex-biased transcriptional patterns, which pointed out to ORs linked to sexual behaviors.

In the second part of our study, we conducted a detailed analysis of the olfactory system of *S. exigua* larvae. We used scanning electron microscopy and in-situ hybridization to identify the number and types of olfactory sensilla present in the antenna and maxillae. Our findings indicate that although *S. exigua* caterpillars host only a few sensilla on their olfactory organs, the number of ORs expressed is high due to each sensillum being innervated by multiple olfactory sensory neurons.

Overall, our study provides valuable insights into the olfactory equipment of *S. exigua*, which will facilitate further studied aimed to deep into the ecology of this species, and in designing new olfactory-based control strategies.

Keywords: olfaction, odorant receptors, *Spodoptera exigua,* caterpillar olfactory system, comparative genomics, gene gain and loss

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## P089. Gross antennal morphology of *Philaenus spumarius* juveniles and behavioural response to olfactory plant cues

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*Philaenus spumarius* (Hemiptera: Aphrophoridae) is the main European vector of the plant-pathogenic bacterium *Xylella fastidiosa.* Despite a large amount of literature available on this species, virtually nothing is known about host plant-finding by *P. spumarius*. The movement towards food sources has been documented both for juveniles and adults, but the mechanisms mediating the host plant location remain unknown.In this study, preliminary investigations of the gross morphology of nymphs' antennae through scanning electron microscopy were conducted, together with Y-tube behavioural assays aimed at evaluating the response of nymphs to olfactory stimuli emitted by alfalfa plants. Nymphs' antennae consist of scape, pedicel and flagellum. Antennal structures are distributed on the pedicel and on flagellomeres from II to V. Different sensory structures were described: basiconic sensilla, sensory cavities, campaniform sensilla and hair sensilla. Although our preliminary observations did not allow to

appreciate the fine structure of the observed sensilla, an olfactory function could be hypothesized for basiconic sensilla spotted along the flagellum, since olfactory pegs also occur in adults' antennae. Results of behavioral assays in the Y-tube olfactometer suggest that nymphs could have detected olfactory cues in the odd Y-tube without orientate themselves towards the olfactometer arm bearing the triggering stimulus. Hence, host plant's volatiles may function as non-directional stimuli. Furthermore, it could be possible that nymphs move toward host plants thanks to a combination of sensory stimuli, for example, olfactory together with visual cues, as evidenced for other Auchenorrhyncha species and suggested for *P. spumarius* adults.

Keywords: spittlebug, Aphrophoridae, host-location, olfaction

## P090. High sexual activity of hungry females in a gift-giving spider: congruence or sexual exploitation by males?

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The females of the nursery-web spider mate with the males in an exchange of nuptial gifts consisting of dead prey wrapped in silk. Mating with hungry females is beneficial to males because these females are more willing to copulate than food satiated females. We hypothesized that hungry females could advertise their foraging needs by pheromones to signal their sexual interest with males who will provide nuptial gifts (the congruence hypothesis). Alternatively, we hypothesized that females do not actively advertise their hunger condition, but, instead, males are able to respond to specific cues (or their absence) in female draglines, where sexually active pheromones are present. We examine male mating behaviour in the absence of visual cues from the female, leaving exclusively draglines of a hungry and fed (or mated/unmated) female in the arena, and we subsequently examined the chemical content of the female dragline with HPLC chromatography using differences in 43 peaks. Female hunger levels did not influence male investment in the production of nuptial gifts. HPLC chromatography also failed to show any differences between hungry and well-fed females. Therefore, we extended the behavioral chemical analyses and found that there are significant differences in the chromatography of female silk between 1. Adult and subadult females, mated and unmated females, and gravid and non-gravid females. We conclude that there is no evidence of male exploitation of female chemical cues, at least if the HPLC method is considered. This work was supported by grant APVV 20–0081.

Keywords: *Pisaura mirabilis*, nuptial gift, pheromones, draglines

## P091. Effects of different foraging strategies in different climate conditions on the reproductive success in the desert harvester ant *Pogonomyrmex barbatus*

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Foraging strategies are crucial for the survival and reproduction of animals. Foraging of ants is manifested through collective behavior, the coordinated behavior of group without central control. To understand how this collective behavior responds to changing environmental conditions, a long-term study has been conducted using the red harvester ant *Pogonomyrmex barbatus* as model organism. Harvester ant colonies have been monitored on a study field site for over 30 years, and their foraging behavior was measured in fluctuating weather conditions. Previous work already shows that the collective regulation of foraging in response to water loss is associated with reproductive success. In this project we are asking how drought conditions have shifted selection pressures on collective behavior. The main food source of harvester ants are seeds, which are also the main source of water. Living in a desert, the ants forage under harsh weather conditions and desiccation risk. We hypothesized that the regulation of foraging in response to humidity has fitness consequences for the colonies in this population. We used restriction-site associated DNA sequencing (RAD-seq) to generate a genome-wide map of polymorphism at 9824 single nucleotide polymorphisms (SNPs). Based on these genotypes, we inferred kinship among 488 colony samples, to detect mother-daughter pairs of colonies. Thereby, we assess the reproductive success of colonies over 20 years. We use this large dataset to test whether colonies that reduced foraging on dry days have higher reproductive success.

**Keywords:** collective behavior, ant, climate, foraging, reproduction

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## P092. Sex in the dark: male investment in nuptial gift production is affected by the visual environment

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The males of the gift-giving spider, *Pisaura mirabilis*, wrap dead prey with silk and then offer it to the female as a form of nuptial gift before copulation. Because the specific external conditions where mating of this species occurs are not well understood, we hypothesize that wrapping with silk would increase the gift visibility in a dark environment in the vegetation. We first investigated the visual properties their resting environment (morning and late afternoon) under natural habitats and compared them with shady environments below the resting places, where they spend most of their activity (and probably also mate). Spiders were randomly divided into dark (red light) and light (white light) environments. Half of the males were experimentally treated to avoid silk production. We predicted that dark environment in captivity results in heavier investment in gift construction and that males unable to wrap are rejected in dark environments more than in light environment where gifts are conspicuous even without wrapping them with silk. Furthermore, male spiders were expected in investing more silk in gift production under dark conditions. External resting places were significantly lighter than the non-resting places, and males invested more in gift production in the dark environment. Unfortunately, treated

males spent extraordinarily much time in attempts to produce gifts, thus it was not valid to examine the rejection rates of these males under various environments. We conclude that the visual environment was a significant driver of male gift construction behaviour. This work was supported by grant APVV 20–0081.

**Keywords:** *Pisaura mirabilis*, nuptial gift, silk, nursery-web spider

## P093. Dietary influences on reproductive behaviour of the black soldier fly

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The black soldier fly (BSF; *Hermetia illucens*) is widely used as an organic waste converter and alternative protein source in livestock feed. Larvae can convert a wide range of organic substrates differing in nutritional quality. Our aim was to investigate whether larval and/or adult diet impact their reproductive behaviour. This species has a natural lekking system, where the male grabs the female mid-air and spirals to the ground while wing-fanning. On the landing surface the male starts abdominal tapping and makes preliminary genital contact. In this courtship sequence, wing fanning is thought to be important for female acceptance. Our study focused on the influence of dietary differences in two life stages of the fly on BSF reproductive behaviour; low and high nutrient diets in the larval stage and presence/absence of feed in the adult stage. The experimental cages were specifically designed to provide the required space for natural lekking behaviour. To promote BSF reproductive behaviour UV, green and blue LED lights were provided in addition to fluorescent strip lights above the cages. We made video recordings of male grabbing of the female, male wing fanning, preliminary genital contact and female acceptance. Courtship duration, mating duration and male-male interactions were also quantified as same-sex courtship behaviour is frequently observed in this species. Our results indicate that adults reared on a low nutrient diet have a longer mating duration than those reared on a high nutrient diet.

**Keywords:** reproductive behaviour, lekking, black soldier fly

## P094. The mystery of disappearing widow spiders in the Negev desert

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Nests of adult female desert widow spiders, *Latrodectus revivensis* and *L. pallidus*, are highly visible in the desert shrubland and preserve a complete record of individual productivity that can be monitored and compared over the years. During 1992-2000, a yearly survey was conducted of nests of *L. revivensis* in the Negev highlands near Sede Boqer, Israel. Two additional surveys were added in 2009 and 2015.

We counted *L. revivensis* nests at the end of the reproductive season (December or January) and recorded the number of egg-sacs present in each. In 1994 nests of *L. pallidus* were added to the survey. A subset of *L revivensis* nests was collected to analyze prey remains, and egg-sacs were opened to count the contents (eggshells or young). The abundance of *L. revivensis* declined sharply in 1994 and did not recover during the following years. There was a weak positive relationship between rainfall in the previous wet season and the number of nests in the following reproductive season, yet nest numbers did not increase in three years following above-average rainfall. There was no significant relationship between previous-season rainfall and the number of egg-sacs/nest, and only a weak positive relationship between rainfall and the number of prey/nest. Spider abundance and productivity were unrelated to the number of prey consumed. Furthermore, *L. pallidus* abundance did not explain the decline of *L. revivensis*. I propose that there was an indirect effect of rainfall on spider abundance via habitat change and subsequent predation risk.

Keywords: long-term survey, rainfall, productivity, habitat, predation

## P096. The role of field-collected cues in the host recognition of twig girdlers (*Oncideres rhodosticta*) on honey mesquite (*Prosopis glandulosa*)

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Conspecific gravid females integrate volatile organic compounds (VOCs) to exploit potential oviposition sites; the role of VOCs has not been examined on twig girdlers, *Oncideres rhodosticta,* and honey mesquite, *Prosopis glandulosa*. To examine the host selection behavior of female twig girdlers, we examined the preference and searching time between healthy and girdled twigs of honey mesquites in New Mexico, USA. Among 21 different volatile organic compounds, female twig girdlers reacted to six chemical compounds from girdled twigs and four from healthy twigs. While females responded to Heptanal, (E)-2-Heptenal, and Linalool oxide on both healthy and girdled twigs, they also reacted to one healthy specific compound and two girdled specific compounds. Female twig girdlers distinguished healthy twigs over girdled twigs with faster searching time. Our finding demonstrates VOCs in the genus *Oncideres* for the first time, providing additional empirical information to help decide on the biological control potential of possible agents during the pre-release risk assessment.

**Keywords:** host recognition, olfactory cues, VOCs, *Oncideres rhodosticta, Prosopis*

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## P097. An integrated approach for the control of *Coroebus undatus* (Coleoptera, Buprestidae)

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Cork is a natural and versatile material extracted from *Quercus suber* trees, which are mostly grown under agro-silvopastoral exploitation. Such systems secure environmental, social and economic sustainability at regional level, in climatic zones and soils deemed inappropriate for other uses. Portugal produces about half of the world cork, exports reaching €1.2 billion in 2022. Cork oaks are regularly attacked by defoliators and xylophagous insects, yet the flathead oak borer *Coroebus undatus* (Coleoptera, Buprestidae) causes serious economic damage to cork, since in some stands over 40% of the trees might be colonized. This collaborative University – Industry project focuses on the bio-ecology, behaviour and chemical ecology of *C. undatus* in Southern Portugal, aiming at the development of efficient control methods, based on the use of semiochemicals. In 2022, a population of *C. undatus* was studied, by monitoring about 200 trees, located in an extensive cork oak system, Évora District. The beetles were trapped within a net covering the tree trunk and transferred to a laboratory culture. Emergences started in early May, a marked protandry being observed, while the sex ratio was biased towards males:  0.63 females/male. Further biological parameters and results of the behavioural tests conducted will be presented. Preliminary analysis of the volatiles, emitted by beetles in different

physiological conditions, as well as by their feeding substrates, were conducted using gas chromatography–mass spectrometry and electroantennographic detection. Antennal responses to some compounds were obtained, pointing to potential applications regarding the integrated control of *C. undatus*.

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## P098. Exploiting the evolution of odorant discrimination in ants to decipher the olfactory code

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Chemical communication is a key component in the life of eusocial insects. It relies, among other factors, on their ability to identify an extensive diversity of ligands using odorant receptors (ORs). Insect ORs are characterized by their massive expansion through gene duplication, where new ORs may develop different ligand specificities, possibly as a result of positive selection and adaptive evolution. Our goal is to infer such events and identify amino acid substitutions that alter OR specificity towards ligands in the carpenter ant *Camponotus floridanus*. We selected candidate ORs that show positive selection after specific-specific duplication in the *C. floridanus* lineage. We then mapped those mutations on a 3D model of insect ORs, and selected candidates for which positive selection acted on an amino acid around the putative ligand binding site. These sequences are now engineered into mutant flies, and tested for their response to different ligands. Comparing the resulting specificity profiles of closely related paralogs shows whether positively selected mutations changed ligand specificity relative to the ancestral protein.

**Keywords:** gene duplication, positive selection, protein structure, olfactory receptors, social insects

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## P099. Effects of the menstrual cycle on human skin odors and mosquito attraction

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Women are frequently underrepresented as subjects in biological and medical research and are sometimes actively excluded from studies to avoid variability associated with the female reproductive cycle. The resulting knowledge gaps can have important implications for human health and wellbeing, particularly in the case of health concerns that disproportionately impact women. For example, there is substantial evidence that pregnant women experience elevated rates of malaria infection, with serious health consequences including reduced birth weight and increased rates of infant mortality. Yet, we currently know little about sex-specific differences in the odor cues that mediate mosquito attraction.

To address this gap, we explored variation in female body odors over the course of the menstrual cycle and its implications for mosquito behavior. We collected skin-odor samples from 16 women during

three phases of the menstrual cycle (menstruation fertile and luteal), and from an equal number of male controls, then tested the relative attraction of the primary malaria vector (*Anopheles gambiae*) to odors from the same individuals over time. Female subjects became significantly less attractive to mosquitoes during menstruation, while no systematic effect of timepoint was observed for male subjects; however, overall variability in attraction observed for males and females was similar.
Building on these findings, we are currently conducting chemical analyses of odor samples collected at the timepoints described above to explore the cues underlying the observed effects of menstruation on mosquito attraction. We are also exploring the variability of odor emissions and mosquito attraction to female subjects over longer time periods.

Keywords: skin volatiles, menstrual cycle, mosquitoes, *Anopheles*, behavior, malaria

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## P100. Searching new tools to improve *Phlebotomus papatasi* control strategies

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Phlebotomine sand flies are important vectors of pathogens of medical importance, such as the *Leishmania* parasites responsible for 700,000 – 1 million new cases of leishmaniasis every year. For developing novel and targeted vector control tools it is important to improve our understanding on the ecology of sand flies across all different stages of their development. It is well known that olfactory cues play important role on insect behaviour. While there are numerous studies investigating the behaviour of adult sand flies in response to attractants or repellents, the ecology of larvae remains comparatively less known. The olfactory bioassays were constructed to investigate the effect of the odors from the natural sources on the behaviour of sand flies (larvae/adults). Gas chromatography mass spectroscopy (GC-MS) equipped with the automated headspace (HS) system was applied to determine the molecular compositions of the volatiles from the study materials, and to identify molecule(s) that possibly produce the attraction or repellency. These molecules could be used to improve attractant/repellent formulations, or/and lead us to the structures for development of new active compounds.

Keywords: sand fly, attractant, repellent, bioassay

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## P101. The smell of drought: Water limitation alters plant volatile emission and parasitoid attraction to herbivore-infested plants

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Intensity and frequency of droughts increase in most European countries. Plant-mediated effects of drought can alter multitrophic interactions by directly influencing performance of herbivorous insects and their natural enemies or indirectly by changing emission of herbivore-induced plant volatiles (HIPVs) and attraction of natural enemies. As drought stress severely alters phytohormonal signaling and plant metabolism, it is difficult to predict its effect on HIPV emission and attraction of parasitoids, which use HIPVS as host-finding cues. Moreover, it is challenging to disentangle species-specific effects from the effect of the mode of drought stress (e.g. intensity, duration). We will present data from two different study systems, 'sugar beet – black bean aphids – *Aphidius colemani*' and 'cabbage – cabbage white caterpillars – *Cotesia glomerata*'. Long-term drought stress resulted in reduced emission of total HIPVs per sugar beet plant, as drought-stressed plants were much smaller than controls. Parasitoid attraction positively correlated with total HIPV emission, resulting in highest attraction to the well-watered plants. When HIPV emission was standardized by plant weight, emission of some compounds appeared to be upregulated in drought-stressed plants, suggesting that short-term drought stress (with no effects on plant size) might result in increased HIVP emission and parasitoid attraction (under investigation).
Short-term drought altered HIPV emission from cabbage, with some compounds increasing and others decreasing upon drought stress, but did not alter parasitoid attraction. We are currently testing if parasitoids focus on those compounds that do not change much upon drought stress and thus act as reliable signals in variable environments.

**Keywords:** drought, HIPVs, plant volatiles, parasitoids

## P010. Do phylogenetic drivers involved in the diversity of tomato volatile profiles shape antixenosis resistance against *Tuta absoluta*?

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Plant volatiles play a pivotal role in interaction with other plants and Animals. Among their different ecological functions, volatile organic compounds (VOCs) are known to convey information used by phytophagous insects to locate host plants. In this study, first we addressed phylogenetic drivers involved in the variation of tomato volatile emissions. We explored the diversity of VOC profiles released by 30 accessions of tomato, belonging to five species, including both domesticated and wild relatives. Second, we performed behavioral assays on 10 accessions with strong differences in VOCs emissions to test the relationships between the diversity of volatile profiles and antixenosis resistance of tomato against *T. absoluta*. Based on the 77 VOCs detected, a redundancy analysis showed a large ecological diversity in plant volatile profiles at both inter- and intra-specific levels... While ancestral species, like *Solanum habrochaites*, release large amount of sesquiterpenes, these VOCs are drastically reduced in tomato that recently evolved in the Galapagos (*S. galapagense*) or that was domesticated *(S. lycopersicum)*. Over the 10 accessions used for behavioral assays, this study confirms that *S. lycopersicum* is attractive towards *T. absoluta*. Among wild relatives, *S. cheesmaniae* includes one accession attractive and one accession repulsive. In addition, two accessions of *S. pennellii* were repulsive. Our results suggest that sesquiterpenes such as (3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, β-myrcen and curcumen could serve as repellent, whereas δ-elemene, indole, p-mentha-1,5,8-triene may help *T. absoluta* to locate host plants. Altogether, this study provides interesting information to better understand plant defenses against insects and improve plant protection in tomato crop.

**Keywords:** plant volatile, tomato, ecological diversity, phylogeny, *Tuta absoluta,* plant-insect interactions

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## P011. How generalist generalist aphid species respond to alien plants?

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Alien invasive plant species are considered as a major threat to global agriculture and food safety. Their introduction in Europe needs continuous monitoring and risk assessment because they may serve as refuges or reservoirs for aphid pests and/or pathogens of conventional crops. In this study, we compared the colonization, by the generalist aphid species *Aphis fabae,* of various alien plant species belonging to the herbaceous stratum (*Reynoutria japonica*, Polygonacea ; *Senecio inaequidens*, Asteraceae ; *Impatiens glandulifera*, Balsaminaceae ; *Solidago canadensis,* Asteraceae ; *Galinsoga quadriradiata* Asteraceae ; Erigeron canadensis, Asteraceae) and frequently present adjacent to vegetable crops. We assessed two components of *A. fabae* behaviour in a context of immediate colonization: (1) its probing behavior using the Electrical Penetration Graph (EPG) technique and (2) its survival and fecundity by using the clip cage technique. We also investigated (3) its colonization ability towards these invasive species over several weeks. The results revealed a gradient in colonization success that ranged from low to none for *A. fabae* on *Solidago canadensis* to high on *Reynoutria japonica*. Our results are discussed in terms of an agronomical point of view and in relation to both the enemy release hypothesis and the behavioral constraint hypothesis.

Keywords: exotic plants, Invasive plants, host plant suitability, Aphididae, EPG, demographic parameters

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## P012. Survival of virgin and mated olive fruit flies of various ages under conditions of food deprivation

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The effect of mating on survival of olive fruit fly adults, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), was studied under conditions of food deprivation at different ages. The role of mating has never been studied before in fruit flies in this context. Upon emergence, adults were placed in individual cages with water and food (a mixture of hydrolyzed protein and sugar). At the 10th day of their life, groups of adults of the same (virgin) or both genders (mated) were transferred to plexiglass cages with water and food. At the end of the 10th day insects were transferred back to their individual cages. To record survival at the absence of food, ten adults of every treatment of a certain age were each transferred to a new individual cage deprived of food. Their survival was recorded every 4 hours. It was found that virgin individuals of both genders survived longer than mated ones in the different age classes studied. Survival decreased with age and in nearly all cases the differences were statistically significant. No statistically significant differences were observed between virgin males and virgin females, as well as between mated males and mated females. Our results suggest that mating is associated with a high cost, manifested as lower survival, following complete food deprivation. The differences between age classes were possibly observed because young adults carry nutritional reserves from the larval stage, which reserves are depleted as adults age.

Keywords: *Bactrocera oleae*, food stress, aging, starvation, mating, lifespan

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## P013. Feeding behaviour of the cabbage stem flea beetle (*Psylliodes chrysocephala*)

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Oilseed rape (*Brassica napus* L.) is an attractive plant resource for a great number of insect species, including a wide range of pests, but also beneficials such as parasitoids or pollinators. A pest species from great economic relevance in parts of Europe is the cabbage stem flea beetle (*Psylliodes chrysocephala* L.). Main damage is caused by beetle's feeding on young plants in autumn and larval feeding in stems in winter. The feeding can cause a lower vitality and survivability of the plants and can lead to yield losses. Control with chemical insecticitides is critical and in the future alternative options like resistant plant varieties are needed. A better understanding of the biology of the beetles will help to develop screening systems for plant breeding and to make predictions for cultivation. We investigated the influence of developmental stage and sex on the beetles' feeding behaviour considering different temperature conditions in climate chambers. We found that newly hatched female beetles had the highest feeding rates under warm temperatures compared to other developmental stages and temperature conditions. However, we also found a high variability in the data, which was lower at later developmental stages and cooler temperatures. Female beetles ate more than males directly after hatching and after the diapause. We conclude that during warm temperatures in autumn, farmers may experience higher damage due to higher feedings rates of the beetles. To develop screening systems, lower temperature or later developmental stages (e.g. after the diapause) may be an option reducing high data variability.

Keyword: plant breeding, beetle ecology, oilseed rape

## P014. Influence of host plants on the development and colonization capacity of two vector insects (Hemiptera: Aphrophoridae) of *Xylella fastidiosa*

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The xylem-limited bacterium *Xylella fastidiosa* causes important damages in several crops in many countries around the world. In Spain it was detected for the first time in the Balearic Islands in 2016 and one year later in the province of Alicante (peninsular east coast). In Spain, two of the three species recognised as vectors of the bacterium in Europe are present: *Philaenus spumarius* and *Neophilaenus campestris* (Hemiptera: Aphrophoridae).The objective of this work was to analyze the suitability of host plants regarding to: 1. The viability of nymphs to reach the adult stage, 2. The female preference to egg-laying, and 3. The attraction of plant volatiles to adults. The assays 1 and 2 were conducted in greenhouse conditions and a Y-tube olfactometer was used to analyze volatile attraction in assay 3.

The results show: 1. Host plants are essential for the development of nymphs. Nymphs of *P. spumarius* reached the adult stage on alfalfa and field marigold (82% of individuals) but no nymphs did it on grasses. Whereas *N. campestris* complete nymphal development on grasses (74%) but not on the other host plants. 2. *P. spumarius* showed no preference for ovipositing on any of the host plants. In contrast females of *N. campestris* lay eggs mainly on grasses. 3. Adults of *P. spumarius* respond to plant volatiles while *N. campestris* seems not to detect these chemicals.

Results in this work provide some valuable information to implement crop cover management as a valid method to control *X. fastidiosa* vectors.

**Keywords**: *Xylella fastidosa*, vectors, host plants, preference

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## P015. Impact of cross-mating on sexual interactions of two strains of *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

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In this study, we examined the impact of lateralization on male mating behavior and success of two strains of *Trogoderma granarium* Everts (Coleoptera: Dermestidae), originating from two different geographic locations (i.e., Turkey and Czech Republic), testing all possible cross-mating combinations. The identification of *T. granarium* was performed through DNA sequencing. For this purpose, three genetic markers (i.e., Cytochrome Oxidase I, large subunit ribosomal RNA, and Cytochrome b) were used. From a behavioral point of view, most males of the Czech strain performed left-biased approaches, utilizing the right foreleg to explore females, achieving higher percentage of successful copulations over the right-biased males. Although more males of the Turkish strain exhibited right-biased approaches, if compared to the left-biased males, the highest proportion of mating success was achieved by left-biased males. When beetle strains were cross-mated, all possible combinations showed population-level left-biased approaches to potential mates, and right-biased exploration of females with foreleg, achieving high mating success. Significant differences were noted in the duration of copulation within each strain (i.e., Czech male with Czech female, Turkish male with Turkish female), while no significant differences were recorded in the duration of each phase during their cross-mating. The fact that cross-mating among strains affects courtship, mating success and laterality of this important stored-product insect pest adds useful knowledge to its basic biology as well as to mass-rearing implementation techniques.

**Keywords:** behavioral asymmetries, cross-mated, lateralization, molecular ecology, successful copulation, stored-product pest

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## P016. Effect of the plant community on the numerical and behavioral dominance of ant species in pineapple cropping systems in Reunion Island

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In Reunion Island, as in other production areas, pineapple cultivation is largely impacted by the Wilt virus complex. Mealybugs of the *Dysmicoccus brevipes* species are the main mealybugs found on pineapple and are vectors of these viruses. However, other species such as ants are involved in this complex pathosystem. Ants usually nurture and protect the mealybugs from predators, while the latter provide honeydew to the ants. This mutualism greatly complicates the regulation of mealybugs by biological control. Indeed, ants can attack or prevent predators from feeding on mealybugs and even reduce the effectiveness of potential parasitoids. In Reunion Island, the ant species involved in this mutualism have not yet been described. In order to characterize the ant communities in pineapple cropping systems, we used an imagery approach that combined in-field image captures and computer vision algorithm to ease the detection and determination of species at play. Associated with these measures, we also determined the plant community at each observation locations. The objective was (i) to determine which ant species are most involved in the frequentation and protection of mealybugs and (ii) to measure the impact of the plant community on the numerical and behavioral dominance of ant species in pineapple systems. We discuss our results in terms of potential management of the ant communities through the plant community allowing a better mealybug regulation.

**Keywords:** *Ananas comosus*, mealybug, behavioral ecology, imagery, ants

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## P018. Exploring West Nile virus ecology in Greece: interactions between hosts, vector, and the environment

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Mosquitoes play a vital role in the transmission cycle of vector-borne infectious diseases, such as West Nile virus (WNV), but the intensity of WNV transmission is dependent on complex ecological interactions between the virus, vectors, and hosts. Variations in viral lineages, vector ecology and abundance, and host availability are some of the factors affecting infection rates and the potential for new outbreaks. Particularly variation in mosquitoes' feeding preference is considered one of the most important parameters, driving intensity and timing of WNV infection. To enhance surveillance and control efforts, it is important to examine this multifaceted phenomenon holistically and over space and time. During 2020-2021, an integrated surveillance study was conducted in Northern Greece and Peloponnese - known WNV foci based on past outbreaks – to provide new insights into the ecology of WNV in the region by studying: vector seasonality, host feeding patterns, and WNV infection rates. Mosquitoes were collected each season (May-September) from urban/semi-urban, agricultural, and natural sites within the target regions to determine presence and abundance of competent mosquito vectors in different ecological settings and their WNV infection rates. Four different mosquito species were found infected with WNV and among those *Culex pipiens* demonstrated the highest infection rates. *Cx. pipiens* was the most dominant species across all ecological settings, demonstrating an opportunistic feeding behavior by feeding on mammals, birds, amphibians, and reptiles. The possible role of all four mosquito species in WNV transmission will be discussed and gaps in knowledge that should drive future research will be identified.

**Keywords:** WNV, vector surveillance, infection rates, mosquito feeding preference, blood meal analysis

## P019. Effect of plant secondary metabolites on the gut microbiota of caterpillars compared with their diet

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The main factor shaping the caterpillar gut microbiota is the species identity; however, caterpillar microbiota can be affected by plant secondary metabolites (SMs). Microbes can help their host to overcome toxic SMs to obtain nutrients from the plants, but their relationship to SMs has been overlooked. To investigate the effect of plant SMs (tannin, tannivin, salicylate) on caterpillar gut microbiota, we sampled nine caterpillar species from oaks  and fed them with an artificial diet (AD) enriched by plant SMs in a concentration gradient (CON, LOW, MED, HIGH) and also spontaneously enriched by microorganisms. Their gut microbiota composition was compared with i) individuals fed by fresh oak leaves (leaf-fed), ii) starved individuals (starved). The SMs concentration played a more important role than its type. Bacterial and fungal microbiota richness decreased with the switch to AD and recovered with an increasing SMs concentration. Higher concentrations of SMs resulted in higher similarity of the microbiota of guts and the AD. Compared to fungi, bacteria responded to the increasing SMs concentrations more strongly, and were, according to null models, assembled less stochastically. Bacterial microbiome composition became more stochastic as the concentration of SMs increased. In fungal microbiome, the presence of SMs in any concentration led to high stochasticity. Leaf-fed caterpillars had rich associations between microbes; with increasing concentrations of SMs in AD, these associations disappear. Metabolic pathways of bacteria in hosts work best in leaf-fed and CON caterpillars, are limited in starved ones, and completely break down in caterpillars fed AD supplemented with SMs.

**Keywords:** bacteria, fungi, artificial diet, microbial associations, SMs concentrations, microbial functions

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## P020. Quantification of host preferences among bryophagous Lepidoptera in Central Europe

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In Central Europe, there are over 60 species of bryophagous moths from the superfamilies Pyraloidea and Gelechioidea, and from a few other groups. For most of these species, little is known about their feeding ecology, except that they (maybe) develop in moss. Our aim was to expand knowledge in this area through extensive moss sampling and subsequent DNA barcoding of the collected Lepidoptera larvae. We sampled mosses from 690 randomly selected 0.25 m$^2$ quadrates across 46 localities. We identified the species of mosses and manually collected Lepidoptera larvae from each quadrate, considering their context of occurrences to assess the possibility of bryophagy. We captured a total of 498 Lepidoptera individuals, of which 263 underwent DNA barcoding. We found that 21 species (419 individuals) consumed bryophytes, and bryophagy was recorded for the first time in two of these species. Bryophagous moth larvae mainly preferred mosses of the genera *Ceratodon, Dicranella and Brachythecium*. Seven species of moths that were recorded in more than 10 individuals were subjected to a more detailed analysis. All of them are polyphagous, utilizing bryophytes across multiple families. However, the preference for bryophytes differed among the species. Our findings provide more precise insight into the habitat preferences of bryophagous moths and show that their densities can vary considerably in mosses.

Keywords: bryophagy, density estimates, DNA barcoding, food-web analysis, mosses, moths

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## P021. Different patterns of winter diapause of aphids

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The life cycle of most aphids includes generations that reproduce parthenogenetically from spring to autumn, and in the autumn a sexual generation are observed, after which the female lays eggs that enter diapause for overwintering. The laying of eggs is the most beneficial and effectual means of winter survival in temperate regions. The process of diapause, ongoing in eggs, in most insects bind at least to partial inhibition of cell growth and division. To date, very little is known about the phenomenon of aphid diapause. The aim of the work was examining the embryonic structures and process of diapause of aphids. The aphids *Brachycorynella asparagi* and *Appendiseta robiniae* were bred in a prepared habitat on asparagus and black locust respectively, in natural conditions. In order to detect cell division during diapause, we immunostained the embryos with Anti-phospho Histone H3 antibody and secondary antibody. Our results showed that the process of embryonic diapause of aphids is complex and also species dependent. On the basis of *B. asparagi* and *A. robiniae*, we showed two different models of aphid diapause. These showed that the process of diapause even between different species of aphids could proceed in a variety of ways, and the cell cycles may be arrested in some species, or mitosis may occur during the entire period of diapause. This feature may be a specific feature for aphid biology of overwintering.

Keywords: aphids, diapause, development, overwintering

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## P022. Emergence of males in the parthenogenetic reproduction of *Marchalina hellenica* (Hemiptera: Marchalinidae) and its mtDNA divergence in Greece

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*Marchalina hellenica* (Hemiptera: Marchalinidae) is a sap-sucking scale insect native in Greece and Turkey that feeds mostly on pine trees (*Pinus* spp.). This species is known for the crucial role in the production of pine honey. Nevertheless, *M. hellenica* causes detrimental effects on pine trees, potentially leading to their mortality in areas it has invaded (Italy, Croatia, Australia). Although *M. hellenica* has originally been reported as thelytokous, the presence of males observed in Turkey and several islands of Greece, perplexes the exact reproduction strategy of this scale insect. The current study aims to investigate the reproduction strategy of *M. hellenica* by analyzing the emergence pattern of male individuals in Greece for two consecutive years (2021 and 2022). It also examines the genetic variation among 15 geographically distant populations using a mitochondrial DNA marker, comparing the results with data from Turkey. Consistent and systematic screening of the population in Thessaloniki revealed the presence of males at a much higher rate than initially thought, highlighting this complementing reproduction strategy to the strict parthenogenesis. We also found a strong genetic affinity between Greek and Turkish populations. While one haplotype was found in Turkey, two different haplotypes are present in Greece, however, these Greek haplotypes do not exhibit geographic continuity. The genetic pattern in Greece seems to have been impacted by human-mediated dispersal.

**Keywords:** Coccoidea, invasive species, parthenogenesis

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## P023. Learning and memory studied using setup suitable for diverse microinsect species

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Miniaturization can lead to various changes in the nervous system, including a considerable decrease in the number of neurons. Features of brain structure associated with miniaturization have been described for many microinsects, but their behavior and learning abilities remain barely studied.

We compared the associative learning capabilities and memory retention in two species of microinsects of different orders. The first one was *Thrips tabaci* (Thysanoptera: Thripidae); the cognitive abilities of insects belonging to this order have never been tested previously. The second species was *Trichogramma telengai* (Hymenoptera: Thrichogrammatidae). We designed an experimental setup for microinsects based on the concept of the Morris water maze. Our setup includes a thermal arena and a LED screen surrounding it. The temperature in the arena is too high for the insects, but the arena contains four spots with lower temperature. At any given moment, only one of the spots is active and the insect needs to locate it. Pairwise comparison of the lengths of the way in every trial shows that after several trials (8 for *Thrips tabaci* and 5 for *Trichogramma telengai*) the insects form an association between the visual stimulus and the target spot. Multiple memory tests have shown that microinsects display both short-term and consolidated memory. Future research will help to determine the limits of brain reduction at which the abilities needed to solve complex behavioral tasks are maintained, which

may lead to the development of modeling biomorphic neural networks.This study was supported by the Russian Science Foundation (project no. 22-74-10008).

**Keywords:** miniaturization, associative learning, memory, microinsects

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## P024. Effect of soy leaf flavonoids on pea aphid probing behavior

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Flavonoids are plant phenolic compounds whose biological activities include participation in plant responses to various stresses of biological and environmental origins, including protection against insect herbivore attack. Flavonoids detected in soybean *Glycine max* (L.) Merr. (Fabaceae) cause various alterations in the metabolism, behavior, and development of insect herbivores. The pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) poses potential threat to soybeans due to the uptake of phloem sap and as a virus vector, but the effect of individual flavonoids on its feeding-associated behavior is relatively unknown. We monitored the probing behavior (stylet penetration activities in plant tissues) of *A. pisum* on its preferred host plant, *Pisum sativum* L. (Fabaceae) untreated (control) and treated with 0.1% ethanolic solutions of flavonoids apigenin, daidzein, genistein, and kaempferol. We applied the electrical penetration graph (electropenetrography, EPG) technique, which visualizes the movements of aphid stylets within plant tissues. None of the applied flavonoids affected the propensity to probe the plants by *A. pisum*. However, apigenin enhanced the duration of probes in non-phloem tissues, which caused an increase in the frequency and duration of stylet mechanics derailment and xylem sap ingestion but limited the ingestion of phloem sap. Daidzein caused a delay in reaching phloem vessels and limited sap ingestion. Kaempferol caused a reduction in the frequency and duration of the phloem phase. Genistein did not affect aphid probing behavior. Our findings provide information for selective breeding programs of resistant plant cultivars to *A. pisum*.

**Keywords:** *Glycine max, Acyrthosiphon pisum,* herbivory, flavonoids, plant resistance

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## P025. Impact of the symbiont *Serratia symbiotica* on *Aphis fabae* behavior

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Symbiotic microorganisms are ubiquitous and play a major role in the ecology and evolution of animals. The aphid-*Serratia symbiotica* bacterium interaction is a relevant model for understanding the mechanisms behind these symbiotic associations. The recent discovery of cultivable CWBI-2.3T *S. symbiotica* strain allowed our team to study its associated biological effects on host aphids. We observed that this *S. symbiotica* strain exponentially colonizes the aphid's entire digestive tract following infection and induces a fitness cost to their host; however, it also seems to offer an immediate protection against parasitoids. The aim of this study was to further the understanding of *S. symbiotica*'s impact on its host by evaluating its effect on *Aphis fabae*'s feeding behavior and locomotor activity. For this study, electrical penetration graph (EPG) was performed on *A. fabae* individuals orally infected by cultivable *S. symbiotica* strain (two conditions: 5 and 10 days post infection (p.i.)) and noninfected individuals (control) on host plant *Vicia faba*. Infected aphids tend to probe less and salivate longer than noninfected individuals. The infection seems to delay the time it takes for infected aphids to insert their stylets into the host plant and increase the total duration of phloem sap intake. Locomotor activity of infected and noninfected aphids, monitored on a target, revealed that infected aphids' velocity is significantly higher than uninfected aphids'. Therefore, infection of the bacteria in the digestive tract seemingly affects the host aphid's feeding and movement behaviors.

**Keywords:** electrical penetration graph, insect-microbe associations, secondary symbiont, locomotor activity

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## P026. The INSIGNIA-EU project

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The INSIGNIA-EU study is a pan-European study, conducted in the 27 EU countries. It describes the pollution status of the environment in the proximity of an apiary. It uses the honey bee colony as an environmental monitor tool because the pollutants found on bees and bee products reflect the pollution status around the apiary. The study shows pesticides, microplastics, heavy metals, and air pollutants. We sample the colonies non-invasively, by applying in-hive-passive samplers. In the 27 EU countries beekeeper citizen scientist in 320 apiaries sample bi-weekly at the same time and in the same way from May till the end of August 2023. This environmental monitoring provides comparable spatial and temporal data. Bi-weekly pollen is collected and analyzed molecularly for its botanical origin. The pollution- and pollen data are statistically analyzed and INSIGNIA spatial and temporal pesticide risk exposure- and pollen availability models are built.

**Keywords:** environment, monitoring, pollutants, citizen scientist, spatial, temporal

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## P027. Annual population cycle of *Maruca vitrata* (Geyer)

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This paper presents the results of field studies on spotted pod borer, *Maruca vitrata*(Geyer), an economically significant insect pest on grain legumes in India and also elsewhere in the world. Understanding its annual population cycle is very much essential for establishment of an effective management strategy. The population cycle of *M. vitrata* was recorded from 2018 to 2022 on unsprayed crop plants at weekly interval throughout the year. The pest appeared on greengram first in its annual cycle, followed by groundnut, cowpea (*Kharif*), pegionpea and the annual cycle ends with cowpea (*Summer*) at MARS, Dharwad. The pest appeared mainly on pods in all the grain legumes and it was on vegetative parts in groundnut. Spotted pod borer average peak intensity was 1.54, 0.26, 0.92, 5.4, and 0 .32, respectively on green gram, cowpea(*kharif*) , pigeonpea and cowpea (*summer*) and which falls on 32nd , 39th ,42nd ,49th and 7th SMW of the year. Further, *M. vitrata* appeared in the pest form mainly on green gram and pigeon pea. Green gram usually sown first in the season followed by other crops by farming community of this region. The pest stayed for minimum period on groundnut with very low population before developing on cowpea (*Kharif*). Their population gradually decreased to disappear on summer cowpea. The pest undergoes 6 to 7 overlapping generations in a year and stayed longer period on pigeon pea and which is its most preferred host. The pest fecundity and survival appears to be low during hot summer at Dharwad . The effective management of *M. vitrata* on green gram may be the best strategy to prevent its population growth further on other crops in its annual cycle.

**Keywords:** population, *Maruca vitrata,* greengram, pigeonpea

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## P028. The influence of olive fruit biometric parameters on *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) oviposition preference among 62 olive cultivars in natural field settings

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This study evaluated the susceptibility of 62 olive cultivars to *Bactrocera o*leae under field conditions. Olives from each cultivar were collected every ten days, from June to October during 2021 and 2022, from the National Olive Germplasm Bank of Greece of the Institute of Olive Tree, Subtropical Crops & Viticulture, in Chania, Crete (Southern Greece). The percentage of infestation was assessed by dissection of all sampled olives under a binocular stereomicroscope to detect the alive and dead preimaginal stages, emergence holes and sterile oviposition punctures. The fruit weight, length, width and maturity index were also assessed. Adults of *B. oleae* were monitored weekly using a network of McPhail traps. Flight curves showed that *B. oleae* had four generations during each sampling period in both years. However, the 2021 population size was almost 33 times lower than in 2022, likely due to unusually high temperatures during summer in 2021. During both years, oviposition signs were observed very early in the growing season (in June). The percentages of fruit infestation varied significantly among tested cultivars with average levels ranging between 0.9 % and 82.1%. Our findings suggest that fruit weight and volume partially explain the susceptibility of olive varieties to *B. oleae.* Specifically, we observed that certain medium and small-sized varieties were significantly more susceptible to *B. oleae* than certain large-sized varieties. It appears that multiple factors may contribute to the susceptibility of different olive cultivars to *B. oleae* attack. This research is supported by the EU-HORIZON 2020 program GEN4OLIVE.

**Keywords:** genotype, olive fruit fly, fruit parameters, susceptibility, cultivar

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### P029. Behavior and development of the parasitoid wasp *Trichogrammatoidea cryptophlebiae* on the natural host *Thaumatotibia leucotreta* and the exotic host *Lobesia botrana*

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The false codling moth (FCM), Thaumatotibia leucotreta, is a major pest of citrus fruit, endemic to southern Africa. The African egg parasitoid Trichogrammatoidea cryptophlebiae is highly effective in the biological control of this pest. In laboratory experiments, it was observed that the parasitoid was able to parasitize eggs of both the false codling moth and the grapevine moth (Lobesia botrana), which is a non-native species in southern Africa. The objective of the research was to study the biology and behavior of the parasitoid, with emphasis on the differences between two hosts: native and exotic. The host species did not affect the duration of parasitoid development, the adult parasitoid's longevity, or the number of parasitoid offspring. The egg size did not influence the choice of the parasitoids to oviposit either one or two eggs per host egg. However, parasitoids that developed in eggs of the FCM were larger than those that developed in grapevine moth eggs. Significant differences were found in the behavior of the parasitoid between the two hosts, as more parasitoids chose to parasitize FCM eggs and initiate the process faster. Finally, a clear preference was found for FCM eggs over grapevine moth eggs in choice experiments. In general, the native host is preferred over the exotic host. However, further investigation is necessary to continue exploring the new association between the parasitoid and the grapevine moth eggs.

**Keywords:** false codling moth, grapevine moth, new association, parasitoid

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### P030. *Halyomorpha halys* in Serbia: should I stay or should I go?

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The Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål), Hemiptera, Pentatomidae (BMSB), one of the most important worldwide pests is a well-known stowaway, which has been spreading to long distances due to human activities and transportation. This polyphagous, invasive species has been monitored in Serbia since its first finding in 2015. Monitoring that started in 2016 by dead-in

pheromone traps (AgBio, Tréce), set in different environments, all over the country, determined the presence of the species in inspected regions. Therefore, since 2018 BMSB was considered a well-established species in Serbia, with an increasing economic impact on agricultural production. Since the beginning of the monitoring, this species was recorded damaging hazelnuts, soybean, cherries, nectarines, and many other plant hosts, including ornamentals. Most of the annual captures (40%) in 2022 were recorded in hazelnut orchards, while 35% were from areas with different cultivated and non-cultivated hosts, mostly different fruit orchards and soybean fields. Since the beginning of monitoring, the decline in the BMSB population was recorded in 2022 for the first time, when 44% fewer specimens were sampled compared to the previous year. Population decrease could be explained with three hypotheses: 1) increased awareness of agricultural producers and increased action in BMSB control, 2) the activity of natural enemies, both predators and parasitoids which are recognizing this alien species as a host, and/or 3) a climate change, a period of long-term drought followed by high temperatures during the summer months, that might negatively affect the development and activity of BMSB.

**Keywords:** monitoring, BMSB, population decrease, damage

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## P031. The impact of soil microplastic on insect diversity and plastic decomposition by insects

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Plastic has been recognized as one of the leading pollutants of all ecosystems. This question has been rarely raised in Serbia, although there is a significant problem with the disposal of communal and agricultural waste. Many wild landfills have been created in past decades in forests near the plasticulture practicing areas and rivers. Plastic materials are degraded by the influence of UV light and various environmental factors, creating smaller particles called microplastics, which are recognized as significantly dangerous to ecosystems. Alluvial planes have been the most endangered due to the horizontal and vertical fluvial activities of the nearby rivers. The influence of soil microplastics on insect communities has not been widely considered and that is why the project EMIPLAST-SoS has been created to investigate insect communities in polluted and unpolluted alluvial soils in Serbia. We compare them in forest and agricultural ecosystems on similar alluvial soils in three locations near three largest rivers in Serbia. Preliminary results from one-season sampling are considered. During the project the influence of the different seasons effects will be investigated. Additionally, we have investigated the rate of plastic decomposition by five insect species in lab conditions (*Plodia interpunctella* Hubner, *Tenebrio molitor* L., *Zophobas morio* Fabr., *Achroia grisella* Fabr. and *Galleria mellonela* L.). *G. mellonela* expressed the highest and the most diverse decomposition of different plastic sources like plastic bags, sponges and styrofoam. The analyses of the obtained plastic residues are still underway.

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 7742318, „Evaluation of the Microplastics in the Soils of Serbia“- EMIPLAST S.o.S.

Keywords: agricultural waste, microplastics, plastic decomposition, insects diversity, *Galleria melonella*, *Tenebrio molitor*

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## P032. Effects of different tomato cultivars on biological parameters of tomato borer *Tuta absoluta*

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The tomato borer *Tuta absoluta* is one of the most destructive and difficult to control pests worldwide. Plant resistance traits could contribute in minimizing crop losses as one of the components of an IPM scheme. In the present study, the effects of four local (Tomatini, Strongyli, Klosaria, Makrouli) and one commercial tomato cultivar (Nisos) were investigated on the growth rate, pupal weight and fecundity of *T. absoluta* progeny on a wild pest population (DRAM 21-6). Pupal weight and fecundity were not affected by the diffident tomato variety cultivars. Tomatini increased the development time of tomato borer, particularly the duration from egg to pupation, while egg to adult duration was significantly increased only compared to Strongili, Klosaria and Nissos. In addition, the effect of insecticide resistance phenotype on oviposition levels in ‘no-choice’ experiments was investigated. Three characterized populations (LAB 2021, DRAM 21-6 and TYMP 21-4, susceptible, moderate and highly resistant respectively) were used for this study, on selected cultivars (Tomatini and Nisos). Oviposition levels on Tomatini were significantly lower compared to Nisos for populations DRAM 21-6 and LAB 2021. In contrast, the insecticide-resistant TYMP 21-4 population exhibited comparable oviposition levels on both cultivars. Tomatini cultivar has traits that could potentially contribute in delaying pest development and oviposition levels, however, there are indication that development of insecticide resistance by T. *absoluta* populations may negatively affect the latter.

Keywords: *Tuta absoluta*, variety, host plant resistance

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## P033. Seasonal dynamics of the clearwing moth *Synanthedon myopaeformis* in an intensive apple orchards

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*Synanthedon myopaeformis* Borkhausen, 1789 (Lepidoptera, Sesiidae) is more and more important pest particularly in the orchard grafted on M9 and some other rootstocks. Attacked trees become exhausted over many years, their reproductive potential decreases and eventually they dry up, which is a big loss for plantations in intensive production that require high investments per hectare, especially if they are in full crop. In this paper, the influence of the apple varieties, the age of the plantations and the sampling period during the growing season of 2019, 2020 and 2021 on the abundance of the clearwing

moth (*Synanthedon myopaeformis*) was investigated. Of all the varieties that are represented in obsereved orchard, the abundance of the clearwing moth was the highest in the Golden Delicious plantation, and the lowest in the Fuji plantation, while the abundance in the Gala, Greny Smith and Breburn plantations was similar. Orchards from 2011 had the highest abundance, while those from 2012 had the lowest. The abundance of adults in traps during 2019, 2020 and 2021 was not significantly different, so the year of sampling had no influence. Maximum flight of adults was recorded in the second half of June, lower but similar numbers were recorded during the first part of June and July. The lowest number of individuals were collected in the second half of May and August. According to developmental biology of this species, the period of maximum flight and number of collected individuals were expected.

Keywords: abundance, golden delicious, plantation, varieties

## P034. Is Guava (*Psidium guajava*) a host for *Drosophila suzukii?*

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*Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is a major economic pest of certain fresh fruit, and many factors contribute to a fruit being a host. The serrated ovipositor of *D. suzukii* females, unique amongst *Drosophila*, increases its potential to infest “thick-skinned” or “hard” fruit. “Thin-skinned” fruit that are harvested when ripe have relatively greater potential to serve as a *D. suzukii* host, relative to “thick-skinned” fruit and/or fruit that are ripened postharvest. For example, small berries (raspberries, blueberries, and strawberries) and sweet cherries, have a greater host potential than “thick-skinned” fruit such as oranges or commercially harvested California peaches, which require a week or more to ripen. This work examines commercially harvested guava as a potential host for *D. suzukii*. We report infestation levels in fruit subjected to hundreds of *D. suzukii* females, as well as the duration of time for required for pupation. Results are presented relative to other fruit types, as well as guavas as a potential overwintering host in the southern United States and Mexico.

Keyword: spotted wing drosophila, tropical fruit, host

## P035. Population of American grapevine leafhopper (*Scaphoideus titanus* Ball, 1932) in different types of vineyards in Eastern Croatia

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One of the most important vectors of the grapevine disease *Flavescence dorée* is the American grapevine leafhopper (*Scaphoideus titanus* Ball, 1932). Our aim was to determine the population size of the adult American grapevine leafhopper in different types of vineyards during 2021 and 2022 in Eastern Croatia. Yellow sticky traps were placed in four cultivated grapevine (*Vitis vinifera* L.) vineyards, one non-cultivated (neglected) and one vineyard with rootstocks of American *Vitis sp.* hybrids (neglected) from June to September. Five yellow sticky traps were placed in each vineyard and changed every two weeks. In September of both years, we collected vector and leaf samples for molecular analysis of phytoplasma. In 2021, there were no targeted treatments against the American grapevine leafhopper, so the population in all vineyards was significantly higher compared to 2022, when targeted treatments were applied. The largest population was recorded in the vineyard with rootstocks in both years, which was statistically significant compared to the other vineyards. Non-cultivated vineyard had a higher population of vector compared to cultivated vineyards, but a high population of beneficial organisms was also noticed there, so the population of the vector did not expand like in the vineyard with rootstocks. Based on this data, we can conclude that American grapevine leafhopper prefer American *Vitis sp.* but despite the large number of vectors (over 600 adults/yellow sticky trap in two weeks) only one positive vector sample was found in 2021 and one positive leaf sample in 2022 for phytoplasma in a vineyard with rootstocks.

**Keywords:** *Scaphoideus titanus*, yellow sticky trap, vineyard, rootstocks, phytoplasma

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## P036. Presence of conspecific affects wing vibration behavior of male olive fruit flies

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Male wing vibration in *Bactrocera oleae* (Diptera: Tephritidae) is associated with courtship and mating. Here, we examined how the presence of another conspecific affects this behavior. A focal virgin male fly was placed in a plastic cup with another virgin male, or virgin female, or mated female and was observed every 10 minutes during the last two hours of the photophase. Male flies were scored for exhibiting the wing vibration behavior and the time duration of signaling was also counted. Additionally, males were scored for aggressive behaviors, like fighting and wing waving. All virgin flies were 8 days old and mated females were 11-12 days old. There were 50 replications per treatment. In the presence of another male conspecific, 66% of males engaged in wing vibration for a mean time of 145.7s. In the presence of a virgin or mated female, 28% and 36% of males were observed wing vibrating for a mean time of 94.7s and 100.5s respectively. The mating status of females did not have any statistically significant effect on the number of males exhibiting wing vibration and the time duration of their signaling. However, between males, wing vibration is observed more frequently and the time duration of signaling is statistically significant longer. Aggressive behaviors were observed between males in alteration with the wing vibration. It is possible that wing vibration has an important role in male-male rivalry in the olive fruit fly.

**Keywords:** *Bactrocera oleae*, wing vibration, male aggressiveness, courtship

# P037. Impact of ozone pollution on the learning and memory of bumblebees

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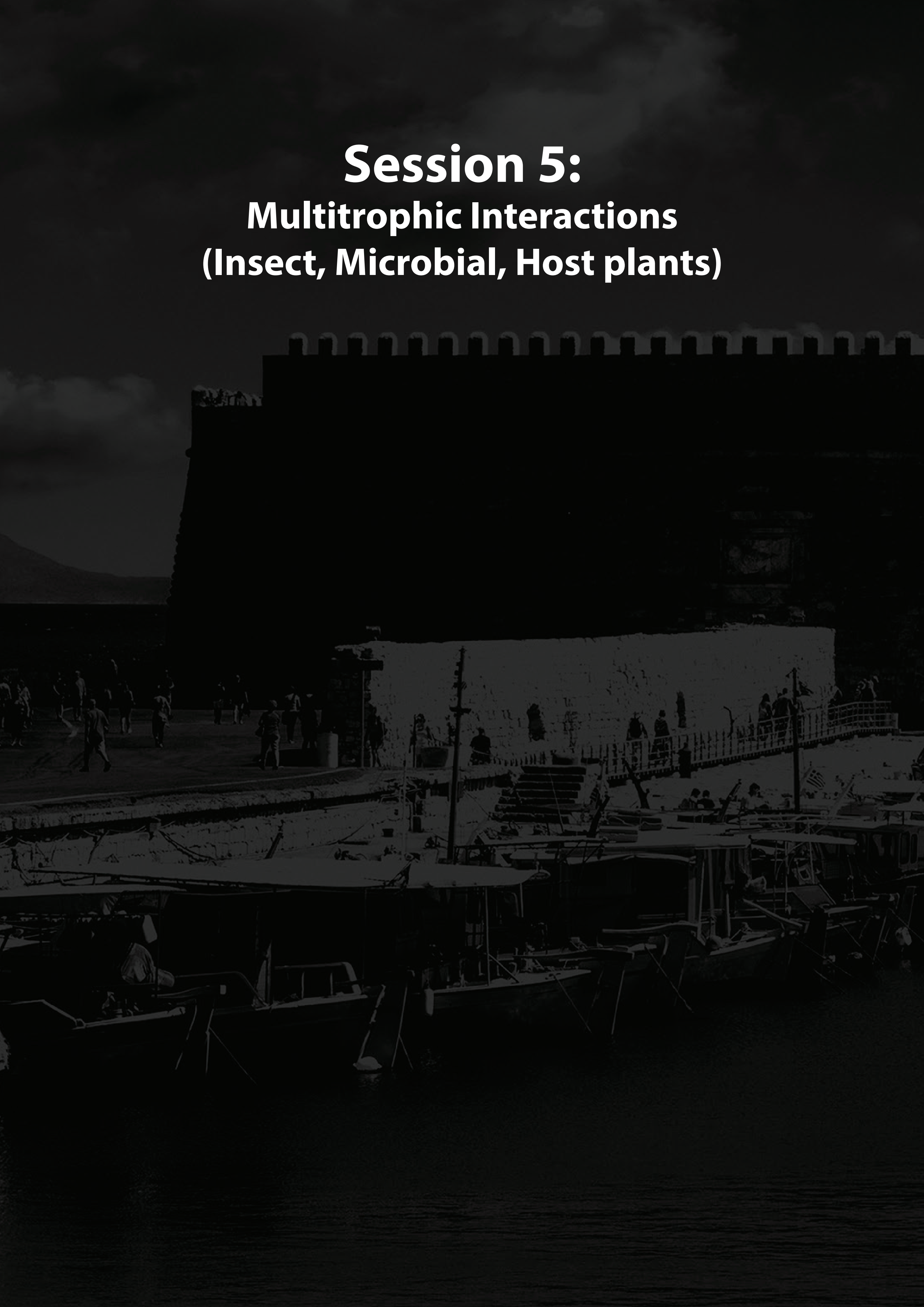
*For the last decades, major drivers of pollinator decline have been identified such as habitat loss and degradation, spread of pathogens, soil and air pollution, and climate change. Especially, it has been recently shown that elevated temperatures alter bee cognition. Besides, little is known about how reduced air quality impacts pollinator cognition. However, this is a major issue that needs to be addressed as global warming is increasing the tropospheric ozone concentration that can alter the cognitive ability of pollinators. These cognitive skills are essential for pollinators to feed and to remember the location of the most suitable floral resources. Ozone pollution has recently been shown to have a significant impact on the olfaction and behaviour of pollinators (i.e., fig wasps and bumblebees). Here we aim to investigate whether cognitive impairments related to ozone pollution could be a factor in pollinator decline by assessing under controlled conditions the effect of realistic ozone episodes on learning and memory in bumblebees. To do this, we will conduct learning and memory tests by associating visual signals with rewarding or repellent molecules. These tests will be carried out on different groups of bumblebees exposed to several realistic concentrations of $O_3$ during various exposure times. The results will allow us to determine whether ozone pollution can alter the cognitive performance of pollinators. If this assumption is verified, these cognitive impairments could have a role in the future structuring of plant-pollinator interactions.*

Keywords: behaviour, bumblebees, cognition, memory, ozone pollution

# Session 5:

## Multitrophic Interactions (Insect, Microbial, Host plants)



## P149. Mycorrhizal symbiosis increases natural enemy attraction upon herbivory: a case study with *Tuta absoluta* / *Nesidiocoris tenuis* in tomato

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The leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a devastating pest in tomato crops, causing important production and economic losses worldwide. The use of biological control strategies, such as releases of the predator *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae), have demonstrated to be an effective alternative pest control strategy. Further, plant inoculation with beneficial soil microorganisms such as arbuscular mycorrhizal fungi can prime plant direct and indirect defenses, leading to induced resistance to insect herbivores. Indeed, recent studies show that mycorrhizal symbiosis induces resistance in tomato plants against *T. absoluta*, increasing larval mortality and reducing plant damage. However, little is known about how mycorrhizal inoculation affects tomato plant indirect defense and *N. tenuis* attraction upon *T. absoluta* infestation. Here we performed different dual-choice olfactometer bioassays with *N. tenuis,* while quantified plant volatile emission. The first bioassay, in which un-attacked plants were unexposed to attacked plant's volatiles, revealed that mycorrhizal inoculation increased *N. tenuis* attraction in response to *T. absoluta* herbivory. The second bioassay, in which un-attacked plants were exposed to attacked plant's volatiles, showed that mycorrhizal plants attracted *N. tenuis* even in the absence of *T. absoluta* herbivory. Mycorrhizal plants thus modulate plant volatile profiles upon *T. absoluta* damage increasing *N. tenuis* attraction, being even primed by neighboring plants' volatiles and able to respond prior to attack. The combination of mycorrhizal inoculation and *N. tenuis* releases appears to be a promising and synergistic strategy for sustainable control of *T. absoluta*.

Keywords: biological control, indirect defense, volatiles, mycorrhiza induced resistance, *Rhizophagus irregularis*, leaf miner

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## P150. Revealing the chemical ecology of *Curculio elephas* (Coleoptera: Curculionidae) through its electrophysiological responses to chestnut plant volatiles

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*Curculio elephas* is an oligophagous pest, attacking several chestnut (*Castanea spp.*) and oak (*Quercus spp.*) species. This weevil is one of the most important pests of European chestnut (*Castanea sativa*) in Europe and it occurs in a continuous range throughout Greece. While, many aspects of the biology and ecology of the chestnut weevil have been studied, scarce research has been conducted to examine the important ecological chemistry behind this highly host-specific plant-insect system. In this study, the aim was to identify the potential volatile organic compounds (VOCs) from chestnut reproductive plant tissues (catkin, nut, and bur) acting as attractants for *C. elephas* females that could ultimately facilitate the development of novel integrated pest management tools. VOCs were sampled *in situ* during Spring and Autumn of 2021 and 2022 in different geographical areas in Greece. For the collection and identification of VOCs, the dynamic-headspace technique combined with gas chromatography-mass spectrometry (GC-MS), was used. Results reveal that chestnut released different VOCs depending on its growth stage, including mainly terpenes, esters, and ketones. The results showed similarity in the chemical composition of the same plant tissue. VOCs provoked electroantennographic responses to antennas of both male and female chestnut weevil adults. Selected compounds were mixed and tested in field trials for attraction of weevil adults. Behavioral responses on both the plant material and individual VOCs will be additionally conducted with the use of Y-tube olfactometer to determine if a compound acts as attractant or repellent.

The research project supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers” (Project Number: 0215).

Keywords: *chestnut, Curculio elephas, electroantennography, VOCs*

## P151. Interaction of olive fruit fly and *Colletotrichum* species infestation on olive fruits

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Olive anthracnose caused by *Colletotrichum* species results to fruit rot leading to premature drop, mummification, and oil quality degradation, especially during severe epidemic years. On the other hand, the olive fruit fly *Bactrocera oleae*, is the most devastating pest affecting olive trees worldwide. Previous studies have provided evidence on the association between *Colletotrichum* spp and *B. oleae* infestation, but the actual impact of *B. oleae* presence on fungal development remains uninvestigated. In this study, we attempted to correlate *B. oleae* and *Colletotrichum* infestations in olive trees, by determining the fungal presence in infested and non-infested olive fruit of two cultivars (Koutsourelia and Koroneiki). Olive fruits from treated and untreated trees of both cultivars were collected and examined for fungal, insect or combined infestation. A combined infestation of *B. oleae* and *Colletotrichum* caused higher quantitative damage to Koutsourelia olives than the disease or pest alone at both treated and untreated fruits. While treated Koreiniki olives appeared to be undamaged, untreated ones were significantly more damaged by a combined infestation than a pest one but less than a fungal damage alone. The association between *Colletotrichum* spp and *B. oleae* infestation is being discussed.

This research has been co-financed by the European and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (T2EΔK-03634).

Keywords: olive fruit fly, interaction, olives, *Colletotrichum*, damage

## P152. *Rhopalosiphum padi* is attracted to Fusarium *graminearium* causing Fusarium head blight, leading to increased pathogen fitness on their shared wheat host

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Fusarium species causing Fusarium head blight (FHB) and the cereal aphids, *Sitobion avenae* (English grain aphid) and *Rhopalosiphum padi* (bird cherry-oat aphid), share wheat as their field host. Both aphids are sap-feeding pests with devastating effects on wheat yield, including virus transmission. We have previously shown that *S. avenae* infestation and prior feeding to infection of wheat by *Fusarium graminearium* results in increased severity of FHB disease and mycotoxins in grain. Volatiles produced by the host when infected by deoxynivalenol (DON) producing *Fusarium graminearum* are repellent to *S. avenae*, and the pathogen-pest interaction results in increased mortality and reduced fecundity of the pest. However, the effects of infection by DON-producing *F. graminearium* on *R.padi's* behaviour and development have not been previously investigated. This study shows the behaviour and life history traits of *R. padi* when exposed to the DON chemotype of *F. graminearum* on wheat ears. In contrast to *S.avenae*, *R.padi* was attracted to plants inoculated with the DON producer. However, forced direct interactions increased mortality and decreased the reproduction rate for *R.padi*. The results suggest that *R.padi* preferentially occupies a separate niche from *S. avenae* or DON-producing *F. graminearium* allowing the pest to co-exist with the pathogen and to share the wheat host without fatal consequences.

**Keywords:** tripartite interactions, aphids, Fusarium, wheat, volatiles

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## P153. Plant-mediated effects of native mycorrhizal fungi against key pests of tomato

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Soil beneficial microbes have long been recognized for their ability to improve plant growth, inhibit pathogens and prime plants against future attackers. Among soil beneficial microbes, arbuscular mycorrhizal fungi (AMF) have been shown to variously affect herbivorous arthropods on aboveground plant parts. These plant-mediated effects do not only vary depending upon the AMF strain but also on the feeding specialization of the herbivore. In parallel, the observed effects could be related to the AMF species, with native AM fungi being better adapted to local conditions as compared to commercial inocula. In this study, we assessed the plant-mediated effects of two native AMF inocula as well as their combinations with commercial rhizobacteria and endophytic fungi on the performance of key pests of tomato namely, the tomato russet mite *Aculops lycopersici*, the whitefly *Bemisia tabaci*, and the two-spotted spider mite *Tetranychus urticae*. We recorded life-history traits such as survival and egg production depending on the herbivore under study on inoculated and control plants. We showed that inoculating plants with native AMF inocula can affect herbivore populations via the plant. Our results

will help in understanding tomato-arthropod-microbe interactions, and developing biocontrol strategies for sustainable pest control.

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**Keywords:** mycorrhizal fungi, native microbes, tomato, tomato russet mite, plant defense, whiteflies

## P154. A step to deeper understand the combined impact of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *Macrolophus pygmaeus* (Hemiptera: Miridae) on host plant traits

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Sap-sucking insects establish intimate trophic relationships with host plants, often mediated by biochemical compounds produced by both organisms and favoured by symbiotic microorganisms. The results of these complex interactions often induce physiological modifications in plants, whose understanding is important to fully penetrate mechanisms of pest damage. Among whiteflies (Hemiptera: Aleyrodidae), *Bemisia tabaci* (Gennadius) is a global pest which attacks a wide range of cultivated plants worldwide, causing significant losses to many crops and being problematic to control due to its rapidly acquired resistance to insecticides. As other sap-sucking insects, it can affect the physiology and development of infested plants. Biological control is an important tool for reducing *B. tabaci* infestations, and artificial releases of the mirid bug *Macrolophus pygmaeus* (Rambur) play an increasingly important role among ecologically safe and effective biological control methods. Nevertheless, due to its zoophytophagy, overall effects of *M. pygmaeus* on plants are not totally understood so far and need dedicated research. To this aim, the impact on eggplant and tomato plants has been assessed comparatively in the work presented here, considering systems composed by "plant + *B. tabaci*" and "plant + *B. tabaci* + *M. pygmaeus*", respectively, and analysing the main morphological and physiological traits of the plants, such as the plant height, dry weight, leaf area and indirect chlorophyll content. The study showed how the negative effects caused by *B. tabaci* to host plants can be significantly reduced by the presence of *M. pygmaeus*, strengthening the role of this insect in the multitrophic system considered.

**Keywords:** sweet potato whitefly, zoophytophagous mirid bugs, plant traits, interactions

## P155. Influence of genetic variability of tomato on the effect of mycorrhiza-induced resistance on *Spodoptera exigua* and its susceptibility to entomopathogens

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Mycorrhiza is a symbiotic association between soil fungi and land plants that enhances plant defense against pathogens and pests. This is known as mycorrhiza-induced resistance (MIR), usually associated to a primed state of the plant for a more efficient activation of responses after a biotic attack. The impact of this metabolic reconfiguration on the susceptibility of phytophagous larvae to entomopathogens is largely unknown and needs further investigation. We evaluated the effect of mycorrhizal tomato plants on *Spodoptera exigua* and its interaction with bacterial (*Bacillus thuringiensis*, Bt) and viral (baculovirus, SeMNPV) entomopathogens. Our results suggested that tomato mycorrhization and previous herbivory increase susceptibility of *S. exigua* to Bt, and has positive or neutral impact on SeMNPV lethality. We then screened 55 tomato varieties to assess the effect of leaf compounds on larval growth and susceptibility to Bt, showing a high heterogeneity in *S. exigua* responses. We selected 6 varieties corresponding to the most extreme phenotypes and performed a new experiment where plants were inoculated with mycorrhizal fungi, treated with methyl jasmonate (MeJA) or a combination of both. The evaluation of the effects of the plant treatments on larval growth parameters showed that all varieties were responsive to MeJA, but only those with low basal resistance displayed MIR, and the association with mycorrhiza did not interfere with the entomopathogenic action of Bt and SeMNPV. Our results reveal that beneficial effects of mycorrhiza are dependent on the plant genotype and show its compatibility with natural entomopathogens in the biological control of *S. exigua.*

Keywords: *S. exigua*, tomato diversity, entomopathogens, mycorrhiza, multitrophic interactions

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## P156. Citrus Tristeza virus tolerant rootstocks trigger dissimilar volatile profiles in *Citrus sinensis* and deterrent responses against the vector *Aphis gossypii*

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Plants emit several volatile organic compounds (VOCs) influencing their relationships with their biotic agents, like viruses and their carriers. In citrus plants, metabolomic profiles can be linked to their response Tristeza virus (CTV), a disease transmitted in Mediterranean countries by the aphid *Aphis gossypii*. Here we investigated the VOC patterns from leaves of varieties with different CTV-contrasting profile: *Citrus aurantium*, a CTV-susceptible rootstock, and *Citrus volkameriana*, Carrizo citrange, and Forner-Alcaide no. 5, three CTV-tolerant genotypes. In addition, we analyzed the VOCs emitted from *Citrus sinensis* plants grafted on the four rootstocks listed above. Furthermore, the aphid preference/avoidance response toward the different combinations was evaluated in a semi-field experiment. Results showed that VOCs emitted and stored in the leaves of the citrus varieties are primarily composed of volatile terpenes. Interestingly, the genotypes showing contrasting CTV-behavior, displayed qualitative and quantitative differences in their profiles. In the specific, a higher level of sesquiterpenes were found in Carrizo citrange, and Forner-Alcaide no. 5. In agreement, the VOCs

recorded on the leaves of *C. sinensis* grafted with the four rootstocks exhibited differences in the abundances and ratios of the compounds emitted. The different profiles probably oriented the *A. gossypii* to prefer *C. sinensis* plants grafted on *C. aurantium* rather than on the other ones grafted on the CTV-tolerant varieties, suggesting a direct role of the VOC in host preference behavior and the susceptibility/tolerance exhibited by the different rootstocks. Future studies will focus to explain the molecular mechanisms involved in the CTV-tolerance through high-throughput approach.

Keywords: CTV, VOCs, cotton aphid, virus vector, *Citrus aurantium*, Carrizo citrange, Forner-Alcaide no. 5, *Volkamer lemon*

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## P157. The predator *Nesidiocoris tenuis* triggers the response of an egg parasitoid to tomato plants infested by *Tuta absoluta*

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The generalist predator *Nesidiocoris tenuis*, an effective biological control agent of numerous pests of the tomato crops, can induce the activation of direct and indirect defence mechanisms of the plant due to its phytophagous behaviour. In southern Europe, *N. tenuis* is frequently released in tomato greenhouses to control the invasive pest *Tuta absoluta*, sometimes combined with another biocontrol agent, the egg parasitoid *Trichogramma achaeae*. In this study, using olfactometer bioassays, we showed that the modification of the volatile chemical profile induced by the plant feeding activity of the mirid made tomato plants more attractive to *T. achaeae,* both in the absence and in the presence of *T. absoluta* eggs or larvae. This result was discussed in relation to difference observed among the chemical profiles of the volatile organic compounds (VOCs) released by tomato plants punctured by *N. tenuis*, without or in combination with *T. absoluta* infestation. A few compounds were found that could play a role in the foraging behaviour of *T. achaeae.* Our data support the emerging idea of an ecological role of *N. tenuis*, in addition to its predatory activity, in making tomato crops more resilient against the attack of pests.

Keywords: biological control, olfactometer bioassays, indirect plant defences, *Trichogramma achaeae*, VOCs

## P158. Beet yellows virus infection of sugar beet promotes performance of its aphid vector *Myzus persicae*

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Since the ban of neonicotinoids in 2018, used to control aphid populations, we observed in France a re-emergence of sugar beet yellowing caused by aphid-transmitted plant viruses. Among the viruses causing important damage to sugar beet is beet yellows virus (BYV), a closterovirus transmitted in a non-circulative and semi-persistent manner. The major BYV vector and object of this study is the green peach aphid, *Myzus persicae*. However, this generalist aphid species it is not well adapted to sugar beet in the field. We confirmed this in experiments under controlled conditions and observed a low survival rate of *M. persicae* adults on non-infected sugar beet plants. However, the survival rate and the aphid population size were greatly increased on BYV-infected sugar beets. This effect is mediated by components of infected plants since aphids, having acquired the virus in 24 hours and then transferred to non-infected plants, did not survive longer than aphids transferred from non-infected plants. This first finding is consistent with the hypothesis that virus effects on host-plant traits can be adaptive and promote host-vector interactions, favorable for transmission. Ongoing experiments will explore in detail the indirect (plant-mediated) BYV effects on *M. persicae* by recording the aphid feeding behavior on BYV-infected or healthy plants. In addition, the effects of raising aphids on BYV-infected sugar beets on aphid growth, mobility and plant choice will be tested. These experiments will be combined with metabolite analyses to identify plant compounds responsible for the observed aphid behavioral and physiological changes.

**Keywords:** aphid, *Myzus persicae*, beet yellows virus, sugar beet

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## P159. Diversity of predators, parasitoids, hyperparasitoids and endosymbiotic bacteria of the black cherry aphid, *Myzus cerasi* (Fabricus) (Hemiptera: Aphididae) in Türkiye

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This research examined the multitrophic levels between predators, parasitoids, hyperparasitoids and bacterial symbionts of the black cherry aphid to allow an essential basis for planning an effective biological control. *Episyrphus balteatus* (De Geer), *Pipiza noctiluca* (Linnaeus), *Eupeodes corallae* (Fabricus), *Paragus tibialis* (Fallen) from Syrphidae (Diptera) were found occurring in *Myzus cerasi* colonies. *Adalia bipunctata* (L.), *Adalia fasciatopunctata revelierei* Mulsant, and *Coccinella septempunctata* (L.) were the most prevalent species among nine Coccinellid predatory species. *Bindoxys heraclei* (Haliday), *Trioxys humuli* Mackauer, *Praon volucre* (Haliday), *Lipolexis gracilis* Förster (Braconidae: Hymenoptera), *Syrphophilus bizonarius* (Gravenhorst) and *Diplazon laetatorius* (Fabricius) (Ichneumonidae: Hymenoptera) were found to be parasitoids and hyperparasitoids of *M. cerasi*.

Additionally, the obligate symbiont *Buchnera aphidicola* was detected in all black cherry aphid populations studied, whereas no facultative symbionts were recorded based on 16S rDNA PCR amplification and Sanger sequencing.

**Keywords:** black cherry aphid, diversity, predators, parasitoids, hyperparasitoids, symbiosis

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## P160. Influence of SBR on phloem sap composition of sugar beet and the behavior of its vector *Pentastiridius leporinus*

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The "Syndrome Basses Richesses" (SBR) is a bacterial disease of sugar beet (*Beta vulgaris*), which reduces sugar content in the beet and causes significant yield losses. The causative agent of the SBR is the γ-proteobacterium *Candidatus* Arsenophonus phytopathogenicus (CAp), which is located in the phloem sap of the host plant and transmitted by the feeding activity of the planthopper *Pentastiridius leporinus* (Hemiptera: Cixiidae). Direct control of the pathogen and its vector is not yet possible and up to now, there are no resistant beet varieties available. A critical barrier to SBR containment and control is the insufficient knowledge of the multitrophic interaction between pathogen, host plant and vector. One aim of our research is the elucidation of the host finding and host acceptance behavior of this planthopper. It is quite unclear whether the vector *P. leporinus* benefits from a sugar beet infection with CAp and is therefore preferably found in the cultivated area. Thus, our study focused on sugar, sugar alcohol and organic acid patterns of phloem sap samples of CAp-infected and healthy sugar beet plants, analysed by using gas chromatography–mass spectrometry (GC-MS). In addition, the feeding behaviour of *P. leporinus* was investigated by using electropenetrography (EPG). These results will contribute to a better understanding of the epidemiology of CAp and might be used to develop innovative and selective vector control approaches to reduce the spread of SBR.

**Keywords:** Syndrome Basses Richesses (SBR), *Pentastiridius leporinus*, *Candidatus* Arsenophonus phytopathogenicus, electropenetrography, phloem metabolome

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## P161. Co-infection with other sugar beet viruses modifies the localization and transmission by aphids of beet yellows virus (BYV)

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one of the crops that are frequently infected by several viruses transmitted by aphids. Some of the viruses infecting sugar beet (the closterovirus BYV and the poleroviruses BChV&BMYV) are restricted to the plant phloem while a potyvirus (BtMV), invades all cells of the plant. The objective of this research was to investigate how BYV co-infections with the other viruses change aphid transmission and virus localization. First, we optimized a detection method, SABER fluorescent in situ hybridization, to localize all sugar beet viruses simultaneously within co-infected tissues and cells. Using this technique, we started with BYV/BChV co-infection. Our results showed co-localization of the two viruses in the same phloem cells. This correlated with a higher transmission of BYV and a lower one of BChV. Changes in viral load could explain lower transmission of BChV but not higher transmission of BYV. Currently, we are testing whether changed aphid behavior on co-infected plants is responsible for higher BYV transmission. Co-infection of BYV&BtMV resulted in different localization of the two viruses in the plant tissue, compared to mono-infection, but this time it correlated with lower transmission of BYV. Other selected double and triple infections are presently tested for correlation between localization, transmission, accumulation & aphid behavior, in order to understand the mechanisms behind the cellular localization and how they impact transmission.

**Keywords**: aphid transmission, co-infection, sugar beet viruses, virus localization, aphid behavior

### P162. Interactions between two host-specific begomoviruses in a common vector and its effect on vector transmission

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Polyphagous insect vectors may acquire more than one virus from various virus-infected plants. The interaction between multiple viruses in an insect vector may contribute to a shift in the disease epidemics. This study examined the interaction of two host-specific begomoviruses, squash leaf curl Philippines virus (SqLCPV) and tomato yellow leaf curl Thailand virus (TYLCTHV), in their common vector, *Bemisia tabaci*. A series of experiments were conducted to study whether the acquisition of one virus affects the acquisition, infection, and transmission of the other. The results revealed that pre-acquisition of SqLCPV or TYLCTHV by whitefly had no effect on the acquisition of the other virus. The interaction of SqLCPV and TYLCTHV in whitefly was assessed by the virus amounts in whitefly's midgut and primary salivary glands (PSGs) that were determined by quantitative real-time polymerase chain reaction. The results demonstrated that pre-acquisition of SqLCPV increased the amount of TYLCTHV in the midgut and PSGs, whereas the amount of SqLCPV in the midgut and PSGs was antagonistic by TYLCTHV pre-acquisition. Transmission assays depicted that pre-acquisition of SqLCPV increased the transmission rate of TYLCTHV, whereas the transmission of SqLCPV was suppressed by TYLCTHV pre-acquisition. Altogether, these results provide a basis for future exploration of the molecular mechanism of synergistic interaction of SqLCPV to TYLCTHV and antagonistic interaction of TYLCTHV to SqLCPV, which would add insights to our existing knowledge of interplay between plant viruses in insect vector.

**Keywords:** *Begomovirus*, *Bemisia tabaci*, mixed infection, vector transmission, virus-virus interaction

## P163. Does fertilization plant-mediated bottom-up effect reach the third trophic level in the tomato-pest-NE tritrophic system?

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In agroecosystems, arthropod communities may be influenced by bottom-up forces (BU) induced by environmental variations (e.g., fertilization) through the modification of plant traits. The way bottom-up forces affect the 2nd trophic level is particularly well documented, but less is known on their ability to affect the third trophic level. Furthermore, plant-mediated bottom-up forces may affect natural enemies directly (plant mediated) or indirectly (through the herbivore). We aim to disentangle 1) if bottom-up effects reach the 3rd trophic level with equivalence between parasitoids and predators and 2) if the diet regime of the predators modify the ability of bottom-up to reach and affect them.
We set-up a lab experiment where variations in fertilization regime were tested as bottom-up factors. The studies were carried out on three different "tomato plant-herbivores-natural enemies" systems. Selected herbivores are frequent pests on tomato systems (*Macrosiphum euphorbiae*, *Bemisia tabaci* and *Tuta absoluta*). The respective parasitoids were *Aphidius ervi* and *Encarsia formosa*, and the respective predators *Adalia bipunctata* (carnivorous), *Macrolophus pygmaeus* (omnivorous) and *Dicyphus errans* (omnivorous). We recorded plant, herbivore as well as various parasitoid/predator traits- survival, development, reproduction and biocontrol ability.
We found strong evidence of diluted bottom-up forces through trophic levels depending on the herbivore types. Parasitoids seem less affected than predators. Predators were able to control the increase in pest populations with increased fertilizer. This work offers perspectives in the framework of Integrated Pest Management on how smart manipulation of plant nutrient solutions might save resources while maintaining efficient biocontrol in crop production.

**Keywords:** predator, parasitoid, tritrophic interaction, biocontrol

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## P164. Effect of *Agrobaterium tumefaciens* on papaya whitefly, *Trialeurodes variabilis* (Quaintance), populations infesting *Carica papaya*

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*Agrobacterium tumefaciens* is a pathogenic bacterium that causes crown gall disease, a plant tumor affecting a wide range of plant species including papaya. Papaya whitefly, *Trialeurodes variabilis* (Quaintance) is the primary whitefly pest of papaya. Initial observations of papaya whitefly populations on papaya with and without crown galls indicated that the presence of the gall did effect whitefly population levels. The effect of *A. tumefaciens* (isolate 29) on papaya whitefly populations infesting papaya was further researched in a replicated randomized complete block design in the greenhouse with the following treatments: untreated papaya, papaya plus Agro, papaya plus whitefly, papaya plus

Agro then infested with whitefly (preventative), and papaya plus whitefly then inoculated with Agro (rescue control). Plant (height, width, number leaves, stem width, gall development) and whitefly (egg, nymph, adult) parameters were taken over time to determine the effect of this pathogenic bacterium on plant health and whitefly populations. Results are presented and discussed.

Keywords: crown gall, papaya, whitefly

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## P165. Plant-mediated effects of beneficial soil fungi against key pests of pepper

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Plants are continuously challenged by biotic stressors among them herbivorous arthropods and pathogenic microbes. Nevertheless, they also interact with beneficial organisms such microbes found in the soil which are known for their ability to improve plant growth, antagonize pathogens and prime plants against future attacks via the elicitation of plant defense responses. In our attempt to identify sustainable pest control tools to substitute chemical control, we assessed the hypothesis that soil microbes can adversely affect aboveground pests of pepper via the elicitation of plant-mediated responses. Hence, we assessed the plant-mediated effects of two *Trichoderma* and one *Rhizophagus* strain on the performance of key pests of pepper belonging to different feeding guilds and feeding styles, specifically the two-spotted spider mite *Tetranychus urticae*, the aphid *Myzus persicae* and the whitefly *Trialeurodes vaporariorum*. We recorded life-history traits such as survival and egg production depending on the herbivore under study on inoculated plants as compared to control plants. Our results show that inoculating plants with different fungal strains can affect herbivore populations via the plant. Overall, our work highlights the role of soil microbes as biocontrol agents in suppressing herbivore populations, possibly via the induction of plant defenses.
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Keywords: aphids, beneficial fungi, pepper, plant defense, spider mites, whiteflies

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## P166. Intraguild predation among the main predator species in Mediterranean pear orchards

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Intraguild predation has a strong effect on the population dynamics and structure of the community of arthropods in ecosystems. Ants are key species in pear orchards due to the mutualistic relationship they establish with honeydew-producing hemipterans and its impact on other natural enemies, such as spiders and predatory mirids. The aim of this work was to assess intraguild interactions among the main predators species in pear orchards, since such interactions may affect pest population suppression. The three-way interaction among the ant *Lasius grandis* (Formicidae), the mirid *Pilophorus gallicus* (Miridae) and spiders (*Oxyopes* sp. and *Philodromus* sp.) was assayed in the presence/absence of each species in a complete factorial design replicated eight times. The arena was a 2-liter clear plastic jar with a pear seedling; ant nests were connected to the arena using a plastic tube. The proportion of *P.gallicus* predated was significantly influenced by both ants (0.7±0.05, mean±SE) and spiders (*Oxyopes* sp: 0.37±0.1 and *Philodromus* sp: 0.36±0.09). Besides, a significant interaction ant∗spiders was found, the proportion of mirids predated being lower than expected when ants and spiders were together in the arena. In addition, the proportion of spiders predated was significantly affected by ants regardless of the presence of *P. gallicus*, and no significant differences were found on predation rates between spider species (*Oxyopes* sp.: 0.81±0.1; *Philodromus* sp.: 0.65±0.1). The impact of ants as intraguild predator of both spiders and predatory mirids could have a significant effect on pest control in pear orchards.

**Keywords:** intraguild predation, biological control, pear orchards, ants, spiders, predatory mirids

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## P167. Plant-mediated interactions between fungi and herbivores

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Plants in natural systems interact with a multitude of microorganisms which can influence herbivores feeding on the host plant. Conversely, herbivores can affect plant-associated microbial communities through plant-mediated effects. Therefore, understanding the reciprocal effects of microbes and herbivores is essential to uncover indirect plant-microbe-herbivore interactions. Our work focuses on plant-mediated interactions between the soil-borne entomopathogenic fungus (EPF) *Metarhizium brunneum* and insect herbivores. *M. brunneum* has been well exploited as a direct insect pathogen. However, recently it has been shown that EPF can also colonize plants endophytically and plant-mediated effects of EPF on insect herbivores are less well understood. We investigated (1) whether inoculation of tomato plants with *M. brunneum* can increase tomato resistance against spider mites (2) whether these effects act synergistically with the effects of other beneficial fungi or with resident soil microbes, and (3) the role of plant secondary metabolites in mediating such microbe-insect interactions. We show that *M. brunneum* enhances tomato resistance to spider mites, but that these effects can be mitigated by resident soil microbes. Co-inoculation with other beneficial plant fungi (arbuscular mycorrhizae and mycoparasitic fungi) showed additive effects of dual inoculations that disappeared in full fungal mixtures. The studied secondary metabolites (phenolics and flavonoids) were unlikely to mediate the observed interactions. In addition, we discuss the effects of EPF on plant growth promotion. Acquiring a more comprehensive knowledge of mechanisms underlying the effects of fungal endophytes on pest resistance and growth promotion is crucial for ensuring efficacy and commercialization.

**Keywords:** entomopathogenic fungi, tomato, arthropod pests, secondary metabolites

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## P168. Multitrophic interactions in tomato and wheat under drought stress

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Biotic and abiotic factors can cause bottom-up effects that can alter trophic interactions (plant-phytophagous-natural enemy). However, these factors can be used to optimize the use of natural resources and control plant pests. In this context, we evaluated in the laboratory the response of *Solanum lycopersicum* and *Triticum aestivum* under the effect of three water regimes (optimal, medium and high drought stress) and two herbivorous insects (i.e., *Phenacoccus solenopsis* and *Sitobion fragariae*). Different plant morphological parameters were measured and choice tests were carried out to assess the behavioral response of two natural enemies (i.e., *Cryptolaemus montrouzieri* and *Aphidius colemani*) to plant emitted volatiles. Besides, the expression level of several plant defense signaling pathways (i.e., jasmonic acid, salicylic acid and abscisic acid) was evaluated. Water regimes and insect pests significantly affected plant morphological parameters and emitted volatiles by triggering multiple olfactory responses on the assayed natural enemies. Similarly, metabolic pathways associated with plant defenses varied significantly in the studied models. Our results could serve as a basis for the future development of plant protection strategies in the framework of Integrated Pest Management.

**Keywords:** blackberry cereal aphid, cotton mealybug, mealybug destroyer, multitrophic interaction, tomato, water stress

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## P169. Interactions between two phloem-limited viruses in their host plant and vector: Implications for vector fitness and virus epidemics

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Infection of a single host by multiple viruses is common in agricultural settings. Virus-virus interactions in mixed-infection can lead to synergistic, antagonistic, or neutral effects. Virus-virus interactions can differentially alter host phenotype and vector fitness than individual viruses. Mixed-virus infection in a vegetable (Squash/tomato) pathosystem in the United States has two facets. Facet 1: Mixed-infection in hosts (squash) due to multiple viruses transmitted by the same vector, whitefly *Bemsia tabaci* Gennadius. Facet 2: Acquisition of multiple viruses from multiple hosts (squash and tomato) by the same vector. Facet 1: *Begomovirus* (*Cucurbit leaf crumple virus, CuLCrV*) and *Crinivirus* (*Cucurbit yellow stunting disorder virus*, CYSDV) interactions in squash and on *B. tabaci* fitness was assessed. Mixed-infection of CuLCrV and CYSDV in squash resulted in a severe phenotype but reduced the accumulation of a component virus (antagonistic interaction). This interaction following mixed infection differentially affected virus acquisition by whiteflies and settling than CuLCrV or CYSDV infection. Facet 2: Combined acquisition of tomato infecting-*Tomato yellow leaf curl virus* (TYLCV) and squash-infecting CuLCrV by whiteflies was examined. Combined whitefly acquisition of CuLCRV and TYLCV enhanced settling towards non-infected tomato and squash plants. Viruliferous (CuLCrV and/or TYLCV) whitefly fitness study conducted on a virus non-host (cotton) revealed that the mere presence of virus in the vector influenced its fecundity positively. Overall, mixed-virus infection in hosts and acquisition of multiple viruses by the vector could potentially exacerbate epidemics than a single virus.

**Keywords**: mixed-infection, vector fitness, virus transmission, epidemics

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## P170. Oviposition responses of *Tuta absoluta* females to herbivore-induced volatiles after *Halyomorpha halys* infestation in tomato plants

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In the field, plants are commonly infested by more than one herbivore, either simultaneously or sequentially. The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera:Gelechiidae), is an important pest of tomato crops worldwide. Moreover, the polyphagous pest *Halyomorpha halys* (Hemiptera: Pentatomidae), has emerged as an invasive pest and among others infest tomato plants. Little is known about how dual herbivory by these two herbivores affect tomato plant volatile organic compounds (VOCs) emissions, and as consequence insect behaviour. Plants release a variety of VOCs that either are attractive to natural enemies of herbivores or repelling to additional infestation by the same or different herbivore species. In this study, we examined the effect on the composition of plant emitted VOCs of sequential infestation of *H. halys* followed by *T. absoluta* on tomato plants. We also aimed to investigate the role of herbivore-induced plant volatiles (HIPVs) after infestation by *H. halys* female adults on the oviposition choice of *T. absoluta* females on tomato plants. We performed two- choice and non-choice oviposition bioassays for *T. absoluta* females on plants infested by *H. halys* and non-infested control plants. For the collection and identification of VOCs, the dynamic-headspace technique combined with gas chromatography-mass spectrometry (GC-MS), was used. The results showed that tomato plants released different VOCs depending on infestation status. Our results showed also that *T. absoluta* females preferred to oviposit on non-infested than infested by *H. halys* plants.

**Keywords:** Pentatomidae, *Tuta absoluta*, tomato, VOCs

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## P171. Aphid settling and feeding behaviour on virus-infected common bean *(Phaseolus vulgaris)* promotes transmission of three viruses.

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Two aphid species, the bean-specialist *Aphis fabae* and the generalist *Myzus persicae*, transmit three important virus pathogens of common bean (*Phaseolus vulgaris*). These viruses, Bean common mosaic virus (BCMV), Bean common mosaic necrosis virus (BCMNV) and Cucumber mosaic virus (CMV), affect bean cultivation. Genetic options for control of BCMV and BCMNV are available but at times futile due to the emergence of recombinant isolates and endemicity of BCMNV on the continent. Further, there are limited studies of CMV in bean. These viruses are aphid-transmissible in a non-persistent way whereby virus acquisition and inoculation onto the plants by the aphids are rapid. We sought to understand the three-way interactions between aphids, common bean, and the three viruses. The aim was to find novel tools or techniques to disrupt aphid-mediated virus transmission.

Using choice-tests, we discovered aphids preferred to settle on uninfected plants over virus-infected plants in 24h observations. However, we recorded that aphid began probing earlier on virus-infected plants, suggesting changes to surface cues on the bean plants that encouraged probing but not settling. We investigated aphid feeding further using electrical penetration graph. Our experiments revealed that aphids encountered feeding difficulty on virus-infected plants. These altered interactions would promote the quick acquisition of viral particles by aphids from the infected plants, while discouraging settling or long-term feeding. This would then facilitate onward virus transmission. These findings have been used to test different pull-push planting configurations in the field to disrupt aphid transmission of plant viruses.

**Keywords:** *Aphis fabae*, *Myzus persicae*, bioassays, electrical penetration graph, viruses

## P172. The love triangle: a new alien bean weevil (Coleoptera, Chrysomelidae) attacking local legumes (Fabaceae) and attacked by local parasitoids (Hymenoptera, Eulophidae) in Israel

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The notorious Afrotropical bean weevil *Decellebruchus atrolineatus* (Pic, 1921) (Chrysomelidae: Bruchinae) was unexpectedly reared in the Community Garden in Rehovot, Israel, from the seeds of the local cowpea (*Vigna unguiculata*). *Decellebruchus atrolineatus* originates from the sub-Saharan and is widely distributed throughout the Afrotropical and in the southern parts of the Palaearctic region (Algeria, Egypt, Saudi Arabia, Yemen), invaded in the Southern and Central America, recorded also from the United Kingdom and Spain, being everywhere a serious pest of cultivated cowpea. Heavy infestation was recorded; close to 100% of the seeds were infested, most of them by 2-3 beetles each. Two species of Chalcidoidea (Eulophidae) were reared from *D. atrolineatus*: 90% of the specimens belonged to *Pediobius bruchicida* (Rondani, 1872), and a single specimen belonged to *Chrysocharis* spp. *Pediobius bruchicida* was already recorded from Israel as a parasitoid of the local Bruchinae; it is an endoparasitoid, attacking the last larval instar or prepupae. New distribution record and new association between *Decellebruchus atrolineatus* and *Pediobius bruchicida* is recorded here for the first time. In spite of being a relatively new immigrant, *D. atrolinaetus* shows a solid population and distinct integration into the local biota. Its appearance in Israel will be examined in the context of the recent shift of distributional patterns of tropical insects into more temperate areas as a result of global warming. *Pediobius bruchicida* should be examined as a potential biocontrol agent of *D. atrolineatus*.

**Keywords:** Bruchinae, *Decellebruchus atrolineatus*, *Vigna, Pediobius,* invasive species

# Session 6:

## Insect Biotechnology

## P213. Plant-mediated delivery of dsRNAs to phloem-feeder leafhoppers

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RNA interference (RNAi) regulates gene expression in eukaryotes and can be exploited in crop protection against pests by exogenous applications of double-stranded RNAs (dsRNAs), although delivery of dsRNAs to sap-sucking insects is still a major challenge for field application. Two phytoplasma vectors, *Euscelidius variegatus* and *Scaphoideus titanus* (Hemiptera: Cicadellidae), were used here as case study to verify whether RNAi can be triggered in leafhoppers by plant-mediated delivery methods. A dye-solution applied to grapevine, broad bean and oat plants by petiole and detached leaf absorption showed systemic distribution in plant via xylem elements. Dissected guts from *E. variegatus* adults fed on dye-treated plants showed specific staining. Consistently, electropenetrography (EPG) recordings described prolonged xylem feeding phases for both species under study. Delivery of dsRNAs was then optimized to silence two gut-specific genes (legumain and natterin) of *E. variegatus* and, as final step, used to silence a systemically expressed gene (ATP synthase β) in both species. Insects fed on dsRNA-treated plants by leaf absorption showed a significant reduction of target gene expression in comparison with insects treated with dsRNAs targeting green fluorescent protein, used as control. Taken together these results demonstrate that the plant uptake of dsRNAs can silence target genes of phloem-feeders fed on those plants. This work paves the way towards innovative alternative control strategies that can potentially replace/integrate insecticide application against sap-feeding and vector species. The method will be also useful in functional genomic studies to decipher the role of vector genes in the transmission of plant pathogens.

Keywords: *Euscelidius variegatus*, *Scaphoideus titanus*, *Vitis vinifera*, RNA interference, vector-borne plant pathogens, phytoplasma vectors

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## P214. Investigating the RNAi target gene function of PSMB5 for managing Colorado potato beetle (*Leptinotarsa decemlineata*)

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The Colorado potato beetle (CPB, *Leptinotarsa decemlineata)* is a devastating coleopteran pest of potatoes in the northern hemisphere, costing tens of millions of dollars in management costs. Due to its adaptive life cycle, allowing for wide distribution of offspring, and the overuse of same class insecticides, rapid resistance has developed to many conventional control methods currently on the market. The use of dsRNA allows for a targeted control method using the RNA interference pathway. A dsRNA targeting proteasome subunit beta type-5 (PSMB5) has demonstrated gene and protein suppression, CPB mortality, and plant protection when

tested in laboratory and field-like conditions. Proteasomes are a multi-catalytic proteinase complex in eukaryotic cells whose function is to maintain protein homeostasis by cleaving, thus inactivating, misfolded or damaged proteins that could impair important cellular functions. The proteasome complex is composed of two sub-structures, the 19S activator regulatory particles and the 20S core particle (CP). The 19S proteasome identifies ubiquinated proteins and aids in delivering de-ubiquinated target proteins into the 20S core particle. The CP contains four homologous 7-ring cylindrical beta-ring structures made of various alpha and beta-subunits, including beta-5 (PSMB5). By targeting this essential beta-subunit of the proteasome, the complex is disrupted and the hypothesis assumes prevention of proteolysis of proteins which negatively impacts the insect from build-up of cell waste. This study aims to validate and further understand the function of PSMB5 by demonstrating the accumulation of ubiquitinated proteins in the insect body as well as validating findings previously demonstrated in other CPB studies.

**Keywords:** RNA interference, functional genomics, pest control

## P215. 3-Carene metabolism: a potential target for RNAi-based *Ips typographus* pest management

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The Eurasian spruce bark beetle (Ips typographus, ESBB) is an important forest pest of Norway spruce (Picea abies) in central Europe causing catastrophic ecological and economical loss. Norway spruce, despite having a well-elaborated chemical defense system consisting primarily of monoterpenes, is successfully infested by ESBB in a coordinated mass attack owing to its complex detoxification machinery. 3-carene is an important monoterpene produced abundantly as an induced defense in Norway spruce. Here we assessed a few spruce monoterpenes for their toxicity on ESBB via fumigation and identified that 3-carene has the lowest LC70 value. Further, the transcriptomics and proteomics study of 3-carene fumigated ESBB revealed 22 common genes between proteo-transcriptomic analysis. We found up-regulation of genes related to detoxification and defense (e.g., cytochrome P450s, dehydrogenases, and hydrolases), whereas downregulation of genes related to body development and chitin-metabolism (e.g., chondroadherin, chitinase, and ecdysteroid kinases). Further validation of proteomics and RNA-seq genes by RT-qPCR showed the results being highly correlated. Furthermore, we used enzyme activity assays (CYP, GST and esterase) to validate these results functionally. In addition, we applied RNAi-based gene silencing method to silence some of the genes involved in development and detoxification. According to these results, we found that when ESBB is exposed to xenobiotic stress, its body development is altered, and the detoxification mechanism is activated instead. This study helps to understand the 3-carene detoxification pathway in ESBB and offers a catalogue of promising target genes for its future pest management.

**Keywords:** *Ips typographus*, 3-carene, RNA-seq, proteomics, metabolomics, RNAi

## P216. Identification of novel targets to control red palm weevil *Rhynchophorus ferrugineus*

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Red palm weevil [Rhynchophorus ferrugineus (Olivier)], is the most destructive pest of palm trees and whilst native to South Asia, has spread globally. This pest insect spends its entire life cycle within the tree, causing severe damage to palm trees leading to heavy economic losses due to decreased date production; in some cases, this insect can destroy whole plantations. Although several synthetic pesticides have been used to try and control this insect, these have been largely unsuccessful. Furthermore, this pest has evolved resistance to these chemicals. This project therefore seeks to identify environmentally friendly and sustainable methods for the management of red palm weevil through use of RNAi-based technologies. RNA interference (RNAi) is a transforming technology that triggers specific gene silencing through delivery of homologous dsRNA fragments. Knockdown of genes responsible for essential physiological or behavioural functions within the insect are likely to lead to increased mortality. Our approach is to target the CNS. Eight different genes have been selected, five encoding ion channels and three encoding neuropeptides. Expression of these target genes in two different life stages (larva and adult) are currently being quantified. This data will inform the selection of genes for further study. Proof of concept will be provided both by injection and oral delivery of dsRNA.

Keywords: red palm weevil, pest insect control, CNS, RNAi, gene knockdown

## P217. Investigating the molecular determinants of Bt-toxicity in *Spodoptera frugiperda* through *in vivo* functional genetics

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The fall armyworm (FAW), *Spodoptera frugiperda* causes enormous annual production losses in major crops. Chemical insecticides and primarily transgenic crops expressing *Bacillus thuringiensis (Bt)* insecticidal proteins (Cry and Vip toxins), that act through binding to receptors expressed in the larval midgut, have been used extensively for its control. However, resistance to Bt toxins has been reported threatening the effectiveness of control measures. Reduced binding of Cry toxins to their respective receptors due to mutations at the binding site, has been proposed as a mechanism of resistance. Nevertheless, *in vivo* functional validation of the role and contribution of each mutation in resistance is lacking in most cases. In addition, not all Cry toxin binding sites have been characterized complicating the elucidation of resistance. We have generated a "pestified" *Drosophila* strain carrying the ABCC2 transporter gene of FAW, which has been linked to Cry toxin pore formation. Through phiC31 integrase system, we introduced two types of mutations in ABCC2, both of which have been identified in Brazilian FAW populations resistant to Cry1F: 1) a deletion of two amino acids (GY deletion) and 2) the P799K amino acid substitution. The Drosophila lines carrying the mutated ABCC2 gene showed increased tolerance to the Cry toxins, present in a commercial Bt formulation. Furthermore, combination of the two mutations in one strain had a synergistic effect. We also apply CRISPR directly in FAW aiming to identify genes that are targets of Cry toxins and enlighten the role of their mutations in Bt resistance.

**Keywords:** fall armyworm, Cry toxins, resistance, pestified *Drosophila*, CRISPR/Cas9

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## P218. Precision guided Sterile Insect Technique in Mexican fly

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*Anastrepha ludens* the indigenous Mexican fly, is considered a devastating pest of the fruit industry in Latin America, California., Arizona and Florida. The enormous economic and agricultural damage has created the necessity for population control and prevention of their spread. Effective strategies from pesticides to the traditional Sterile insect technique (SIT) has been applied through the years to suppress *A. ludens* population, but each of them has carried different risks (resistance, accidental release, fitness cost, mating competitiveness). Despite the many attempts, *A. ludens* continues to be a huge economic threat to crop production. Precision guided sterile insect technique (pgSIT) is a new strategy based on programmable CRISPR gene targeting to generate female lethality and sterilized males that can be used to suppress populations. Here, we present a novel attempt for pgSIT application in *Anastrepha ludens*. We demonstrate the optimized pgSIT tools that have been already successfully used in Drosophilidae and *Aedes aegypti*, and we describe an efficient way to generate pgSIT in other tephritid species that are not considered as model organisms.

Keywords: Mexfly, pgSIT, CRSPR, Tephritids

## P219. The construction of an infectious clone based on a covert-infecting RNA virus in Euschistus heros (Hemiptera: Pentatomidae) for VIGS

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The Halyomorpha halys virus (HhV) is a +ssRNA virus first detected in the *Halyomorpha halys* transcriptome, grouped in Iflaviridae (Picornavirales). This species was also reported in *Euschistus heros* transcriptomes with high number of transcripts. The study of covert-infecting viruses in insects have only recently received more attention, with increase in their discovery through high-throughput sequencing methods. Although this type of virus presents little prospects for pest control in its wild-type form, their use as vectors for virus induced gene silencing (VIGS) is a potential approach for host target-gene silencing. Thus, this study aims to provide evidence on HhV transmission in *E. heros*, and to obtain a clone with the HhV complete genome. Two sets of primers were used for HhV detection through RT-PCR in different life stages, and in an experiment to assess whether HhV can also be transmitted horizontally. The 5’-end of the HhV genome was amplified through 5’-RACE, cloned and sequenced. The sequence was used to design primers for whole genome amplification. The results indicate that HhV persists through all life stages of *E. heros*, and might be spread both vertically and horizontally. Moreover, the complete HhV genome was successfully amplified from *E. heros* cDNA and cloned into an expression plasmid. This marks a significant progress towards constructing an infectious clone of HhV, which can aid in the study of virus-host interaction, and facilitate the construction of a VIGS vector for RNA interference (RNAi)-based studies.

**Keywords:** RNA virus, Iflavirus, stinkbugs, RNAi

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## P220. Pathogenicity of *La Jolla Virus* in *Drosophila suzukii* following oral administration

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*Drosophila suzukii* (*D. suzukii*) is an invasive pest insect that causes severe and widespread damage to soft fruit crops. The special thing for *D. suzukii* females is that they have a large, pointed and serrated ovipositor, which makes it possible to lay their eggs in ripening instead of rotten fruits. Due to wide host plant rage and high tolerance for different environments devastating harvest loss can occur yearly.
Due to climate change worldwide, the pest emerges earlier in the year and stays longer, which leads to longer infestation of soft-shelled fruits.
Still the most common method of pest control is the use of broad range chemical pesticides. Which effects pest and beneficial arthropds alike, and are in general harmful to animals and environment. So, there is an

urgent need for sustainable and host specific pest control. The development of an effective biocontrol product would require the characterization of virulence following oral delivery and stability under different pH and temperature conditions reflecting realistic exposure scenarios.

We previously described a strain of *La Jolla virus* (*LJV*) found in moribund *D. suzukii* specimens in Germany. Here we describe the pathogenicity of *LJV* following oral delivery to *D. suzukii* adults and larvae. The oral infection of *D. suzukii* adults with *LJV* reduced survival in a concentration-dependent manner, whereas the oral infection of *D. suzukii* larvae caused the arrest of development during pupation. *LJV* remained stable and infectious following exposure to a broad pH range and different temperatures. We, therefore, demonstrated that *LJV* is promising as a candidate biological control agent against *D. suzukii*.

Keywords: *Drosophila suzukii*, La Jolla virus (LJV), *Iflavirus*, biological pest control

## P221. Development of a screening system for the identification of antiviral compounds that target the IRES of insect-specific dicistroviruses

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To avoid recognition by the immune response and to increase their coding potential, RNA viruses have evolved unconventional gene expression strategies, such as the use of internal ribosomal entry sites (IRESs) that are adopted to avoid cap-dependency during translation. Dicistroviruses, a family of picorna-like RNA viruses, include pathogens of insect pests of medical and agricultural importance but are also known to infect beneficial insects and arthropods such as the honeybee and shrimp. A dual luciferase reporter system was constructed in which firefly luciferase mRNA was translated by a cap-dependent mechanism while Renilla luciferase mRNA was dependent on the IRES from the type species Cricket paralysis virus. Over-expression and silencing experiments showed that the initiation factor eIF4A (DEAD-box helicase) stimulates IRES-dependent mRNA translation while UPF1 (RNA helicase involved in nonsense-mediated mRNA decay) has an inhibitory effect. The IRES construct was also more sensitive to stress conditions but not adversely affected by baculovirus infection. Future studies will examine the effects of inhibitory drugs of translation factors and extend to infections by CrPV virus. Our aim is to identify compounds that can accelerate or inhibit viral infections in insect pests and beneficial insects, respectively, and that are safe for human health and the environment.

Keywords: *Dicistroviridae*, Cricket paralysis virus, IRES, antiviral compounds, proviral genes, mRNA translation

# Session 7:

## Symbiosis and Insect Pathology

## P222. Desiccation tolerance in *Ceratitis capitata* eggs and the effect of *Wolbachia* on stress response

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The Mediterranean fruit fly, *Ceratitis capitata* (Medfly), is an economically important agricultural pest feeding on a variety of fruits. Environmental stress resistance and adaptations are key traits that contribute to species dispersal and potential to invade new habitats. Symbiosis can alter host's behaviour and resistance to different stresses. Notably, in Medfly, an artificial symbiosis with *Wolbachia* has been established, manipulating reproduction and imposing effects onto behaviour and stress response.
Desiccation tolerance is important since the underlying mechanisms would allow them to withstand periods of drought and low humidity, common in Mediterranean regions. However, limited information is available on the egg stage anhydrobiotic capabilities of Medfly. Herein, we study the desiccation tolerance and desiccation-response profile of Medfly eggs by implementing a series of survival assays at different time points. Our aim: to investigate any threshold of tolerance that could hold potential for anhydrobiosis under the conditioning of the individuals and/or eggs. Furthermore, we compare the desiccation-response profile between *Wolbachia*-infected and non-infected laboratory populations to test whether *Wolbachia* is correlated with protection against desiccation stress or increased vulnerability.
The mechanisms underlying desiccation tolerance are not fully understood, but it is believed that they involve production of protective proteins, accumulation of trehalose, activation of stress response pathways, reduction in metabolic activity, and changes in membrane fluidity. The investigation of desiccation tolerance of Medfly eggs could be of immense importance in the survival and dispersal of this pest in arid environments. Understanding these mechanisms could lead to new strategies for controlling its spread.
*The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "3rd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 7615|* **DESICCOME** : DESIccation toleranCe in metazoans using comparative transcriptomics

**Keywords:** medfly, symbiosis, anhydrobiosis, survival essays

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## P223. Understanding the antagonist effects of Actinomyces in honeybee *Apis mellifera* against the American foulbrood *Paenibacillus larvae* and European foulbrood *Melissococcus plutonius* through biochemical analysis

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The American foulbrood (*Paenibacillus larvae* AFB) and European foulbrood *Melissococcus plutonius* (EFB) are major bacterial diseases affecting honeybees, leading to a decrease in viability of the hive, decreasing honey production, resulting significant economic losses to beekeepers. Few experiments, have examined the antibacterial effectiveness of Actinobacteria against diseases that affect bees. In this study a series of biochemical test is submitted to understand the antagonism effect of the isolated strain against the American and European foulbrood. The work done allowed the isolation of 19 Actinobacterial strain against *Paenibacilus larvae* and 11 Actinobacterial strain against *Melissococcus plutonius*. The isolated bacteria where most effective against *Paenibacilus larvae* than *Melissococcus plutonius*. Using a series of biochemical test to identify the Actinobacterial profile including their capacity to ferment the sugar, capacity to catabolize the sugar, the presence of different enzyme like oxidase, catalase and lipase, the ability to grow on different pH and the capacity of salts degradation. Most of the Actinomyces wereGram + bacteria with different bacterial characterization including enzymes presence and ability of growth in different pH. The effective strain P 16 F and P G15 against American Foulbrood had same profile with one different characterization. The isolated bacteria with high efficiency against European Foulbrood were M G10, M S8. The effective strain against European Foulbrood had the same profile with two different characterizations. This work was undertaken by WP III, of the PRIMA- SafeAgroBee project.

Keywords: *Apis mellifera*; actinobacteria; pathogenic microorganisms; antagonistic effect, *Paenibacillus larvae*, *Melissococcus plutonius*

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## P224. The impact of symbiosis disruption in pentatomid stink bugs affects pest control

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Obligate associations of insects with bacterial symbionts are crucial for insect ecology and evolution. Interrupting these symbioses has been proposed as a method to contain pest populations, with a target shift from insect to symbiotic bacteria to avoid insecticides. The family Pentatomidae in Hemiptera is regarded as a major target for symbiont-oriented control strategies. Indeed, insects in this family harbor an obligate gut symbiont, which is vertically transmitted via egg surface contamination by the mother. In the environmental phase, the symbiont can be easily reached by anti-bacterial treatments, resulting in impaired symbiont acquisition by the newly-emerged generation. Knowledge of the insect response to the dysbiotic condition generated by the interruption of obligate symbioses is essential to understand the potential success of symbiont-targeted control. We provided evidence for a differential response to symbiosis interruption in different pentatomid species depending on the variable interactions among the host, the primary symbiont and the overall gut microbiota. The brown marmorated stink bug *Halyomorpha halys* is strongly dependent on the primary symbiont, and hence the dysbioisis is extremely lethal for this species. In contrast, the survival of the southern green stink bug *Nezara viridula* does not rely on the dominance of its primary symbiont within the gut microbiota; accordingly this species is poorly affected by symbiosis interruption. Such a difference in the insect- primary symbiont-microbiota association may have

a significant impact on the success of symbiont-targeted control programs in the field and must be taken into account in the implementation if this strategy.

**Keywords:** symbiotic control, Pentatomidae, *Halyomorpha halys*, *Nezara viridula*, dysbiosis

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## P225. Revealing insect resistance mechanisms in a host-parasitoid interaction

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Insects are subject to infectious organisms and defend themselves with an innate immune system, as they are generally thought to lack acquired immunity. The innate defense is divided between humoral immunity, which consists in multiple anti-pathogen molecules, and cellular immunity, which is based on hemocytes. Those cells are able to discard larger invaders, either by phagocytosis or encapsulation. The latter is used against large foreign bodies, and consists in enclosing them in a multi-layer cellular shell, which will produce toxic compounds and melanin in addition to preventing interactions with other tissues. Parasitoid eggs, when triggering immune system, are killed by this phenomenon. Our study focuses on the host/parasitoid couple formed by the Lepidopteran stem borer *Sesamia nonagrioides* and the Hymenoptera *Cotesia typhae*. Parasitism success of a *C. typhae* strain is low on the French *S. nonagrioides* population due to encapsulation. To study the dynamic of capsule formation and the targeted egg or larval stages, we used several methods and compared their results. First, we dissected non-resistant hosts to characterize parasitoid larval development. We then dissected resisting hosts to retrieve and observe capsules under microscopy, especially because they do not melanize and are hard to detect. We labelled their hemocytes with immunohistochemistry techniques to identify their populations, and finally, we checked for the structural arrangement of the capsules with histology sectioning. Together, those methods gave us a broad understanding of capsule formation dynamics and of the cell populations involved.

**Keywords:** parasitoid, host resistance, encapsulation, insect immunity, hemocytes

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## P226. Endophytic and entomopathogenic presence of *Beauveria bassiana* in kudzu bug, *Megacopta cribraria*, and kudzu, *Pueraria montana*, in the southeastern United States

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Kudzu bug, *Megacopta cribraria*, first reported in the United States (U.S.) in 2009, spread rapidly throughout the southeastern region of the country. Kudzu bug is problematic to homeowners and farmers, as it is found in urban structures, where it is disruptive to homeowners, and it feeds on soybean, where it reduces crop yield. In recent years, kudzu bug populations have declined in many previously-infested areas. These local and regional declines are influenced by at least two natural enemies, a naturally-occurring entomopathogenic fungus, endophyte, and saprophyte, *Beauveria bassiana*, and an accidentally-introduced egg parasitoid, *Ooencyrtus nezarae*. Collectively, these two natural enemies reduce overwintering kudzu bug populations by about 97%. Seasonal changes in saprophytic presence of *B. bassiana* may influence its' endophytic and entomopathogenic presence in kudzu and kudzu bug. Research demonstrated that, throughout the growing season, *B. bassiana* increases in entomopathogenic presence in kudzu bugs and endophytic presence in kudzu in late summer to fall (colonizing up to 23% of a vine by mid-August). The pathway of transmission of *B. bassiana* to kudzu bug is unknown, but it is likely due to interactions with kudzu, soybean, and the soil associated with both systems. Further research on mechanisms of dissemination and the interactions among these species, as well as soybean, is underway and is essential to better improve the use of *B. bassiana* to manage kudzu bug populations. The purpose of this poster is to provide information on distribution and seasonality of *B. bassiana* in kudzu and its impact on kudzu bug populations.

Keywords: kudzu, kudzu bug, entomopathogens, biological control, *Beauveria*, fungus

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## P227. Influence of secondary symbionts on host plant utilization and choice in the whitefly *Bemisia tabaci*

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Microbial symbiosis is recognized as a major source of biological and evolutionary innovation in eukaryotes. However, the extent to which symbionts contribute to their hosts' ecological adaptation and subsequent diversification is not fully elucidated yet. The whitefly *Bemisia tabaci*, a major agricultural pest, is a suitable model to address these questions. It harbors a nutritional 'primary' endosymbiont, allowing it to thrive on plant sap, and up to seven 'secondary' endosymbionts (S-symbionts), whose roles remain poorly known. Here, we compare the phenotypic and metabolic responses of whiteflies reared on host plants with different amino acid contents, and address whether S-symbionts influence *B. tabaci* capability to choose, feed and oviposit on those plants. Whiteflies' oviposition rate, free amino acid profile and symbiotic load were found to be plant dependent, suggesting a critical role for the plant nutritional properties. Using genetically homogeneous whiteflies harboring different cytotypes (mitochondria and symbionts), we demonstrated that cytotype affects *B. tabaci* amino acid profile on the least favorable plant and can mitigate its deleterious effect on whiteflies' oviposition rate. Plant choice experiments showed that, according to their cytotype, whiteflies exhibit no plant preference for oviposition, or avoid unfavorable plants. Thus cytotype influences both *B. tabaci* plant utilization and selection. As cytotypes harbor different S-symbionts' combinations, we propose that these symbionts mediate whiteflies' suitable plant range, possibly through their impact on their hosts' amino acid metabolism and dietary requirements. Our findings provide new insights into the influence of endosymbionts on their hosts' adaptation to the ecological niche and diversification.

**Keywords:** *Bemisia tabaci*, plant utilization, plant choice, cytotype, symbionts

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## P229. Effects of a plant virus and endosymbionts on aphid resistance to parasitoids

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Aphids are important vectors of plant viruses and also form well-characterized associations with non-pathogenic endosymbionts. Consequently, they are a frequent study system for work exploring microbial effects on host phenotypes and ecology. Yet, few studies to date have explored interactions between aphid symbionts with potentially divergent fitness interests. Here we explore how interactions between pea aphids (*Acyrthosiphon pisum*) and parasitoid wasps (*Aphidius ervi*) are influenced by pea enation mosaic virus (PEMV) and by the presence of facultative aphid endosymbionts. Previous work has shown that aphid susceptibility to parasitism can be influenced both by both endosymbionts and virus-infection, but their combined effects have not previously been explored. We examined how aphid interactions with parasitoids differed across aphid lines harboring one of five different facultative endosymbionts (or only the obligate endosymbiont *Buchnera aphidicola*) on PEMV-infected and uninfected fava bean plants. While our initial results do not reveal effects of either virus or endosymbionts on rates of parasitoid attack or parasitoid emergence from aphid mummies, we observed effects of both virus infection and endosymbionts on post-attack resistance: PEMV-infection decreased rates of mummification (regardless of the endosymbionts present), while overall survival and reproduction following attack were also influenced by particular endosymbionts, including *Regiella insecticola* strain R5.15, which has previously been reported to enhance resistance to parasitoids. Attacked aphids harboring this strain also exhibited increased longevity, but only on virus-infected plants. These findings show that both virus-infection and endosymbionts influence aphid interactions with parasitoids, with potential implications for disease transmission and biological control.

**Keywords:** gut microbiome, tritrophic interactions, symbiosis, pathogens, parasitoids

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## P230. Effects of diet on the expression of immune genes in the house fly (*Musca domestica*)

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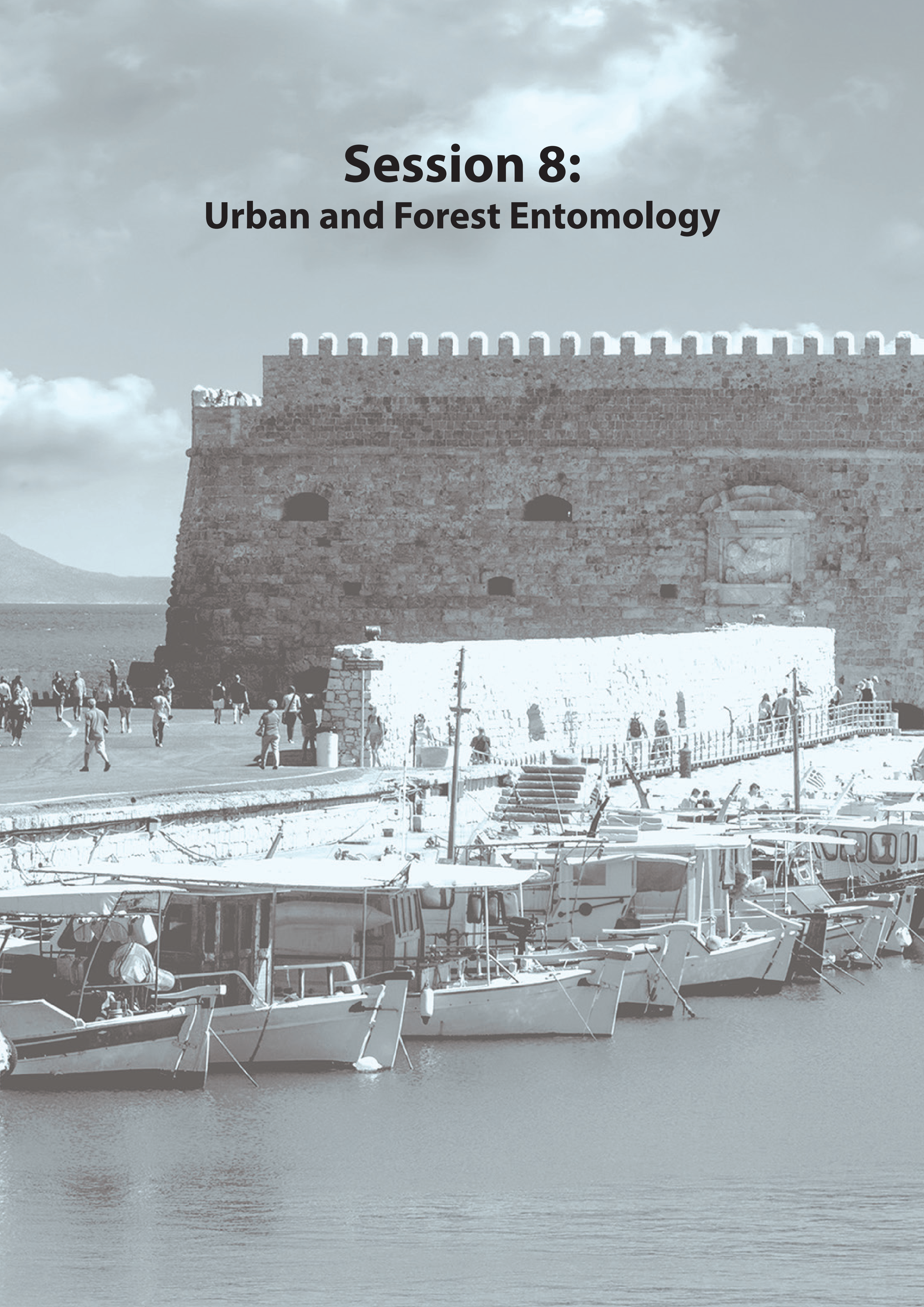
Insect meal is considered a sustainable alternative to soy or fishmeal as feed for agricultural stock, especially because some insects can be reared on waste from the food industry. However, the nutritional composition of the industrial food waste can vary substantially. Therefore, it is important to understand the effects various food wastes as insect rearing substrate can have on the health of production insects. The innate immunity of insects is known to be influenced by their diet, with both high protein content and high sugar content linked to upregulation of immune genes in *Drosophila melanogaster*. Expression of antimicrobial peptides is significantly affected by diet in black soldier flies (*Hermetia illucens*), even in the absence of an infection. The housefly is of particular interest for rearing on waste streams, as this fly is well-adapted to living on organic waste. Species-specific literature on the effects of diet on the immunity of houseflies is however still lacking. We studied the effects on the immune response of housefly larvae when reared on three different diets (sugar rich, fat rich and control) for up to 12 generations, through qPCR on a panel of genes coding for antimicrobial peptides. We will present the (preliminary) results of these experiments, to give insight into the adaptation of the immune system of housefly larvae to their diet, and which diets are preferable when optimizing health of houseflies in mass rearing facilities.

**Keywords:** insect immunity, dietary effects, mass rearing, housefly

# Session 8:

## Urban and Forest Entomology



## P038. Successes, limitations, and opportunities for biological control of invasive species in forests and urban areas in the Southern Appalachians, Tennessee, USA

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Prior to the 1980s, many of the problematic non-native insect pests and weeds in the United States (U.S.) originated from Europe (such as spongy moth, cereal leaf beetle, and alfalfa weevil). Since then, many of our new invasive species (e.g., emerald ash borer, hemlock woolly adelgid, and kudzu bug) have originated from Asia. In fact, about 10% of our most severe invasive species are from China. These invasive species are particularly problematic in the southern Appalachians, which is primarily composed of forests. The Great Smoky Mountains National Park, which is the most visited National Park in the U.S. with about 14 million visitors annually, is in the southern Appalachians and is not immune from these destructive invasive species. Evidence of tree mortality is evident throughout the Park. Forests and urban areas are currently impacted by emerald ash borer and hemlock woolly adelgid, which pose environmental, economic, and safety concerns. Some (e.g., Asian tiger mosquito) pose serious health concerns. Because of the difficulty in chemically treating vast areas of forest or urban areas, biological control becomes an important and viable option in integrated pest management programs directed against these species. However, use of biological control is limited by lack of available promising agents, ability to rear large numbers of those agents for release, and evaluating field performance and establishment. Adequate funding for these projects also is a limiting factor. This poster examines successes, limitations, and opportunities for biological control of insect pests in forests and urban areas in the southern Appalachians.

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## P039. Identification of the introduction pathways of invasive ants of the *Tapinoma nigerrimum* group in the Metropolis of Montpellier

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Biological invasions often have negative consequences for both human activities and local biodiversity. The *Tapinoma nigerrimun* complex includes two species (*T. darioi, T. magnum*) that have an invasive character and the capacity to form supercolonies. These ants are native to the Western Mediterranean area, but have been recently reported as invasive in more northerly areas as well as in Mediterranean cities such as Montpellier (France). The objective of this study is 1) to identify the role of plant nurseries in the spread of these two species and 2) to identify the origin of the populations found in the metropolis of Montpellier. Colonies were sampled in various plant nurseries and their surroundings. Since the species can hardly be differentiated morphologically, specimens were genotyped using 15 microsatellite markers. The genotypes obtained were compared with the genetic profiles of populations from Spain and Italy, two areas where *T. darioi* and *T. magnum* are supposed to originate from, and where a large proportion of the Mediterranean plants sold in French nurseries come from.

This type of investigation allows, on the one hand, to know if the origin of the plants in the nursery is correlated to the origin of the ants associated with them (in other words, if the paths of introduction can be easily traced or if they become blurred as a result of colonies displacements), and on the other hand, to know what the sources of introduction of these ants are, in order to put in place appropriate management and control measures.

**Keywords:** ant, biological invasions, plant nurseries, urbanization

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## P040. Using knowledge of urban multitrophic interactions to implement urban plant protection in green space design

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Urban green spaces (UGS) are essential for sustainable development as they provide habitats for species that otherwise are threatened by ongoing landuse changes and as they provide important ecosystem services (ES) for humans. Function of UGS is dependent on good plant health and a suitable species community, which could be enhanced by design and management of the vegetation system. There is, however, poor knowledge of the presence and needs of species groups that have important functions in the urban ecosystem e.g, parasitoids and invertebrate predators. In this interdisciplinary project, we thus aim to study plant-pest-parasitoid interactions in UGS and survey human attitudes and perception to components of the vegetation system that increases pest resilience to develop UGS designs that enhances both biodiversity and pest control as an ES, while still preserving aesthetics. Preliminary data from pilot studies (inventory of biodiversity and experimental assessment of predation on clay larvae) indicate a large variation between UGS in both presence of higher trophic levels and predation pressure.

**Keywords:** urban ecology, multitrophic interaction, sustainable pest management, one health, landscape archtitecture

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## P041. Non-chemical control of *Lymantria dispar* in three European countries

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The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) is a polyphagous species that infects forests in the Europe, Asia, Africa and North America. It constitutes a severe environmental problem as it is a voracious eater that defoliates entire trees and causes health problems to humans and animals. The LIFE eGymer project aims to utilize non-chemical control by developing and implementing e-traps, novel trapping devices, mass larval trapping and mating disruption techniques to remotely monitor and effectively control *L. dispar* in three European countries (Slovenia, Spain and Greece). Based on our results in 2022, there are specific trap devices that capture more *L. dispar* adults than others, but the ranking of traps varied according to the geographical zone. Mating disruption also proved very promising for the next years, given that there was a significant reduction in the trap captures, as compared with the control areas. The overall results clearly illustrate that there are good potentials for the techniques that have been evaluated here to be used in area-wide management strategies to mitigate *L. dispar* expansion.

The LIFE eGymer project is co-funded by the LIFE Programme of the European Union under contract number LIFE20 ENV/GR/000801 and with the contribution of the Green Fund.

Keywords: *Lymantria dispar*, non-chemical control, pheromone traps, mating disruption

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## P042. Title: Comparing Urban Heat island effects on the pine processionary moth development in two regions with contrasted climates in France

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Climate change affects ecosystems worldwide, triggering responses such as range shifts and changes in survival and phenotypes. Yet, the extent of species' adaptive capacities remains poorly understood,

particularly when they strive along urbanization gradients, which may alter the effects of climate change due to Urban Heat Island (UHI) effect. The pine processionary moth (PPM), *Thaumetopoea pityocampa*, is one of the few species for which a causal relationship between climate warming and range shift has been determinedly demonstrated. Its development is also known to be highly plastic in this species depending on local environmental conditions. We used this model species to explore phenology variability in response to temperature deviation along a gradient of urbanization from urban to forested areas in two different bioclimatic regions: (i) degraded ocean climate and (ii) Mediterranean climate. We monitored larval development, pupation processions and adult flight activity during 2022-2023.

We tested the hypotheses that (1) PPM larvae develop faster in urban areas compared to forested areas within each bioclimatic region, and (2) urban environments in degraded ocean climate are the most favourable environments for PPM survival due to warmer winters. Contrary to these putative beneficial effects, negative effects of urban warming may be observed under the Mediterranean climate, as urban environments may become too warm with climate change.

This ongoing project aims at highlighting the importance of studying urban warming as it may drive changes in PPM phenology that heighten or decrease the risks of human exposure to its airborne urticating setae.

Keywords: *Thaumetopoea pityocampa*, Urban Heat Island, phenology variability, gradient of urbanization, human health, France

## P043. The Life Oak Processionary Caterpillar project – Is biological control possible?

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The Oak Processionary Caterpillar (*Thaumetopoea processionea*, OPC) feeds gregariously on Oak trees (*Quercus*). During the development the caterpillars produce bag-like silk nests filled with hairs, shed skins and excrements. These defensive hairs, which are spread by wind, are toxic and induce allergic reactions when they touch the skin, eyes, or are inhaled by humans. There is a painful, red rash on the skin which is extremely itchy. In recent years, the distribution range of the OPC spread (North) over Europa and population densities increased considerably resulting in increased nuisance, especially in more densely populated areas. When infestation is low, control measures include the manual removal or burning of nests. This, however, is very labour intensive. When the OPC population levels get too high, control switches to spraying with pathogenic *Bacillus thuringiensis* or nematode solutions. These, however, are not specific and will also kill non-target insects or other invertebrates that come in contact.

The European Life-project (https://oakprocessionary.life/) aims to develop and promote alternative control techniques. The three main options that are explored are: 1) natural verge management to increase nectar rich flowers which in turn attract more natural enemies (parasitoids and predators), 2) attract more caterpillar feeding birds (mainly tits) by installing nest boxes, 3) breeding and releasing the Forest Caterpillar Hunter, *Calosoma sycophanta* (Carabidae). The preliminary results are not clear yet, but our results at least show that, at certain sites, natural enemies are able to kill more than 90% of the OPC population.

The LIFE Oak Processionary project is co-funded by the LIFE Programme of the European Union under contract number LIFE19 ENV/BE/000102.

**Keywords:** *Thaumetopoea processionea*, non-chemical control, parasitoids, predation

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## P044. Species and density of pests occurring in container seedlings of *Dendropanax morbiferus* in a tree nursery in Korea

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This study was conducted to obtain basic data for producing healthy seedlings by investigating the types, and occurrence period of pests that damage container seedlings of *Dendropanax morbiferus* tree in nursery pavements. From 2020 to 2022, the types and densities of pests were investigated for 1 and 2-year-old tree seedlings in two nurseries in southern Korea. From March to November, the survey was conducted at two-week interval, and damages caused by two pests (*Tetranychus urticae* and *Austroasca vittata*) were observed. There was a difference in the occurrence period or the maximum damage period according to the survey area, the age of the seedlings, and the year. In the case of first-year seedlings, damage by the *Tetranychus urticae* occurred from July when three or more leaves were formed, but damage occurred from May to October in second-year seedlings. The peak occurrence period of the *Tetranychus urticae* was August in the 1-year-old seedlings, but it varied between May and July in the 2-year-old seedlings. The damage of the *Austroasca vittata* was confirmed only in July, and the damage rate was over 58% in two-year-olds and over 38% in one-year-olds.

**Keywords:** *Austroasca vittata*, *Dendropanax morbiferu*, *Tetranychus urticae*, tree nursery

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## P045. Urbanization reduces gut bacterial microbiome diversity in a habitat specialist ground beetle

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Urbanization is transforming natural environments, creating networks of modified land types. These urbanization-driven modifications lead to the local extinction of several species, but the surviving ones also have to face novel selection pressures, including exposure to pollutants, habitat alteration, and shifts in food availability and diversity. Based on the assumption that the environmental pool of microorganisms is reduced in urban habitats due to habitat alteration, biodiversity loss, and severe pollution, we hypothesized that the diversity of bacterial microbiome would be reduced in urban hosts compared to their rural counterparts. Investigating the gut bacterial communities of a forest specialist ground beetle, *Carabus convexus*, in rural vs. urban forest stands by next generation high-throughput sequencing of the bacterial 16S rRNA gene, we identified 1163 bacterial operational taxonomic units. The composition of the *C. convexus* gut bacterial microbiome was significantly different between rural and urban individuals. The microbiome diversity (evaluated by the Rényi diversity function) was higher in rural than urban adults, similar in urban females and males but significantly higher in males than females in the rural habitat. This sex-specific difference in gut microbial diversity could emerge because males with greater mobility are more likely to acquire more facultative bacterial symbionts from their environment than the less mobile females. Our findings demonstrate that urbanization significantly altered the composition and diversity of the gut bacterial microbiome in *C. convexus*. The study was supported by the National Research, Development and Innovation Fund (OTKA K-131459).

**Keywords:** carabid, habitat alteration, microorganisms, sequencing urban, rural

## P046. How to find the right host- Primary attraction and host selection of the bark beetles *Ips typographus*

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Proceeding climate change caused substantial damages in many forests in recent years. Increased occurrence of climate extremes such as storms, fires or droughts has led to a greater susceptibility of trees towards biotic agents, like the bark beetle *Ips typographus* in Norway spruce forests. While many aspects of the beetle's life cycle are well understood, there are still large knowledge gaps in interactions between beetles and host trees, in particular concerning the selection of appropriate host trees by pioneer beetles. These beetles need to identify trees with reduced defences to survive attack and establish the first brood of the population. It has been suggested that beetles might follow kairomone signals from trees to evaluate the physiological condition of potential hosts. Accordingly, beetles may differentiate between healthy and weak trees based on the tree volatile organic compounds emissions. We have conducted field measurements of volatile emission profiles of healthy and weakened trees to investigate the potential effect of monoterpenes, a dominant group of conifer volatiles, on primary attraction. We discuss potential host selection mechanisms and present our methodical approach for quantification and qualification of volatile signals and present first results of volatile emissions of weak and healthy spruce trees, as likely selection cues for pioneer beetles in search of suitable hosts.

**Keywords:** bark beetles, *Ips typographus*, primary attraction, olfactory signals

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## P047. Fungi associated with fir beetles (*Pityokteines vorontzovi* and *P. curvidens*), their behavioural attraction and putative functions

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Fir beetles (*Pityokteines vorontzovi*, *P. spinidens,* and *P. curvidens* ) are among the most aggressive bark beetles in Europe. In most bark beetles symbiotic associates, in particular fungal ectosymbionts, are crucial for a successful colonization of trees due to their tree virulence, direct nutrition and detoxifying abilities of plant-defensive compounds. Despite the economic importance of fir beetles, fungal associates of fir beetles are poorly known and their potential functions remain enigmatic. Here we studied fungi from two fir beetle species (*P. vorontzovi* and *P. curvidens*) at three distinct locations in the black forest using culture-dependent and metabarcoding techniques. Fifty strains of 35 fungi were identified. The five core symbiotic fungi (*Geosmithia sp*, *Ophiostoma piceae*, *Cladosporium cladosporioides*, *Penicillium brevicompactum* and *Graphilbum fragrans*) were selected for further investigations. Growth tests at different temperatures reveaeld differences in fungal temperature preferences and potential niche differentiation. A novel behavioral bioassay was applied to investigate the beetles' responses to fungal volatiles and gustatatory cues. Neither *P. vorontzovi*, nor *P. curvidens* showed significant preferences for any fungus based on its volatiles, however, phloem media colonized by three of the fungal species (*Geosmithia sp*, *Ophiostoma piceae* and *Cladosporium cladosporioides*) were significantly preferred by beetles for boring their tunnels. Such decisions could be the result of beetles' recognition of specific fungal nutrients and/or detoxification products of plant-defensive compounds. Future studies are required to test the functions these fungi have for beetle nutrition and whether this preference for fungus-colonized substrate is also present during host-colonization in nature.

**Keywords**: bark beetle, fir beetle, fungal symbionts, behavioural bioassay, *Pityokteines vorontzovi, Pityokteines curvidens*

## **P350.** Resistance against *Leucoptera sinuella* (Lepidoptera: Lyonetiidae) among hybrid clones of *Populus* spp. in central Chile

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*Leucoptera sinuella* (Lepidoptera: Lyonetiidae) is a leaf miner specialist on Salicaceae recently introduced to Chile and Argentina, where it is causing economic damage to poplar plantations. A field survey in a poplar nursery naturally infested showed that regardless of the poplar hybrid taxon, high variability in resistance was observed among clones within families for oviposition and leaf-mining damage. A group of susceptible and resistant hybrid poplar clones was then selected for a laboratory evaluation of oviposition (antixenosis) and leaf-mining damage (antibiosis) on potted, rooted shoot cuttings. The concentration of condensed tannins (CTs) and salicinoid phenolic glucosides (SPGs) of the leaves of the selected clones from the laboratory study was also measured. Total oviposited eggs were positively correlated with leaf area, with the lowest oviposition on TMxT 11372. The lowest percentage of mined leaf area was obtained for TMxT 11372, TMxT 11463 and TDxD 17574, but surprisingly no correlation between percentage of mined leaf area and concentration of CTs and SPGs was found. Resistant poplar hybrids of our study could be suitable for breeding programs aimed for *L. sinuella* integrated pest management.

**Keywords:** oviposition, severity, antibiosis, tannins, salicinoids

# Session 9:

## Medical and Veterinary Entomology

## P209. Body condition, diet, and protozoan infection in the insect vector *Mepraia spinolai* captured during contrasting environmental conditions.

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*Mepraia spinolai* (Hemiptera: Reduviidae) is an endemic triatomine species from the semiarid-Mediterranean ecosystem of South America. Its hematophagous feeding habits and high frequency of infection with the protozoan parasite *Trypanosoma cruzi* (Trypanosomatida: Trypanosomatidae), which causes Chagas disease in humans, have turned it into the main sylvatic vector in Chile. Our goal was to evaluate the association between epidemiologically relevant variables in natural populations of *M. spinolai* and environmental conditions. We compiled data regarding stage, weight, length, *T. cruzi* infection and diet of *M. spinolai* populations from the same study site, in two years that showed average (203.2 mm) and reduced (17.2 mm) total annual precipitation. The environmental variables evaluated included maximum and minimum temperature, rainfall, normalized difference vegetation index (NDVI), and camera trap fauna sightings. There were significant differences between years modulated by developmental stage in the body mass index, diet diversity and frequency of infection of the triatomines, with environmental differences in the potential prey availability. All of these variables presented lower values during the reduced precipitation year. The most frequent vertebrate species in the triatomines' diet also varied according to the sampling period, in concordance with the fauna sightings only during the average precipitation year. These results would allow to suggest the relevant variables involved in host-vector parasite transmission dynamics. Funding: ENL01/21; FONDECYT-1221045; ANID - Programa Becas - Doctorado Becas Chile 2019 72200391 and 72200094.

**Keywords:** Triatominae, kissing bug, Chagas disease, Chile, *Trypanosoma cruzi*, blood-sucking insect

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## P210. Two consecutive years studies on sand fly species distribution in Lombardy region (Northern Italy)

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Sand fly surveillance is essential for risk assessment of emerging infections caused by protozoa of the Leishmania genus and viruses belonging to the Phlebovirus genus, the most significant group of viruses transmitted by sand flies. Within the context of vector-borne disease surveillance programs of Lombardy region, information on composition and density distribution of sand flies from June to the beginning of October 2021 and 2022 were acquired. The collections were performed in the 12 provinces of the region by using modified CDC traps baited with $CO_2$ ($CO_2$–CDC traps). A total of 1377 sand flies were captured,

including males and females. Regarding species identification, *Phlebotomus perniciosus* was the most abundant species (52%), followed by *Phlebotomus perfiliewii* (16%), *Phlebotomus mascitti* (11%), *Sergentomia minuta* (6%), *Phlebotomus neglectus* (5%), *Phlebotomus papatasi* (5%), and *Phlebotomus ariasi* (5%). The quali-quantitative analysis of the data showed that composition of sand fly populations did not change between seasonal periods and surveillance years. Regarding the two most abundant species, *Phlebotomus perniciosus* had two peaks in weeks 28 and 32, whereas *Ph. perfiliewi* had two peaks in weeks 26 and 29. Concerning altitudinal gradient, it was shown that in both surveillance seasons the greatest amount of specimens were sampled within a range of 301-450 m above sea level. These preliminary results may be useful in future monitoring and control programs aimed at reducing the risk of Leishmania and Phlebovirus infection.

Keywords: Sandflies, *Phlebotomus perniciosus*, Italy

## P211. Characterization of chitin and chitosan derived from the diptera *Hermetia illucens* for application in cosmetic and pharmaceutical fields

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Chitin and chitosan are natural polymers of great technological and economic interest, having numerous applications in different fields. Currently, chitin is extracted industrially from fishing waste. The debate on the sustainability of this resource and the steady increase in market demand have prompted a search for alternative sources. Insects are gaining great interest, particularly bioconverting insects such as *Hermetia illucens*. Currently, *H. illucens* is reared for protein feed production. Its farming also generated large amounts of chitin-rich waste biomass (exuviae from moulting processes and dead adults) that could be exploited as a source for the polymer extraction. Chitin extracted from these biomasses had yield, chemical characteristics and purity similar to that commercially available. Pupal exuviae were the richest biomass and also the most easily collected from insect farm. From chitin, chitosan was produced by heterogeneous and homogeneous deacetylation; the two methods showed significant differences in deacetylation efficiency, yield, deacetylation and crystallinity degree in support of the heterogeneous method. Spectrometric, diffractometric and morphological characterization of different chitosans confirmed their similarity to the commercial polymer, from which they vary in lower viscosity and molecular weight. The chitosan biological properties useful for biomedical and cosmetic applications were also evaluated. IC50 values showed good radical scavenging activity of *H. illucens* chitosans; all chitosans, particularly heterogeneous ones, were able to reduce the expression of IL-6, IL-8, IL-1α and TNF-α, proving to be good anti-inflammatory agents. Furthermore, all chitosan samples positively modulated the expression of the antimicrobial peptide HBD-2, demonstrating an indirect antimicrobial activity.

Keywords: chitosan, indirect antimicrobicity, black soldier fly

## P296. Promotion, exploitation and results by Mosquito Alert ITALIA: the story of a successful citizen science project.

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Mosquito Alert is a non-profit citizen science project, coordinated by different public research centres with goals to: 1) monitor the spread of Aedes invasive species and Culex spp. (transmitting global diseases such as dengue, or West Nile virus fever) through georeferenced photos of adult mosquitoes and 2) increase the awareness of citizens about public health problems related to mosquitoes. During 2-years implementation of Mosquito Alert in Italy, 18,323 users downloaded the App and turned on the sampling effort monitoring. Among the 8,201 mosquito photos received, 5,168 were identified by experts as mosquitoes at species/genus level: 36.8% as *Culex* spp., 58.1% as *Aedes albopictus*, 0.3% as *Ae. koreicus* and 0.1% as *Ae. japonicus*, 0.1% as either *Ae. koreicus* or *Ae. japonicas*, and 4.5% as other autochthonous mosquito species. Users' tentative identifications matched with those of the experts in 77% and 62% of the cases for Aedes invasive species and Culex spp., respectively. Both *Ae. albopictus* and *Cx pipiens* were reported from all Italian regions, while *Ae. koreicus* and/or *Ae. japonicus* were reported from northern regions only. Temporal trends of relative frequencies of the identified reports of the two most abundant species reflect their known seasonality. Interestingly, 11 *Ae. albopictus* females were reported in winter months in 6 Italian municipalities. The added value and potential of the involvement of citizens in providing large scale data on mosquito species and in complementing conventional entomological monitoring will be discussed.

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## P297. Deciphering the molecular interaction between the Asian Tiger Mosquito *Aedes albopictus* and the Chikungunya virus

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The tiger mosquito *Aedes albopictus* becomes infected with the Chikungunya virus (CHIKV) after ingestion of a viraemic blood meal. Afterwards, the arbovirus starts its journey inside the mosquito, that involves overcoming immune barriers, such as midgut and haemocoel, and infection of salivary glands before transmission to new hosts. Using RNAseq we analyzed gene expression variations in midgut and carcasses collected from CHIKV-infected and uninfected *Aedes albopictus* at 1 and 5 days post infection (dpi). Differential expression (DE) and gene enrichment analyses showed modulation of different mosquito immune pathways (e.g. ubiquitination, RNAi) starting from the early stages following CHIKV infection whereas, at a later stage, after viral dissemination into the hemocoel, we mainly found expression of LRRs (Leucine Rich Repeat), production of AMPs (antimicrobial peptides) and activation of the melanization cascade. Five candidates were selected from the list of DE genes for validation of RNA-seq results. Analysis by RTqPCR of dissected female tissues/organs confirmed the enriched expression of a LRR, the AMP Holotricin, and a PO activating factor in the haemolymph, the midgut-specific downregulation of a trypsin, and revealed a strong induction of the Vitellogenin Receptor in the ovary.  Overall, our study highlighted mosquito immune responses and factors modulated by CHIKV, as well as strategies developed by the virus to survive, as is the case for the downregulation of trypsin which may counteract enzymatic degradation during blood meal digestion. Functional analyses are in progress to further clarify the role of identified genes in the interactions between mosquito and CHIKV.

**Keywords:** viral infection, vector biology, molecular interaction, gene expression, mosquito immunity

## P298. Implications of sublethal concentrations of *Origanum vulgare* essential oil and its dominant constituent carvacrol on life-cycle traits of *Aedes albopictus* and *Culex pipiens* biotype *molestus* (Diptera: Culicidae)

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*Aedes albopictus* is the most invasive mosquito species worldwide and a field vector of Dengue, Chikungunya and Zika viruses. *Culex pipiens* biotype *molestus* is an anthropophilic member of *Cx. pipiens* mosquito species complex that transmits West Nile Virus. Both mosquito species occur in Europe where their management relies mainly on larval control with limited number of approved synthetic or microbial insecticides. Over the last decades, a growing number of plant-derived Essential Oils (EOs) have been tested as low-risk mosquito larvicides acting through various modes of action. In the laboratory, we evaluated the effects of $LC_{50}$ concentrations of EO from *Origanum vulgare* and its dominant constituent carvacrol on life-cycle traits of *Aedes albopictus* and *Cx. pipiens* biotype *molestus.* Short-term (24h) exposure of 3rd-4th instar larvae to $LC_{50}$ concentrations of the oregano oil and carvacrol caused significant delayed mortality to surviving larvae until adulthood, morphological abnormalities in surviving larvae and pupae, and failure in adult emergence in both mosquito species, indicating potential insect growth inhibition properties of the tested materials. Larval stage of *Ae. albopictus* was significantly prolonged after carvacrol treatment. Larvicidal treatments with carvacrol significantly reduced the longevity of *Cx. pipiens* biotype *molestus* males and resulted in larger *Ae. albopictus* males. No further remarkable effects on life-cycle traits of emerged adults were recorded. Our findings suggest that carvacrol and carvacrol-rich oregano EO are effective larvicidal agents at doses lower than the acute toxic ones supporting a more sustainable perspective for their use against mosquitoes.

**Keywords:** mosquitoes, larvicidal, sublethal effects, growth inhibition, carvacrol, oregano oil

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## P299. Developing a push-pull systems to control *Culicoides* biting midges

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Biting midges of the genus *Culicoides* are vectors of veterinary relevance, as they can transmit a plethora of pathogens such as bluetongue virus and African horse sickness virus. Novel control techniques aim at using push-pull systems to alleviate the impact of insect vectors upon hosts. Spatial repellents are employed to keep the insects away from farm animals, while traps baited with attractants collect them. In our project, we investigate the repellency potential of novel candidates (plant-derived compounds), in tube bioassays

and via video-tracking. Two of these compounds exert a highly repellent effect against lab reared *Culicoides nubeculosus* and the mosquito species *Aedes aegypti* alike. Subsequent experiments aim at upscaling to semi-field and field conditions. Future steps aim at identifying attractive volatiles that can be employed to bait traps. The focus will be oriented towards the exploitation of the biting midges' sugar-feeding behavior, i.e., to analyze plant volatiles for attractiveness towards biting midges. Once identified, the best repellent and the best attractant will be tested together in a push-pull system.

**Keywords:** attractants, control, *Culicoides*, repellents, vector

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## P300. Food for thought (Tiger mosquito- *Enterobacter* version)

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The Asian tiger-mosquito, *Aedes albopictus,* is a vector of several diseases, including Yellow and Dengue fever, as well as Zika and Chikungunya. Current vector control methods either rely on chemical insecticides or on methodologies that are partially effective, costly, and time-consuming. Methodologies inducing sterility in natural populations, through releasing males, are reviving to suppress natural populations and subsequently reduce both disease transmission, and biting nuisance. Among them, the Sterile Insect Technique (SIT), relies on the mass production and release of sterile males to mate with the females of natural populations. Success of SIT depends on the mass production of competitive sterile insects. Consequently, a balanced larval diet, during the mass production, can ensure efficient males' production is necessary. The main factors that affect the choice of a diet are repeatability in production, performance (in terms of rearing efficiency and biological quality of males to be released), cost, and unhindered availability of the appropriate ingredients. Considering the available literature and recent advances in the field, we used as reference the "CAA" larval diet, consisting of cat food, yeast, and fish food (developed by Centro Agricoltura Ambiente "G.Nicoli"). The main goal of this study was to evaluate the use of biomass derived from an available *Enterobacter sp.* strain, previously shown to have beneficial effects in the artificial rearing of different Diptera, as either additive or replacement of specific ingredients. Our data show that *Enterobacter*-based larval diets can support the efficient production of *Ae. albopictus* reared for SIT purposes.

**Acknowledgment:** Within the framework of the projects: «moSquITo»: Innovative approaches for monitoring and management of the Asian tiger mosquito with emphasis on the Sterile Insect Technique (TAEΔK06173- National Recovery and Resilience Plan, "Greece 2.0" & EU Funding – Next Generation EU); "Establishing Genetic Control Programmes for *Aedes* Invasive Mosquitoes"; IAEA- Programme of Coordinated Research Activities "Identification and Characterization of Temperature Sensitive Lethal Genes and Response to Thermal Shock of SIT Target Species"(IAEA-CRP Contract No: 23372)

**Keywords:** *Aedes albopictus*, disease vector, *Enterobacter*, larval diet, SIT, tiger-mosquito

## P301. *Anopheles* mosquito species from Greece: species identification and insecticide resistance status

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Mosquito species of the genus *Anopheles* are abundant in many areas in Greece, with some known to be competent vectors of malaria, posing a potential health risk.

In the present work, we recorded the *Anopheles* species of larval breeding sites from six different areas of Greece. Bioassays contacted to monitor their susceptibility to certain insecticides. The recommended by World Health Organization (WHO) method has been used for the evaluation of the efficacy of the larvicidal agent *Bacillus thuringiensis israelensis* (Bti) against mosquito larvae and the CDC bottle assay method for the response of adults to pyrethroid insecticides. Furthermore, molecular testing took place for species identification and the detection of known insecticide resistance alleles (kdr mutations).

The primary vector for malaria transmission *Anopheles sacharovi* was observed in one of the six sampling locations, among other less important but competent malaria vectors (*An. claviger*, *An. algeriensis*, and *An. plumbeus*) which were also present. A variation in Bti response was observed, among six different *Anopheles* populations assessed, possibly associated with the extensive use of Bti in mosquito control programs. No differences were observed in the bioassays with CDC bottles in response to pyrethroid insecticides. The results of the bioassays are in line with molecular testing, which showed the absence of kdr mutations in *Anopheles* populations from Greece. The findings suggest the importance of continuous mosquito surveillance for effective and sustainable malaria vector management.

Keywords: malaria vectors, *Anopheles*, Greece, resistance, bti

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## P302. A platform for gene drive-inspired local control of insect pests

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At Biocentis we are developing tools for genetic control of the world's most dangerous insects. Our founders developed the first gene drive technologies for population suppression. Leveraging key developments in this field and building upon successes in the malaria mosquito, we are tackling non-malaria disease vectors, such as the yellow fever mosquito (*Aedes aegypti*) and agricultural pests, such as the spotted-wing *Drosophila* (*Drosophila suzukii*). Our approach is inspired by gene drive but has been refined to allow precision control that is not only species-specific, but also limited in its capacity to spread in space and time. The technology targets disruption of the master-regulator of sex determination in insects,

the *doublesex* gene, to prevent reproduction. Unlike previous attempts at genetic control using CRISPR, the *doublesex* gene appears to represent the Achilles heel of insect biology – with extreme sequence constraints that act to preclude resistance and thus extend the life of a carefully designed genetic control element. The Biocentis lab has now demonstrated the feasibility of this approach in several insect pests, with mathematical models suggesting a substantial advantage over existing interventions. The solution we envision is a sustainable, cost-effective, and safe alternative that could boost ongoing efforts to safeguard human health and food security

**Keywords:** gene drive, genetic control, commercial biocontrol

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## P303. Baseline characterisation of Southern African malaria vectors for paratransgenesis potential

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Residual malaria transmission is a challenge for South Africa's malaria elimination agenda. This low-level, persistent transmission of malaria is sustained by outdoor biting species with zoophilic tendencies. One of these species is the major malaria vector *Anopheles arabiensis*. This exophilic and exophagic species is difficult to control by conventional vector control interventions. As such, alternative control methods are needed. In general, *An. arabiensis* is comparatively poorly studied. The same is true for the major malaria *An. funestus*. This extends to novel interventions such as paratransgenesis. Here we report two key steps towards the implementation of paratransgenesis for these species. The first is a comparative description of the microbiota of various tissues in laboratory reared *An. funestus* differing in resistance phenotype. The second is a comparison of the gut microbiota laboratory reared, wild and F1 *An. arabiensis*. The microbiota was characterised using 16s rDNA sequencing on an Illumina platform. Alpha and beta-diversity as well as overlapping species and relative abundance was assessed. In *An. funestus*, the salivary gland microbiome was significantly different from that of other tissues. There was a strong conservation of species in the gut and reproductive organs. Composition and diversity were affected by the resistant phenotype. When comparing wild to laboratory-reared mosquitoes there was a significant increase in diversity and abundance in the wild adults. Despite the differences, in all cases, bacterial species suitable for paratransgenesis were found in all populations. These findings lay the foundations for future paratransgenesis interventions in Southern Africa.

**Keywords:** symbionts, diversity, Next Generation Sequencing, vector, malaria

# P304. Structural determinants of ORco ligands antagonizing odorant receptor function for mosquito vector control

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Insect odor receptors (ORs) are heteromeric ligand-gated cation channels composed of an obligatory, highly conserved receptor subunit, ORco, and one of many variable subunits, ORx, in as yet undefined molar ratios. When expressed alone in various cell culture systems, ORco forms homotetrameric channels gated by ORco-specific ligands acting as channel agonists. In previous studies, we identified a number of small ligands acting *ex vivo* as specific ORco channel antagonists, orthosteric or allosteric relative to a known agonist binding site, and cause severe inhibition of the olfactory function in mosquitoes. Therefore, we sought to design a virtual screening protocol to identify candidate ORco antagonists that could be used as vector control agents. Initially, based on the previously characterized compounds, we developed a pharmacophore describing the 3D arrangement of orthosteric antagonist features necessary for blocking ORco's biological response. A new collection of 49 volatile organic compounds (VOCs) was both computationally and functionally screened. The pharmacophore's sensitivity was 86%, while its specificity was 57%, leaving room for improvement. Consequently, we calculated a set of 2D descriptors for all pharmacophore hits and generated a support vector machine (SVM) capable of discriminating between true (*ex vivo* confirmed) orthosteric antagonists from false positive hits. The statistically estimated cross-validation out-of-sample misclassification rate of the SVM was 3.13%. The validity of employing the two-step *in silico* protocol to identify ORco orthosteric antagonists is currently under investigation on a third collection of natural compounds with a combination of *ex vivo* activity inhibition and *in vivo* repellence assays.

**Acknowledgements:** The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty Members & Researchers and the Procurement of High-and the procurement of high-cost research equipment grant" (Project Number: HFRI_FM17_637).

**Keywords:** mosquito ORco co-receptor, mosquito repellents, pharmacophore, support vector machine, virtual screening, volatile organic compounds

## P305. Molecular Identification of blood meal source in collected blood-fed mosquitoes in Greece, for the period 2017-2021

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Blood seeking, an essential process in the reproductive cycle of most mosquitoes, holds a key role in the transmission of pathogens to hosts and is influenced by various genetic and environmental factors. The purpose of this study was to investigate the origin of the blood meals of adult mosquitoes species belonging to medical importance genera, collected in Regional Units in Greece, the period 2017-2021, during entomological surveillance programs. A total of 414 mosquito samples were analyzed for their feeding habits using molecular techniques. DNA extraction was performed with the NucleoSpin Tissue, Mini kit for DNA from cells and tissue (Macherey-Nagel). It was then amplified by PCR, using two (2) primer sets, one targeting a portion of the 16s rRNA gene common to vertebrates and another specific for mitochondrial DNA animal sequences. Identification of the host species was based on sequencing of the PCR products obtained, searching the sequences in the NCBI database (BLAST). A diverse range of hosts were identified, highlighting that most mosquito species studied, mainly fed on various mammalian species (i.e. human, dog, cat), followed by certain bird species. The genus Culex is the most widespread one, including species that feed opportunistically on both humans and animals and therefore constitute competent vectors of pathogens causing zoonotic diseases. Mosquito blood feeding behavior has profound implications in disease transmission, vector control, and entomological surveillance. Thus, a thorough understanding of mosquito feeding patterns and preferences, is imperative and should be independently assessed through blood meal and preference analysis assays.

**Keywords:** Culicidae, bloodmeal analysis, molecular identification, pathogens

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## P306. *Culex pipiens* biotypes, host preference and resistance mutation in the Attica Region

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*Culex (Cx.) pipiens* mosquitoes are main vectors for various pathogens such as West Nile Virus (WNV), Usutu virus, Rift Valley fever virus, Japanese encephalitis virus, Sindbis virus, Tahyna virus and Dirofilarial worms.

The different biotypes of the *Cx. pipiens* (*pipiens* and *molestus*) can be competent to transmit pathogens to humans and animals, and especially the hybrids of the two forms can act as bridge vectors for the WNV. The Attica peninsula is on the eastern edge of Central Greece, encompasses the city of Athens (the capital of Greece), and hosts, approximately, half of the population of the country. In this region, different land uses can be found and in the last twenty years, different vector-borne diseases have been identified either native, like WNV and malaria, or imported, like Dengue. In the current study we collected blood-fed *Culex pipiens* mosquitoes from February 2022 to February 2023, employing 55 BG-sentinel traps in the Attica region (equipped with BG-Lure and constant flow rate of CO2 -gas cylinders). Our results are providing valuable information for the consistency of the different biotypes of wild *Cx. pipiens* and their host preferences in the Attica region, as well as of the frequency of kdr alleles associated to insecticide resistance phenotype.

**Keywords:** *molestus*, hybrids, blood fed, kdr mutations

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## P307. Mosquito as a potential vector of Lyme disease

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Lyme disease is a worldwide vector-borne infection. It is caused by spirochetes of the *Borrelia burgdorferi sensu lato* (s.l.) complex, which are transmitted by ticks of the genus *Ixodes*. Although ticks are generally considered the primary vectors of the disease, there are several studies reporting that *Borrelia* spirochetes are also present in other arthropods, such as mosquitoes. However, direct evidence of whether mosquitoes are vectors of *Borrelia* spirochetes is still missing. In this study, we are investigating the capacity of mosquitoes belonging to the genera of *Aedes* and *Culex* to acquire and transmit Lyme disease spirochetes to mammalian hosts. To do that we utilized two models: 1. *in-vitro* membrane feeding system and 2. *in-vivo* mouse model. Using an artificial membrane feeding system, we were able to infect *Aedes aegypti* sp. with Borrelia with a 100% efficiency. Subsequently, we observed a gradual decrease in infection rates during the post-blood meal digestion. We hypothesize that Borrelia spirochetes elimination during the off-host phase might be caused by mosquito digestion or immunity. Compared to in-vitro feeding, mosquitoes fed on *Borrelia*-infected mice became infected at a lower rate. Moreover, testing of the ability of *Aedes aegypti* sp. to transmit Lyme disease spirochetes showed that mice were not infected even after being bitten by *Borrelia*-infected mosquitoes. Our findings suggest that Lyme disease is unlikely to be transmitted by mosquitoes in nature because of the rapid clearance of *Borrelia* spirochetes in mosquitoes.

**Keyword:** Lyme disease, *Aedes aegypti*, vector competence

# P308. Assessment of non-live formulation of *Chromobacterium* sp. Panama (Csp_P) with an attractive sugar bait for the control of malaria vector mosquitoes

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Mosquito control remains the most effective method of malaria control. Chemical insecticides in the form of indoor residual spraying and insecticide treated bednets are broadly used for mosquito control. Despite the success of conventional chemical insecticides, off-target effects, insecticide resistance, and environmental concerns has resulted in the need of alternative mosquito control methods. Attractive toxic sugar bait (ATSB) is an environmentally friendly mosquito-specific method designed to minimize the spread of insecticides in the environment. Our previous studies have shown that *Chromobacterium* sp. Panama (Csp_P), a soil bacterium, has adulticidal and larvicidal activity against multiple mosquito species. In this study we evaluated a novel non-live preparation of Csp_P to be delivered to mosquitoes via ATSB. Our results show the new Csp_P formulation combined with the sugar bait efficiently kills indigenous insecticide resistant *Anopheles* species in Burkina Faso in a semi-field setting. Mosquitoes fed on Csp_p also become more susceptible to other chemical insecticides. Overall, Csp_P ATSB is an attractive candidate to be deployed as a novel mosquito control tool.

**Keywords:** mosquitoes, Malaria, biopesticide, insecticide, bacteria, bait

# Session 10:

## Invasion biology and climate change

## P173. Non-destructive methods for detection of fruit flies' infestation in fruits

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Monitoring and detection of insect infestation in fruits and vegetables is critical for sustainable agriculture. Infestation by true fruit flies (TFF) at early stages is difficult especially at early stages of infestation. Inspection for infestation is usually destructive for a large proportion of the commodity. Since, insect herbivory can elicit changes in host plant chemistry and so in volatile emission, in this study, the aim was to determine infestation-specific volatile organic compounds (VOCs)-indicators emitted by fruits (peaches, pears, apples, oranges and mandarins) after the infestation by TFF, namely *Ceratitis capitata* (Wiedemann), *Bactrocera dorsalis* (Hendel) and *B. zonata* (Saunders) (Diptera: Tephritidae). VOCs emitted from non-infested and TFF-infested fruits were collected by the dynamic headspace sampling technique, analyzed by Gas Chromatography-Mass spectrometry (GC/MS). VOCs were also used as a training and validation set for the e-Nose system in order to be evaluated as a potential technology for detection of hidden infestation. Results showed that specific esters were TFF species specific for peaches. In case of pome fruits, esters increased along with the procession of fruit ripening and quantitative differences were observed between non-infested and infested fruits. In citrus, the monoterpene, limonene and the sesquiterpene, valencene were the main VOCs detected in both non-infested and infested fruits. *E*-(*β*)-ocimene and homoterpene *E*-4,8-dimethylnona-1,3,7-triene (DMNT) were found in significant quantities in infested fruits. Different statistical models were developed from the results of e-Nose, that required validation both in laboratory and in field on the potential for adoption of this non-destructive method for detection of tephritid infestation.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 818184.

Keywords: fruit flies, VOCs, E-nose, *Ceratitis capitata, Bactrocera dorsalis, Bactrocera zonata*

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## P174. *Bactrocera dorsalis* (Diptera: Tephritidae) in the Indian Ocean: a tale of two invasions

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An increasing number of invasive fruit fly pests are colonizing new grounds. With this study we aimed to uncover the invasion pathways of the oriental fruit fly, *Bactrocera dorsalis* into the islands of the Indian Ocean. By using genome wide SNP data and a multi-pronged approach consisting of PCA, ancestry analysis, phylogenetic inference and kinship networks, we were able to resolve two independent invasion pathways. A western invasion pathway involved stepping-stone migration of *B. dorsalis* from the east African coast into the Comoros, along Mayotte and into Madagascar with a decreasing genetic diversity. The Mascarene islands (Reunion and Mauritius) on the other hand were colonized directly from Asia and form a distinct cluster. The low nucleotide diversity suggests that only a few genotypes invaded the Mascarenes. The presence of many long runs of homozygosity (ROH) in the introduced populations are indicative of population bottlenecks, with evidence of a more severe bottleneck for populations along the western migration pathway than on the Mascarene islands. More strict phytosanitary regulations are recommended in order to prevent further spread of *B. dorsalis*

Keywords: *Bactrocera dorsalis*, phylogeography, invasive species, Indian Ocean, pest species

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## P175. Using socioeconomic variables to predict the distribution of invasive termite species in the Asian Pacific region?

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Invasive species are a growing concern to ecologists and ecological managers. Economic losses are massive and will keep increasing in the decades to come through three drivers: climate change, global trade and socioeconomic changes. The Asia-Pacific region (APAC) is a major driver of the global economic growth. It is also one of the biggest exporters of invasive alien species. The high level of trade with and within the APAC region promotes the spread of invasive species, including termites worldwide. If termites play an important role in ecosystems, several species are also significant economic pests, mainly in urban areas by attacking the wooden structure of buildings, but also crops, production forests and natural forest habitats. Acting before invasion drastically reduce ecological and economic costs associated to the introduction, establishment and spread of invasive species, but it is difficult to predict effectively which species will invade which regions. Species distribution models (SDMs) are a valuable statistical approach for both understanding species distribution and predicting range expansion after introduction. Out of 18 invasive termite species present in the APAC, twelve of them were selected based on their data availability and their intensity of damages. The SDMs all show that these twelve invasive species present in the APAC could currently settle in many densely populated regions in tropical, subtropical and to a lesser extent in temperate regions. Adding general circulation models for different scenarios and periods show that climate change will increase the distribution of most species, especially in a fossil-fueled world.

**Keywords:** termites, invasive species, pest species, SDM, species distribution modelling, Asia Pacific

## P176. Monitoring and control of the predatory hornets *Vespa Velutina nigrithorax* and *Vespa orientalis* in Italy

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Hornets are predatory species that feed on many insects, with some of them showing a marked preference for honeybees. In recent years, some hornet species invaded countries where they were not native, while others extended their geographical range in their native regions.
The Asian hornet *Vespa Velutina nigrithorax* arrived in Europe in 2004 and it has since then spread to many western European countries. The species reached Italy from the border with France between 2012 and 2013 and it has now been reported in 5 regions of the North-west and further spreading south and east.
*Vespa orientalis* is a Mediterranean species accidentally transported to numerous non-indigenous localities in Europe. In Italy, it was endemic to the southern regions, but in the last few years it has been reported in several central-northern regions and in the Sardinia Island.
The recent spread of *V. velutina* and *V. orientalis* represents a threat to both wild and managed pollinators and it is causing serious damage to Italian beekeeping. Monitoring and containment measures must necessarily follow different paths, depending on whether the species is alien (*V. velutina* is included in the EU list of invasive alien species) or native (*V. orientalis*).
Here, we report the current situation of the two species in the Italian peninsula and propose an integrated approach for their monitoring and control based on a trapping scheme in apiaries coupled with a citizen science reporting project, followed by nest neutralization and reduction of the number of hornets in front of the hives.

**Keywords**: *Vespa Velutina*, *Vespa orientalis*, beekeeping, monitoring, EU regulation

## P177. Effect of thermal acclimation on the metabolome of the Mediterranean fruit fly

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The Mediterranean fruit fly, *Ceratitis capitata*, is a global threat to fruit and vegetable production and holds an impressive record of invasion events around the world. Currently, *C. capitata* expands its geographic distribution to more temperate and cooler areas. Medfly invasion success is facilitated by its high phenotypic plasticity that allows the survival of the pest under the influence of stressful environmental and temperature conditions. Several studies have shown that prior exposure to certain thermal regimes leads to the acclimation of the fly to new temperature conditions, thus, contributing to a higher thermal tolerance of the insect. Research on other fruit flies has shown that cold acclimation leads to certain adjustments in the metabolic profiles of the studied pests. However, the physiological and molecular mechanisms underlying thermal plasticity and tolerance in the medfly are yet to be investigated. Under the framework of the project MEDFLY (grant number: HFRI-FM17-4289, and partially supported by REACT (grant number: 101059523)), we used targeted metabolomics (HILIC-MS/MS) to determine the effect of acclimation at four temperature regimes (10, 20, 25 and 30°C) on the metabolic profiles of *C. capitata* adults originating from three distinct geographic regions (Crete-Greece, Lechonia-Greece, Dubrovnik-Croatia). Our results demonstrated that both the geographic origin and acclimation regimes lead to significant changes of the medfly metabolome, shedding light to the metabolic mechanisms involved in *C. capitata* thermal plasticity.

**Keywords:** thermal plasticity, metabolic profile, medfly

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## P178. First report of pepper fruit fly (*Atherigona orientalis*) in Greece on commercial pepper crops

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The pepper fruit fly *Atherigona orientalis* (Schiner 1968) (Diptera: Muscidae), is a cosmopolitan tropical pest which has been recently recorded in several European countries. The larvae live and feed on decaying fruits and vegetables, but sometimes even in vertebrate and invertebrate carrions, dung and faeces. Accordingly,

the pepper fruit fly has long been viewed as a minor pest, with only recently being acknowledged as a primary pest of pepper fruits. In April 2022, infested pepper fruits from an organic greenhouse in the Tympaki rural area (Central Southern Crete, Greece) were transferred to the Laboratory of Entomology (Hellenic Mediterranean University, Heraklion Crete, Greece) due to extensive damage by unknown insect pest. In May 2022, a second infestation of pepper fruits with a similar appearance of symptoms was reported in a greenhouse at the area of Stomio, in Ierapetra (Southeastern Crete). Specimens from both locations were examined under high magnification stereoscope and were placed in rearing boxes to complete their development. Species identification was based both on morphological and molecular markers. In the greenhouse pepper crop in Tympaki, *A. orientalis* larvae were found together with the larvae of the Mediterranean fruit fly (*Ceratitis capitata*) in the interior of the pepper fruits. In Stomio, the infestation was attributed purely to *A. orientalis*. The occurrence of the new pest has significant implications in the pest management schemes implemented in pepper crops since none of the current control practices for the major pepper pests are known to be effective in controlling diptera species.

**Keywords**: fruit fly, pepper, Diptera, *Atherigona orientalis,* Greece, infestation

## P179. Insect pest invasions in mainland France since 2000

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Damage from alien insect pests is increasing all over the world with the expanding trade of agricultural produce. To date, there is no sign of saturation in the accumulation of invasive agricultural pests worldwide. In order to assess the threat these species pose to agriculture and forestry, it is necessary to track insect introductions over time and space.

Here, we aim to provide a comprehensive overview of insect pest introductions in mainland France since 2000. First reports of insects in a territory often come from various sources. We collected records of new introductions from our diagnostic activities in the framework of the national official surveillance, by reviewing the literature, by gathering unpublished scientific data and by monitoring amateur forums.

We list 178 insect species introduced in mainland France between 2000 and 2022 that are invasive and have caused, or are likely to cause, damage to crops or forests. The estimated date of introduction, the area of origin and the plants affected are given for each species. We focus on the species that pose the greatest risk and examine the evolution of the number of invasions, the taxonomic diversity, the geographical origins and the pathways at risk. We believe it is important to maintain an updated list of these species so that the evolution of biological invasions in agricultural or forest environments can be monitored over time. Understanding the patterns of introductions is also an important prerequisite for designing policies to mitigate the impact of invasive agricultural pests.

**Keywords:** invasive insects, introductions, reports, agricultural pests, phytosanitary risk, France

## P180. Ecological niche modelling in predicting response to climate change: the case of *Eupoecilia ambiguella* (Lepidoptera: Tortricidae) in Europe

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Grapevine is one of the main crops of Europe, having a huge economic, social, cultural, environmental and landscape impact. There are indicators pointing to the fact that Europe is warming up even faster than many other parts of the world. In addition, within Europe, manifestations of climate change will vary between countries and sectors of activity. Therefore, it is of great interest to obtain predictions on the future geographical distribution of important grapevine pests under different climate change scenarios. Taking into account this situation, the objective of this study was to assess the impact of climate change on the distribution of the European grape berry moth *Eupoecilia ambiguella* in Europe, using the Ecological Niche Modelling (ENM) considering the Representative Concentration Pathways RCP 4.5 and RCP 8.5, and the "present- 2050" period. A persistence of the suitable environment for *E. ambiguella* is foreseen in regions of Europe with humid climates and/or microclimates, in a more attenuated way in those in which temperature increases and precipitation decreases. On the other hand, losses of environmental suitability for this moth are estimated throughout Europe, being its distribution area restricted to the northernmost regions and some areas of the center. Taking into account only countries where viticulture constitutes an economically relevant sector and *E. ambiguella* is a key pest (Germany, Italy and Switzerland), very small increases in surface of environmentally suitable conditions are predicted for the moth (≤ 1% in the worst scenario, RCP 8.5).

Keywords: European grape berry moth, grapevine, Ecological Niche Modelling, Representative Concentration Pathways

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## P181. New localities in Türkiye and new hosts in the world of invasive alien species: *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae)

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*Corythucha arcuata* (Say, 1832) is an alien invasive species originating from North America. It was detected for the first time in Bolu in Türkiye and has spread rapidly to the Black Sea, Central Anatolia, South-eastern Marmara and Eastern Mediterranean regions. This report examines the preliminary results of the new distribution and new host records of the pest in the Western Mediterranean region. The study was carried out in 19 locations in Afyonkarahisar, Burdur and Isparta provinces from 2017 to 2022. *Quercus cerris*, *Q. infectoria*, *Q. ithaburensis*, *Q. trojana*, *Q. vulcanica* and *Q. robur* were the tree species investigated in these locations. In addition, *Q. frainetto*, *Castanea sativa*, *Acer pseudoacacia*, *A. negundo*, *Pyrus eleagnifolia*, *Sorbus torminalis*, *S. umbellata*, *Ulmus minor*, *Rubus* sp. and *Rosa canina* species were also examined in the Botanical Garden, Isparta. The results indicated that the species has expanded its distribution area in the Western Mediterranean Region and reached southern parts of the region. Species were identified in eight (50%) of the locations examined. The occurrence of the pest was high in Isparta-Campus area, Isparta-Botanical Garden and Eğirdir Kasnak Oak Nature Protection Area. *C. arcuata* was recorded for the first time in *Q. infectoria* and *Q. vulcanica*, an endemic oak to Türkiye. The pest was only found on *Q. robur* in the three provinces. Oak forests are of ecological and economic importance in the country. The monitoring and detection of the important pests may be invaluable for the health and sustainability of these ecosystems.

Keywords: pest, oak forests, oak lace bug, endemic tree species, *Quercus vulcanica,* Türkiye

## P182. Supercooling capacity and acute cold stress of *Ceratitis capitata* (Diptera: Tephritidae) populations across the Northern Hemisphere

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*Ceratitis capitata* (Diptera: Tephritidae), holds an impressive record of successful invasion events promoted by globalization in fruit trade and human mobility. Additionally, *C. capitata* is gradually expanding its geographic distribution to cooler temperate areas of the Northern Hemisphere. Cold tolerance of *C. capitata* is a crucial feature promoting population establishment and hence invasion success. To elucidate the interplay between invasion process in northern hemisphere and cold tolerance of geographically isolated populations of *C. capitata*, we determined (a) the supercooling capacity (SCP) of immature stages and adults, and (b) the response to acute cold stress as far as survival of adults was regarded. To assess the phenotypic plasticity, the effect of acclimation to low temperatures on acute cold stress survival was also examined. The results revealed that there is no correlation between SCP and climate variables. SCP was much lower than the lowest temperature individuals may have ever experienced in the respective habitats. For acute cold stress of adults, the results revealed that low temperature acclimation was positively related with survival after acute cold stress, except for females originating from Thessaloniki (Greece). Adults from South Arava (Israel) were less tolerant after acute cold stress compared with those from Heraklion (Crete, Greece) and Thessaloniki (Greece). Plastic responses to cold acclimation were population specific, with the Israel population expressing lower levels compared to the two Greek populations. These results set the stage for asking questions regarding the evolutionary adaptive processes that facilitate range expansions of *C. capitata* into cooler temperate areas of Europe.

Keywords: *Ceratitis capitata,* supercooling capacity, cold response, cold treatment, dispersal potential

## P183. *Popillia japonica* Italian outbreak management using natural strains of Biological Control Agents (BCAs)

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*Popillia japonica* Newman (Coleoptera: Scarabaeidae), a priority pest for the EU, was first detected in northern Italy in 2014. In the larval stage, *P. japonica* lives just below the soil surface, in an area that can also be colonized by two of its most feared natural enemies: entomopathogenic nematodes (EPNs) and entomopathogenic fungi (EPF). Since 2017, numerous natural EPN and EPF strains were collected in the Italian outbreak area from soil infested by *P. japonica* larvae. Previous virulence laboratory analyses carried out with EPNs and EPF showed that the indigenous strains *Hetehorabditis bacteriophora* (POP 16) and *Metarhizium robertsii* (17/T02), respectively, achieved the best results in grubs mortality. These two strains of indigenous BCAs were tested in open field trials to assess their efficacy in containing *P. japonica* larval population and their persistence in the soil. Since September 2021 these tests have been performing in three meadows with different irrigation management (not irrigated, semi irrigated, and irrigated meadows), in the Piedmont region. The analysis of the soil samples, collected at different times after treatment, showed a significant reduction in the number of *P. japonica* larvae in the plots treated with EPNs with respect to plots treated with EPF. As regards persistence, the EPNs and EPF strains used were also isolated after the winter season and after one year, with differences depending on the formulation used. In conclusion, our preliminary results suggest a good persistence of EPNs and EPF, but a higher efficacy of EPNs in the containment of *P. japonica* larvae.

Keywords: entomopathogenic nematodes, entomopathogenic fungi, pest management, Italian outbreak, grub

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## P184. World-wide invasion of *Popillia japonica* reconstructed based on complete mitochondrial genomes and nuclear snp markers

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*Popillia japonica* is a coleopteran of substantial economic interest, as it can attack numerous wild and cultivated plant species. Native to Japan, the pest has invaded large areas of the USA, Canada, Azores, Northern Italy and Ticino, and it is considered a priority for control in the European Union. We determined the complete sequence of the mitochondrial genome in 86 individuals covering the entire distribution of the species and developed a panel of 295'396 biallelic unlinked nuclear single nucleotide polymorphisms (snps) in 83 individuals based on full genome resequencing. Phylogenetic analysis supports a primary division between South Japan and Central/North Japan, with all invasive samples coming from the latter. The origin of invasive USA samples is incompatible, time-wise, with the introduction of a single lineage, with multiple Japanese lineages having been introduced and only one accounting for most of the population expansion. Colonization to Italy and the Azores are supported as two separate invasions stemming from the USA population, and samples from Ticino appear as an expansion from the Italian population. Within major areas, snp analysis identifies some substructure in Japan and between islands in the Azores, whereas the USA and Italian+Ticino populations are characterized by extensive mixing and no substructure. Demographic analysis identifies a population expansion followed by a period of contraction pre-invasion. The arising scenario is compatible with previous reports and adds a time and demographic dimension to available reconstructions. Full results of the mitochondrial datasets and preliminary analyses of the nuclear snp dataset are presented.

Keywords: *Popillia japonica*, colonization, invasion, mitochondrial genome, snps

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## P185. Long-Lasting Insecticide-treated Nets: a new strategy to control the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae)

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*Popillia japonica* is a scarab beetle native to Japan that in the course of the last century has spread into the US, Canada, The Azores, Italy and Switzerland. Due to its potential harmfulness to agricultural crops this insect is currently included in the EU priority pest list. *Popillia japonica* is highly polyphagous, able to feed on more than 400 plant species, and highly mobile, capable of flying for several kilometers every day.
Recently, a control strategy has been developed based on the technology of Long-Lasting Insecticide-treated Nets (LLINs). *Attract-and-kill* devices (A&K)- consisting of a net impregnated with a pyrethroid and standard pheromone and kairomone dual-lures- have been tested and extensively employed in Italy to limit the spread of this pest. In the present study we show the results of lab and field experiments in which we evaluated the effectiveness of two different LLINs as well as the duration of effectiveness of an *attract-and-*

*kill* device when exposed to field conditions for the entire flight period. Moreover, we evaluated the best ratio of A&Ks per hectare. Results show that in laboratory trials LLINs are highly effective in killing the beetles (90%- 100% mortality); furthermore, we verified that the tested LLIN remain effective after the first month of exposure to field conditions, despite a performance reduction of about 30%. Finally, we observed that 1 A&K per hectare is effective in reducing the population of *P. japonica* in a given area.

**Keywords:** attract-and-kill, insecticidal net, insect trap, pest management

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### P186. Damage cost implications of the Japanese beetle (Popillia japonica) in Europe: a synthetic control group approach

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The Japanese beetle (Popillia japonica), an insect native to Japan, is an invasive species in Europe that was detected for the first time on continental Europe in 2014. Countries infested by the beetle include Portugal (Azores), Italy and Switzerland. It is a quarantine pest that has been nominated as a priority pest candidate in the European Union. The beetle is considered a major threat to agriculture as it can feed on over 300 different host plants, including economically important crops. In light of the pest's potential negative impact on agricultural production, this study employs a synthetic control group (SCG) approach to empirically analyze the effects of the Japanese beetle on the production of main host crops such as maize, blueberry and grapevine in the infested Italian regions Piemonte and Lombardy. The synthetic control method is particularly useful when it is impossible to observe the outcome of the treated unit (= regions infested by the beetle) in the absence of the intervention (= infestation), making it difficult to identify the causal effect. The statistical approach involves creating a synthetic control unit that closely matches the pre-infestation characteristics of the treated unit, and then comparing the post-infestation outcomes between the two units.

**Keywords:** yield damage, pest infestation, invasive species

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### P187. Producer willingness to pay for biological pest control under *popillia japonica* infestation across Europe

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The study uses the Japanese beetle (Popillia japonica) infestation in Europe as a case study in order to understand producer preferences for biological and synthetic pesticides in the context of pest infestation. We conduct a discrete choice experiment and analyze the importance of different product attributes like control efficacy, application rate as well as environmental and health impacts. The assessment compares grape wine farmers in Italy, France, Germany and Austria who have been affected by the invasive Japanese beetle to varying degrees. In our model, we control for the farm´s intensity of infestation and proximity to infestation zones to understand how infestation relates to willingness to pay. As we specify the monetary attribute as willingness to pay for research and development, we expect that those currently affected by the pest will be willing to pay more only for new methods with a high probability for high efficacy levels given the expected time lag until product availability. We also hypothesize that the respondents´ management practices, use and attitude towards biological pesticides may affect willingness to pay for biological pest control products. The results are expected to provide important insights for management strategies against the Japanese beetle as well as European policy targets like the EU´s Farm to Fork Strategy and its aim to reduce the risk and use of chemical pesticides in the EU by 50% by 2030.

**Keywords:** Japanese beetle, discrete choice experiment, pesticides, biocontrol, grapevine, pest infestation

# Session 11:

## Biodiversity and Conservation

## P309. Regimes of fire and its relationship with aboveground arthropod communities

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Mediterranean-type ecosystems are highly resilient to disturbances such as fires, herbivory pressure and droughts. However, the new climate scenario, together with changes in the land use, jeopardize its response capacity. Prescribed burnings and targeted grazing in firebreak areas are effective fire prevention strategies. Both tools are traditional pastoral practices with low implementation costs compared to other forest fire preventive techniques. Little is known about the combined effects of fire and herbivores on the diversity and function of Mediterranean mountain.
We tested the effects of coupled and decoupled regimes of pyric herbivory on the response of a spectrum of arthropod taxonomic groups, as well as on plant and microbial soil functional diversity. Some groups of arthropods suffered and some benefited from pyric herbivory. The functional biological diversity represented by arthropods requires that these organisms be considered as ecological management indicators.

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**Keywords:** pyric herbivory, wildfire, aboveground arthropods, soil functionality

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## P310. Flowering plant mixture in sown patches for conservation of wild pollinators in apple orchards

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The study examines a scheme to provide floral resources for insect pollinators in apple orchards potentially contributing to their presence inside the orchard. For this reason, a flowering plant mixture, (*Coriandrum sativum*, *Eruca sativa*, *Lathyrus sativus* landrace, *Vicia sativa*, *Vicia faba* landrace) was sown in patches inside apple orchards and compared to weed flora patches. Moreover, attraction of the *V. faba* landrace to bees was compared with a commercial variety. The pollinator taxa recorded on the sown and weed flora patches were honey bees, wild bees, syrphids, bee flies. The sown mixture attracted more diverse bee taxa (*Bombus, Hylaeus, Sphecodes, Nomada, Xylocopa* only on sown mixture*; Andrena, Anthophora, Eucera,* *Halictus, Lasioglossum*, Megachilidae on both; *Systropha* only on weed flora) and in greater numbers, but it did not influence pollinators' abundance on apple flowers. The most abundant pollinator of apple was *A. mellifera* but wild bee taxa common to the patches were also recorded (*Andrena*, *Anthophora, Bombus, Xylocopa, Lasioglossum*, Megachilidae). The *V. faba* landrace attracted more bee visits and genera (*Apis, Anthophora, Eucera*) compared to the commercial variety (only *Eucera*). The results support the benefit of groundcover management with patches of a suitable flowering mixture including landraces on conservation of pollinators in apple orchards.

Part of this work was supported by the Hellenic Entomological Society of Greece, scholarships for PhD students 2021-2022.

Keywords: flowering patches, pollinators, agro-ecosystems, floral resources, ecosystem services, landraces

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## P311. Assessing different anthropogenic pressures on the diurnal Lepidoptera biodiversity of Aspromonte National Park

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The study of insect biodiversity can be a valid tool for understanding the conservation status of specific environments. Diurnal Lepidoptera, because of their close relationship with angiosperms and their sensitivity to sudden environmental changes, are used to assess the impact of environmental disturbance on specific areas. This study reports the results of a monitoring activity aimed at using diurnal Lepidoptera as bioindicators to assess the conservation status of Calabrian populations. Both qualitative and quantitative composition of these pollinators were studied in different scenarios and environments located in the National Park of Aspromonte (Calabria, Italy). Ten transects, 500 m long, located in different environmental contexts (intensive and extensive grazing, cultivated field, burned area, natural environments) were surveyed twice a week from May to September and the different species of flying adults were annotated. The results obtained, although related to the first period of observation, highlighted the negative impact of human activity on the structure and composition of the lepidoptera studied. During the monitoring, 43 species belonging to 5 families were recorded. In the most degraded environments (grazed and burned areas), which had a low abundance of flowering plants, the biodiversity of butterfly species was lower than in environments where human pressure was limited or absent.

Keywords: pollinators, anthropic activity, biodiversity, butterfly

## P312. Species diversity of the leafhoppers and planthoppers, known or potential vectors for phytoplasma, in the Moldova Hills wine region

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The leafhoppers and planthoppers group (Auchenorrhyncha: Hemiptera) comprises several pest species of economic importance for grapevine through their ability to act as vectors for plant pathogens such as phytoplasma. The paper presents data on the leafhoppers and planthoppers present in vineyards in the Moldova Hills wine region in Eastern Romania, which are reported in the literature as confirmed or potential vectors of grapevine phytoplasma. The field survey was conducted in a network of seventy-seven vine plots from 2016 to 2022. Yellow sticky traps of 30x20cm where used for insect monitoring from June to October each year. A total of twenty six species confirmed or potential vectors for grapevine phytoplasmas were recorded. The most abundant species were *Neoaliturus fenestratus*, *Scaphoideus titanus*, *Fieberiela florii* and *Reptalus quinqecostatus* known to be associated with Stolbur and *Flavescence dorée* phytoplasmas in European grapevine. Two diversity indices Simpson-Gini and Shannon-Wiener were calculated to measure the diversity of sampled species, and the equitability derived from the Shannon index and the other from the Simson index were used to measure the species evenness.

**Keywords**: Auchenorrhyncha phytoplasma vector, species diversity, Romanian grapevine

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## P313. Abandonment and intensification: How changes in grassland management affect pollinator communities. Lessons from LIFE PollinAction

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Extensively managed grassland ecosystems are a biodiversity hotspot for plants and pollinators. However, in Europe, socio-economic changes have led to a sharp decline in extensively managed grasslands, which have undergone two major changes: the introduction of large-scale intensive agricultural practises on the most productive grasslands and the abandonment of marginal grasslands. Understanding how changes in grassland management affect pollinator communities is increasingly important for their conservation and the provision of pollination service. In this study, we compared extensively managed lowland grasslands (not fertilised and mown twice a year), intensively managed grasslands (fertilised and mown three times a year) and abandoned grasslands (not fertilised and mown once every two years) in terms of spatial attributes (i.e., area of the grasslands and the length of woody edges along their perimeter), plant community attributes (i.e., number of flowering entomophilous species and number of flowers) and pollinator community attributes (i.e., richness of pollinator orders and probability of presence of individual orders). Intensively managed grasslands were characterised by larger areas and more scattered woody edges, while abandoned grassland had smaller areas than extensively managed grasslands; moreover, richness of flowering plant species and the number of flowers were significantly higher in extensively managed grasslands. The richness of pollinator orders was significantly higher in extensively managed and abandoned grasslands than in intensively managed grasslands. Specifically, the probability of Coleoptera and Lepidoptera occurrence was lower than expected on intensively managed grasslands, underlining that among the changes in extensive grasslands, agricultural intensification had the worst consequences for pollinator communities.

**Keywords:** agricultural intensification, grassland management, plant community, pollinator community, biodiversity, pollination

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## P314. A bibliographic review of the ants of Greece and Cyprus: drawing a historical and biogeographic picture

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Ants currently hold globally more than 14000 species. Recent research indicates that there are various ant fauna hotspots around the world, one of which is the Mediterranean area. In light of several recent projects studying the ant faunas of both Greece and Cyprus, the authors performed a thorough review of all past published works mentioning ant species from the two countries. A data matrix holding details of the ant species name that was originally used, the publication details as well as any information for the metadata for each species (i.e. collection locality, time, method, collector, identifier, etc.) were also recorded. In numerous cases, information was extrapolated from relative records of other publications indicating the same collecting events for other taxa. Locality details were georeferenced when possible and relating higher administrative information populated the data matrix. Altitudinal data was also approximated using Google Earth Pro. The final dataset provides information on the occurrence, biogeography, seasonal phenology and even collection efforts history of ants for the two countries. We find periods of intense efforts but also long periods of fragmentary collection attempts. Moreover, there is a shift of collection points from the south to the north of Greece with no apparent changes for island locations through time. Recent systematic efforts from scientists visiting Greece and Cyprus as well as a more thorough taxonomic work with new tools, significantly increased the number of species the two countries currently hold.

**Keywords:** ants, references, occurrence data, biogeography, history

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## P315. The two BeeNet monitoring networks for the evaluation of Italian agroecosystems

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The Beenet project, funded by the Italian National Rural Network, aims to monitor the health of the Italian agroecosystem through two monitoring networks: the honeybee network and the wild bee biodiversity network. The first is composed by over 350 beehive stations, distributed across the whole national territory. The monitoring includes the state of health of the colonies both in terms of growth and development during the year, and in relation to the presence of pathogens and the contamination of bee bread with pesticide residues. The second network evaluates the number and diversity of wild bees along 24 transects located in 11 regions and positioned in two different agro-environments, one intensively cultivated and one semi-natural.

Concerning the pathological data, the analyses highlighted the ubiquitous presence in Italy of *Nosema ceranae* and the viruses DWV, CBPV and ABPV. Among trypanosomatids, *Lotmaria passim* was found only sporadically in bee samples, while *Crithidia mellificae* and *Crithidia bombi* have never been found.

The results of bee bread analyses show that two-thirds of the samples contain one or more active ingredients, many of them contain between two and five and some of them over ten residues. In addition, almost a third of the positive samples contain active compounds highly toxic to bees and only 20% contain only active ingredients with low toxicity for bees.

The results of the wild bee biodiversity network highlight that semi-natural agroecosystems have a higher biodiversity (both in terms of species richness and individual abundance) of wild bees, compared to intensive agroecosystem.

**Keywords**: honeybees, wild bees, monitoring, biodiversity, agriculture

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## P316. Assessment of biological soil quality in organic farming systems in Southern Italy (Caserta, Campania)

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The Soil Biological Quality Index based on the microarthropod community (QBS-ar) is a qualitative index exploiting the presence/absence of different edaphic groups to assess soil biological quality and the impact of different agronomic soil management on soil fertility. This study compared different organic systems (vineyard, hazelnut grove, non-productive chestnut grove) in the Caserta area during the spring period. The systems had different soil management, involving specific tillage. For every grove 6 replicates (each one

including 3 sub-replicates) were provided. The surveys covered the spring season involving two sampling periods: the first in mid-spring (April 20-May 6) and the second in late spring (May 25-June 10).
The results showed significant differences according to both sampling period ($P<0.05$) and arboretum type ($P<0.0001$), but not due to the interaction between the two factors. QBS-ar values were significantly lower in the second sampling period than in the previous one, showing that higher temperatures tend to reduce soil moisture and consequently the animal component. Among groves, the largest differences were found between the forest-chestnut grove (average QBS-ar: 219.7 ± 16.4) and the two productive tree systems (QBS-ars ranging from 151.2 - 86.7). Furthermore, the QBS-ar of the vineyard's grassed inter-rows was significantly higher (T1 = 151.2 ± 3.9; T2 = 133.3 ± 27.7) than that of the ungrassed inter-rows (T1 = 131.8 ± 3.0; T2 = 86.7 ± 15.6) and the hazelnut grove (T1: 120.6 ± 7.96; T2: 110.7 ± 10.2), indicating that soil tillage and frequent mechanical activity can negatively affect soil biological fertility.

**Keywords:** biodiversity, soil fertility, arthropods, QBS-ar, organic orchards

## P317. Arthropods as functional biodiversity in different sugarcane agrosystems in Reunion Island: study methods and first results

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Key arthropods present in sugarcane crops were collected and determined in Reunion Island, a French overseas department, using 2 type of traps: at the soil and the plant level on 32 plots across different agricultural landscape and altitude, which give 64 traps to observe and analyze for a period of almost 12 months of sugarcane crop, from the plantation of cutting setts or ratoons to the harvest. These plots are located in the region of Petite Ile which gathers different types of agricultural landscapes, from the lowlands of the region to the highlands at a maximum of 1000 m altitude. The project and the methodology used are presented and described: type of traps, collection of individuals at regular intervals, replacement and refilling of traps, identification of different groups of arthropods present in the containers, etc. All this manipulation in field and laboratory is time consuming and needs the help of taxonomists. Apart from morphological identifications of samples collected whenever is possible, the function of each group was specified as pests, parasitoids, predators, and soil insects. A thorough database is currently under construction to be able to further study the impact of agricultural practices, surrounding landscape, and altitude on the arthropod diversity and abundance in different sugarcane agrosystems.

**Keywords:** sugarcane pests, natural enemies, functional biodiversity, trapping methods

## P318. Insect decline – Evaluation of potential drivers of a complex phenomenon

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Insect decline is a complex phenomenon, resulting from a range of causal factors, which act at different spatial and temporal scales, with different impacts on different insect groups. There is a variety of data sets which describe changes in insect populations, however, a broad data basis providing systematic insights into factors causing declines is lacking. Thereby, correlation analyses can help to identify potential factors associated with declines. Here, we present a detailed analysis of the development of potential drivers of insect decline over the last decades in Germany where a decline of insect biomass has been measured in previous studies, and of changes in the states of these potential drivers.

Factors investigated included landscape structure, land use change, grassland management, cropping practices, pesticide use, climate, and light pollution. Data about the developments of these parameters over the last decades were obtained from statistical data such as cropping statistics and market research, and long-term sequences of satellite imagery. The results of the correlation analysis suggest that habitat loss caused by changes in land management practices and land cover, as well as intensification of land use are factors frequently correlating with declining tendencies. This is to our knowledge one of the first studies where all of these factors were systematically retrospectively investigated. With this extensive dataset we will be able to correlate the often-mentioned habitat drivers (landscape change, monotonicity of agricultural structures, loss of fragmented structures) of insect decline combined with other potential stressors (pesticides, urbanization, night light).

Keywords: Landscape transformation, habitat loss, pesticides, climate change

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## P319. Construction of DNA taxonomy references of mayflies inhabiting Japan

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The development of high-throughput parallel DNA sequencing methodology (next-generation sequencing) has greatly advanced the DNA-based analyses to characterize bulk biota samples (unfractionated specimens without morphological identification), and to define the species present in environmental DNA. For identifying species based on DNA sequence comparisons, it is mandatory that high-quality reference data (DNA data obtained from precisely identified specimens) are available. Every effort, such as those by the projects of the international barcode of life promoted by iBOL (https://ibol.org/), is made to expand the reference databases. Our research group has been making continuous efforts to collect aquatic insects (especially focusing on Ephemeropteran species) from the central Honshu Island of Japan, and to construct a database that consists of DNA data, digital images of specimens showing morphological characters, and sampling information. Physical specimens (source materials for DNA extraction) are securely stored in our laboratory or deposited in museums. Here we report the current progress of our reference collection of the aquatic macroinvertebrates in Japan, and the results of metabarcoding based on the DNA taxonomy data.

Our collections are available on the web site J-amir (http://www.b.s.osakafu-u.ac.jp/~mkato/J-amir_home.htm)

**Keywords:** DNA barcode, Ephemeroptera, molecular taxonomy, reference collection

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## P320. LIFE SPAN Saproxylic Habitat Network: planning and management for European forests (LIFE19 NAT/IT/000104)

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Traditional forest management, focused on the maximization of continuous wood production, has brought the reduction of structural heterogeneity, deadwood volume and microhabitat availability, and consequently flora and fauna diversity. This is even more true for saproxylic biodiversity, i.e. that represented by species that, at least in one stage of their life cycle, are linked to decaying or dead wood or to other saproxylic organisms. LIFE SPAN project's main aim is to demonstrate that it is possible to combine traditional forest management practices, with the creation of a biodiversity protection network, without compromising the management's economy. Through an innovative forest planning and management approach, always attentive to the economic sustainability of the proposed interventions, management solutions aimed at the conservation of habitats and species of Community interest linked to dead wood will be implemented and monitored. The main tool of the project is the creation of Saproxylic Habitat Sites (SHS) in the productive forest matrix. Intervention will be carried out to increase the presence of deadwood, microhabitat, structural heterogeneity and gaps in 25 SHS in Italy (Cansiglio Orientale Forest) and in 18 SHS in Germany (University Forest Sailershausen). Saproxylic vertebrate (e.g. woodpeckers and bats) and invertebrate (e.g. beetles and true bugs) species will be monitored. The interventions are expected to trigger a rapid evolution of processes beneficial for biodiversity, which will naturally promote the increase in ecosystem services.

**Keywords:** insects, biodiversity, dead wood

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## P321. The atlas of mitochondrial genetic diversity for Western Palaearctic butterflies

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Butterflies are a fascinating group of insects and their study has become increasingly important in the context of global biodiversity conservation efforts. This group is the most extensively studied with mtDNA sequencing, which has proven particularly useful for species identification (DNA barcoding), detection of cryptic species, reconstruction of phylogeographic phenomena, and evaluation of matriline dispersal. The atlas of mitochondrial genetic diversity provides a full set of resources for the study of Western Palaearctic butterflies: (1) an updated checklist comprising 555 species; (2) a curated dataset of ~32,000 COI sequences for 534 species; (3) seven indexes of intraspecific genetic variation (IGV); and (4) an atlas with species-level maps illustrating their phylogeographic patterns. Version 2.0 of the atlas of mitochondrial variation with recently updated taxonomy and sequence data is available on GitHub (www.github.com/leondap/iodatabase), together with the IOdatabase project as an R package.
We hope that this resource will enhance the research on butterfly diversity and evolution in the Western Palearctic.

**Keywords:** phylogeography, barcoding, Papilionoidea

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## P322. Farmers' opinions and perceptions on pollination services: Evidence from a multi-country study in the Mediterranean Region

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Pollination constitutes an ecosystem service of particular social importance and its contribution to sustainable agriculture has been acknowledged in the literature. This study is designed to investigate farmers' knowledge, opinions and expectations with regards to pollination services, taking into account the declining rate of insect pollinators in many European countries, which is considered the main limiting factor for the development and expansion of such services. To meet this objective, an online survey was conducted with the use of a structured questionnaire, which was addressed to farmers from 13 countries of the Mediterranean basin (France, Greece, Algeria, Croatia, Slovenia, Lebanon, Cyprus, Portugal, Jordan, Morocco, Italy, Malta and Turkey). The questionnaire included specific Likert-scale questions for attitudes, motivations and perceptions of farmers regarding aspects related to pollination services, such as climate change, proposed practices, cooperation with beekeepers, training-information-consultancy, governmental financial aid, pollinators efficiency and crop protection. The survey also aimed at recording famers' personal profiles – in combination with basic characteristics of their farms – and demographic attributes. For the purpose of the main analysis, the collected data were analyzed by means of descriptive statistics methods. The results demonstrate that farmers consider pollination services important for their crop yields while they also pinpoint the insufficient training and advisory support as well as the lack of subsidized pollination programs in the majority of the countries. These findings provide useful suggestions for the integration of policy strategies towards the enhancement of pollination services across the Mediterranean Region, considering farmers' needs and particular characteristics.

**Keywords:** questionnaire survey, pollination services, climate change, insect pollinators

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## P323. Conservation of the saproxylic beetles in Romanian Carpathians (LIFE ROsalia)

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Romanian Carpathians are considered a biodiversity hotspot in Europe, with large forested areas, including old-growth forests. Past forestry practices, such as selective logging resulting in high forest grading and removal of ancient or decaying trees. These practices led to forest habitats with few veteran trees, and a small amount of deadwood, which protected saproxylic beetles rely on for completing their complex life cycles. Moreover, saproxylic species are considered pests under traditional forestry practices, as they reduce the value of timber. As such, Romanian forestry practices have actively sought to reduce the amount of

deadwood in an effort to decrease the presence of saproxylic species, thus effectively isolating stands with a high diversity of saproxylic beetles. In 2020, European Commission LIFE Programme financed the project LIFE19 NAT/RO/000023 LIFE ROsalia Conservation of saproxylic beetles in the Carpathians. The project aims to stop and reverse the loss of protected saproxylic beetles (*Rosalia alpina Osmoderma eremita*, *Cerambyx cerdo*, *Morimus funereus*, and *Lucanus cervus*) in the Carpathians by demonstrating conservation actions for increasing the connectivity of favorable habitats in Natura 2000 site ROSCI0208 Putna-Vrancea (Eastern Carpathians, Vrancea County, Romania), and transferring and replicating best management practices in other Romanian Natura 2000 sites (e.g. creating a next generation of veteran trees in areas lacking suitable habitats and providing artificial and natural deadwood habitats to facilitate reproduction and dispersal). LIFE ROsalia will be implemented until 2025 by the EPA Vrancea, University of Bucharest, Putna-Vrancea Natural Park Administration, and Association for Biodiversity Conservation (see www.liferosalia.ro).

Keywords: saproxylic beetles, habitats directive, EU Biodiversity strategy for 2030, Natura 2000, LIFE Nature

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## P324. Shrubs as a reservoir for natural enemies in Mediterranean agroecosystems

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The establishment of green infrastructures to enhance functional biodiversity is an increasingly common practice in agriculture. Their purpose is to promote ecosystem services by acting as a reservoir of arthropods useful for biological control. This work aimed at assessing the abundance and diversity of the main groups of natural enemies on Mediterranean shrubs. For this purpose, 37 plant species were sampled between 2019 and 2021 at 7 different locations in southern Spain. At each location, two samplings were carried out between April and June and two between October and February to cover the maximum blooming period. In each sampling, a total of 10 branches from different specimens were randomly selected for each plant species. Shrubs were beaten with sticks and arthropods were collected using entomological funnels (diameter 30 cm). The main predatory arthropods were Araneae (42.52%), Hemiptera (37.64%), Thysanoptera (11.90%), Coleoptera (5.01%) and Neuroptera (2.93%). The dominant spider families, Theridiidae, Philodromidae, Araneidae and Salticidae, were abundant on *Helichrysum stoechas*, *Asparagus* spp., *Lavandula dentata*, *Thymelaea hirsuta* and *Tetraclinis articulata*. Hemipterans were mainly represented by the families Anthocoridae and Miridae, and they were abundant on *Ononis natrix, T. hirsuta*, *Dittrichia viscosa* and *Ballota hirsuta*. Predatory thrips were represented almost exclusively by the family *Aeolothripidae*, abundant on *Helianthemum* spp. and *Anthyllis cytisoides*. Coleoptera, were represented almost exclusively by family Coccinellidae, and they were abundant on *Prunus dulcis, T. hirsuta, Pistacia lentiscus and Punica granatum*. Lastly, Neuropterans were represented by the family Chrysopidae and mainly found on *P. dulcis*, *P. granatum* or *P. lentiscus*.

Keywords: predators, biological pest control, ecosystem services, green structures, biodiversity

## P325. Wild bees and other pollinators of my place: a drawing competition as a learning tool

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LIFE4POLLINATORS is an EU LIFE project aiming at improving pollinator conservation through progressive changes in human behavior and practices across the Mediterranean. A major goal of the project is to improve information and knowledge on native wild pollinators, i.e. insects, and their partners, the entomophilous plants. The project utilizes a series of educational tools to promote awareness to different key stakeholders, albeit at a scale of diffusion limited to particular events and small group of people. To enhance promotion and increase awareness, we set up a drawing competition for school children at a panhellenic scale, an action concerted by the Greek Ministry of Education and engaging schoolteachers in the process. Suggested by the title (*Wild bees and other pollinators of my place*) and following a few instructions, children were asked to depict the plant–pollinator relationships they encounter in nature. The effort targeted to four age categories (two for elementary, two for high school) while a fifth one regarded special schools. The response was unprecedented for such a competition, with 3100 drawings received from all over the country until January 2023. The drawings, which are now under assessment by eight assessors representing different disciplines (ecology, education, museology, photography, painting), constitute an extremely interesting material from the artistic, educational, psychological and ecological viewpoints. Evidently, the entire effort has ended up with conservation lessons learned by the participated children, which need be further disseminated. The poster will comprise the results of the analysis of the drawings received from all over the country.

**Keywords:** public awareness, education about pollinators, LIFE4POLLINATORS project

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## P326. Transforming nature into wonderland: creating an artificial Mediterranean flower–bee garden as a learning tool

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The project LIFE4POLLINATORS aims at improving pollinator conservation through progressive changes in human behavior and practices across the Mediterranean. To this end, we created a series of educational tools in order to promote awareness aiming at different key stakeholders. Effort was made to focus on children, especially schoolchildren, with the scope to eradicate the fear for bees and other pollinating insects that are considered as 'disgusting' and 'dreadful'.
To make children more aware about the importance of insects as pollinators and highlight the aspects of insect mutualistic relationships with flowering plants, we created an artificial garden encompassing handmade models of flowering plants and their major pollinators observed in the Aegean region and the Mediterranean in general. All model creations are oversized nature mimics made of recycled material, mainly paper pulp. The garden assembles 13 plant species (21 plants) bearing flowers of diverse pollination syndromes; and 13 insect species of different pollination guilds (bees, hoverflies, beetles, butterflies, and hawkmoths) that are associated with the modelled plant species. All models are x5 their natural stature, so they can stimulate children explore the insect and flower body parts, at the same time make them feel the grandeur of the pollination relationship. The assemblage, accompanied by a scenario, and assorted with comic sketches and drawings of the natural system, is going to be soon installed in a Museum for pedagogical purposes for children of different age. The poster will depict parts of the garden and present the idea and educational aims of the project.

Keywords: plant–pollinator relationships, mutualisms, education, LIFE4POLLINATORS project

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## P327. Differences between co-occurrence networks of indigenous and exotic arthropods in the Azorean archipelago

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Island biotas are under imminent threat from introduced species and having an insight into the processes by which these species affect indigenous communities is of high conservation importance. The arthropod fauna in the native forests of the Azores is well-studied and ongoing long-term monitoring allows the assessment of the impact of exotics. Here, using an archipelago-wide, long-term dataset, we generated co-occurrence networks between arthropod species (including insects and spiders) and investigated the topological differences in these networks between indigenous and exotic species. Network properties, such as connectance and centralities, pinpointed differences between indigenous and exotics, with most exotic species being little connected, and thus, exotic networks having a large proportion of unconnected species. The resulting decreased connectance and the increased modularity with the increase of the proportions of exotics in the networks suggest that most exotics have a too low prevalence to show associations with other species, and only a few dominants drive co-occurrences. Moreover, the proportion of negative links, as indicators of competition, did not increase with the increase of exotics in the habitats. This suggests that exotics provided distinct functional roles in native forest communities. However, when networks consisting of only indigenous species were investigated, connectance decreased and closeness increased with the increase of exotics, indicating processes of network degradation. Although our study provides evidence for the impact of introduced arthropods, this impact seems to be obscure, suggesting that introduced species blend into natural communities by occupying empty niches when they colonise native forest remnants.

Keywords: community change, ecological network, biological invasions, island ecology

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## P328. Interaction between introduced psyllids and native and exotic ants in eucalyptus plantations

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Commercial interest in eucalyptus wood has driven massive plantations outside its native distribution. With the tree, its natural pathogens and parasitoids were progressively introduced. We hypothesised that ants exploit the resources offered by the introduced psyllids (Homoptera) parasitising the eucalyptus as they do with aphids. We expected this interaction to be higher for introduced ants than for the native ants due to their adaptation to new resources. We expected plantations located close to human settlements to show lower native ant biodiversity but a higher presence of introduced ants than those located distanced due to the modification of their surroundings and the higher likelihood to colonise modified areas by invasive species. We studied the presence of ants on eucalyptus trees and the ant diversity on the ground in eight eucalyptus plantations (four close and four far from settlements). We analysed at the laboratory the attraction to psyllids of two native and one invasive ant species. We found native ant species over psyllids clusters in the sprouts of the eucalyptus. A trend suggested a higher use of eucalyptus resources in remote plantations. We did not find differences in the ant diversity of plantations according to their distance from the human settlements. Invasive ants were absent in the sampled areas, but in the laboratory experiment we found that this species interacted more with psyllids than these others observed over eucalyptus on the field. Our results suggest that the use of honeydew of introduced psyllids by ants depends on local adaptation.

Keywords: *Ctenarytaina eucaliptii*, *Cenarytaina spatulata*, culture, *Glycaspis brimblecombei*, *Gonipterus scutellatus*, *Mycosphaerella* spp

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## P329. Landscape effects on whiteflies explained by local trophic interactions and the consequences of land-use landcover

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Despite consensus on landscape processes moderating biodiversity patterns and services, key questions remain unresolved and challenges of practice persist, which limits effective biodiversity conservation. Addressing current challenges requires knowledge about the distribution of diversity across the landscape (community structure and assembly), and associated distribution of services and species interactions resulting or partitioned within different land-use contexts at multiple scales. In the state of Georgia, USA, a diversity of crops grow year round within a wetland and pine agroforestry matrix, and a major row crop is cotton at over 400 thousand hectares grown. This understudied diverse landscape creates an ideal context for studying both landscape and within field habitat effects on predatory arthropod communities and associated interactions with common pests and alternative prey insects. Here we will present a combined set of data for landscape level crop diversity effects and within field habitat management effects of cover crops on communities and trophic interactions (determined by molecular gut content analysis). Whitefly abundance was negatively correlated with crop diversity, with a potential for alternative prey, aphids, to alter the interactions between natural enemies and whiteflies. At the local scale, cotton planted into winter cover crops reshaped early season natural enemy communities and associated interactions. Combined, our results suggest insecticide use a large scales alters whitefly populations, and at the local scale both land-use patterns around fields and alterative prey moderate natural enemy effects on whitefly biocontrol.

**Keywords:** MGCA, molecular trophic interactions, natural enemies, predator-prey interactions

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### P330. Bringing insects to new audiences

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The Royal Entomological Society has a strategic priority to increase public understanding of insect science, as part of its vision to ‘enrich the world with insect science’. In this talk we will share some of our innovative approach to outreach activities from 2023 that have increased this engagement, including events for British Science Week, RHS Chelsea Flower Show with designer Tom Massey, Insect Week 2023, and Big Bang Science Fair. Many of these are run in collaboration with partners such as specialist insect societies as well as those working with wildlife and natural history. In this talk we will share some of the successes of these campaigns and events, and how we are attracting support from other institutions. We will describe the lessons learned from these activities, that are helping us plan future opportunities to increase public engagement, and increase understanding of the sector.

**Keywords:** outreach; science communication; public engagement

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## P331. Effect of field margin management on diversity and activity density of ground-dwelling spiders in orange orchards

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Spiders are generalist predators of various insect pests of agricultural crops. In the present work, the diversity and activity density of ground-dwelling spiders were studied in seven orange orchards (Argolis, Greece) with different field margin management. Spider samplings were conducted using pitfall traps along the field margins in three crop years (2020, 2021, and 2022). In 2020 no intervention was made in the field margins. In 2021, aromatic plants were established as hedgerows in one of the field margins in five orchards (rosemary x 2 orchards, savory, oregano, sage), whereas the field margins of two other orchards were used as control, i.e., bare soil and weed vegetation. A total of 67 species of ground spiders belonging to 17 families were collected. In all cases, the families with higher activity density were Lycosidae, Gnaphosidae, and Theridiidae. Rosemary and savory hedgerows showed the highest diversity and activity density of spiders, respectively. Their potential contribution to the biological control of citrus pests is discussed.
Financial support has been provided by PRIMA (grant number 1812/PLANT-B), a programme supported by the European Union

**Keywords:** spiders, aromatic plants, weeds, citrus

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## P332. Can flowering plants support insect-based ecosystem services in Korla fragrant pear orchards?

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Korla fragrant pear, *Pyrus sinkiangensis,* is a premium species of pear with high market value. Cultivation of this pear in Xinjiang, China, is characterized by low pollination success and high pesticide input. There is a need to strengthen the services of pollination and insect biological control, and it is believed that diversification of the vegetation of the orchard floor may help to support a more abundant and diverse insect community providing ecological benefits to growers. Here we study which native and non-native flowering plant species have potential as cover plant in orchards to enhance both pollinators and pest natural enemies. We assessed which insect species utilize 39 different plant species in a replicated field experiment in Korla, Xinjiang, in 2020 and 2021. Insects were quantified using sweepnetting and direct counts on plants. Densities of ladybeetle larvae were highest on plant species carrying high aphid population densities, e.g. cotton, *Gossypium hirsutum.* During flowering, the highest densities of ladybeetle adults were observed on dill, *Anethum graveolens*, fennel, *Foeniculum vulgare,* and rucola, *Eruca sativa*, due to their beetle-attractive flower resources. These three plant species also had high densities of hoverflies, honeybees and wild bees. Plant-insect networks differed greatly before, during and after flowering. Further work is needed to evaluate the compatibility of planting these service plants in orchards with farmers' practices and needs.

Keywords: beneficial plants selection, natural enemies, pollinators, plant-insect associations, flowering time

## P333. Thrips on red raspberry in South Norway

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Thrips receive an increasing amount of attention in Scandinavian raspberry production, reflecting more frequent observations of both thrips and their suspected damage. In general, thrips damage can result in distorted and scarred fruits. Furthermore, thrips are also known as vectors of plant viruses. Preliminary results suggested that *Thrips fuscipennis* Haliday, a thrips species found on raspberry, could potentially transmit the pollen-borne raspberry bushy dwarf virus (RBDV). However, which thrips species occurring in raspberry was not well documented. Therefore, this study aimed to identify thrips present in flowers of cultivated raspberry, *Rubus idaeus* L. in South Norway. Adult thrips were collected from nine sampling sites in the counties Vestland and Viken during June – July 2022. Most of the samples were taken in 'Glen Ample', as it is the dominating cv in Norway. Thrips samples were stored in 70% ethanol and mounted using Hoyer's solution. The specimens were identified based on their morphology. Five species of thrips were found, *T. fuscipennis*, *Thrips major* Uzel*, Thrips flavus* Schrank*, Thrips vulgatissimus* Haliday and *Thrips brevicornis* Priesner. Most of the identified specimens were *T. flavus*, followed by *T. fuscipennis* and *T. vulgatissimus*. Among them, only *T. fuscipennis* has previously been reported in cultivated raspberry, but all five species are associated with the flowers of many plants. *Thrips tabaci* Lindeman, which is present in southern Norway and previously found in raspberry, was not found. A further study of the potential of these thrips as direct pests or vectors of raspberry viruses should be carried out.

Acknowledgements
The research leading to these results has received funding from the EEA / Norway Grants and the Technology Agency of the Czech Republic within the KAPPA Programme (TO01000295).

Keywords: raspberry virus, *Rubus idaeus*, species composition, Thysanoptera, virus vector

## P334. Improving knowledge and conservation of wild bees populations in the regional natural parks of the French Nouvelle-Aquitaine region

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Despite their large contribution to the pollination service, the current situation of wild bee populations remains poorly known on the French territory, primarily due to the lack of studies. Indeed, more than 60% of the French wild bee species are data-deficient species, making it difficult to set up conservation actions. In view of this statement, French government implemented the Governmental Action Plan "Pollinisateurs", itself declined at a regional level in the Nouvelle-Aquitaine region. In this context, the five regional natural parks of this region have decided to work together, in partnership with the INRAE BIOGECO research laboratory and two associations (OPIE and ARB NA), to develop a plan of action aimed at improving wild bee conservation, the European LIFE project "Wild Bees" (LIFE19 NAT/FR/000975). Capitalizing on the richness and diversity of habitats within these vast territories, one of the main objectives is enhancing knowledge on the diversity and ecology of wild bees species on a broad range of open grassland habitats of high conservation value (lowland hay meadows, heather moorlands, calcareous grasslands …). Furthermore, linked with the restoration of a favorable dense habitats network through ecological engineering work on these open grasslands, the project aims the assessment of their impact on wild bees populations. Thus, since 2022, wild bees have been monitored in 75 open grassland sites. The studied sites as well as the first data will be presented.

**Keywords**: wild bees, conservation, knowledge, regional natural parks, "wild bees" LIFE project, Nouvelle-Aquitaine

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## P335. Conservation of *Parnassius apollo* in Poland, Czech Republic and Austria (Project LIFE APOLLO2020)

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Actual data clearly indicate negative trends in the number of insects in Europe. Over 30 % of taxa show regression and *Parnassius apollo* is among the most affected species. An important reason for the species decline is habitat fragmentation and loss, mainly due to the changes in land use, invasions of alien plant species, and the expansion of urbanized areas and roads. *P. apollo* acts as an umbrella species for protection of biodiversity on the ecosystem level and habitats' mosaic. Conservation attempts for *P. apollo* reintroduction have been carried out in project countries since the beginning of 20th century, but now some of these populations are on the verge of extinction. The main goal of the project is to (1) restore or strengthen the populations in three functional areas: Sudetes, White Carpathians, and Austrian Alps in the Continental bioregion, (2) initiate the process of creating metapopulations in these areas, by creating at

least 40 "stepping-stone sites" to secure species migration, (3) create three additional breeding farms in all three functional areas to anchor the population in natural conditions, as well and (4) raise awareness and social commitment to the conservation and maintenance of species' habitats by establishing 50 “Apollo Gardens” with involvement groups of locals and volunteers and organising youth education workshops. The project is therefore the first, test and pilot action for further population development in the bioregion.

**Keywords:** *Parnassius apollo*, biodiversity, conservation, repatriation

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## P378. Mesofauna diversity and soil carbon sequestration: Case of study of Inga-Cocoa agroforestry system in the Ecuadorian Amazon

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Plants diversity is a well-known driver of invertebrate communities. This study was conducted to establish if Inga-Cocoa agroforestry system host a greater diversity of mesofauna and enhances the carbon capture in soil. The landscape included in this research were inga-cocoa agroforestry, cocoa monoculture, and amazon rainforest as an undisturbed control. Samples consisted of 5 x 10 x 20 cm. soil monoliths, were searched for 40 minutes for mesofauna, founded organisms were labelled and preserved in isopropyl alcohol for later identification. Finally, the soil samples were analyzed in the laboratory. Diversity was measured using Shannon, Simpson, and richness index; and, soil organic carbon content was measured using Walkley and Black method. Identified 17 taxonomic orders were found, being hymenoptera and crassiclitelata the most common. As for the biodiversity indices studied and soil carbon content, no statistically significant differences were found, the means between the three landscapes were: Richness index=1.84±0.23, Shannon index=0.47±0.08, Simpson index=0.94±0.05, Organic carbon content=7.72±0.89%, Organic matter content=9.13±0.47%. Although we did not find statistically significant differences, clear trends in favor of the inga-cacao system can be observed, this allows us to affirm that further investigation of mesofauna composition and arthropod diversity, together with the carbon content of the soils, is worthwhile. Our research was a preliminary approach to the scientific evaluation of the association of these two tropical species, from an ecosystem service delivery point of view.

**Keywords:** agroforestry, biodiversity, arthropods

# Session 12:

## Social Insects and Apidology

## P336. All altitudinal zones are important for the pollination systems of Mount Olympus

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Global change is expected to severely impact mountain systems, especially within the Mediterranean. In this context, pollinator diversity and services are key ecosystem elements to be affected. To address current knowledge gaps and to meet future conservational needs, we investigated the insect pollinators and their interaction networks with plants along the altitudinal gradient of Mt. Olympus, a legendary landmark and biodiversity hotspot in Greece. Across three years (2013, 2014, 2016), we explored ten sites on the north-eastern slope (327-2596 m a.s.l.), and we analysed the response of α- and β-diversity of pollinators, flowering plants, and interaction networks along the altitudinal gradient. We found 333 insect pollinator species, among them species new for Greece (*Bombus quadricolor, Melitta tomentosa*), including 22 bumblebee taxa, rendering Mt. Olympus one of the most pollinator-diverse mountains in the Mediterranean. Alpha-diversity of all pollinators, and specifically of bees, non-bumblebee bees, bee flies, and butterflies showed linear declines with altitude; in contrast, hoverflies and bumblebees showed unimodal patterns, peaking at high altitudes, probably owing to their tolerance of high-altitude conditions. Beta-diversity of all pollinators, and specifically of hoverflies, bees, bumblebees, and butterflies showed linear increases with altitude. The high dissimilarity and uniqueness of pollination networks along the altitude, which is probably a result of high biodiversity and endemism of Mt. Olympus, is driven by species turnover and the formation of new interactions between new partners. Our findings highlight the importance of all altitudinal zones for the diversity of plant–pollinator communities, conveying important historical, ecological, and conservational implications.

**Keyword:** α-diversity, β-diversity, altitudinal gradient, Mount Olympus, pollinator guilds, pollination network

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## P337. Composition and diversity of bee (*Hymenoptera: Anthophila*) species found in flowering apple orchards in Latvia

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Latvia is a country in a temperate climate zone by the Baltic Sea. It is located between 55°40'N and 58°05'N; the maximum altitude is 312 m a.s.l.; ~52% of the territory is covered by forests, while ~30% is agricultural land. The composition of bee species was studied in eight apple orchards during their flowering period in 2020–2022. In the study, we used three-color pan traps (white, blue, yellow) and Malaise traps, which were kept in the orchards for six sunny days during apple tree flowering. Captured bees were removed from the traps in the evening of each day. In total, we have observed 92 bee species: 30 Andrenidae, 26 Apidae, 31 Halictidae and 5 Megachilidae species. *Andrena haemorrhoa* (proportion 21.45%) and *Apis mellifera* (18.79%) were the most common species. Another 15 species can be considered relatively frequent, the proportion of which was in the range of 1–6%. They were *Andrena nigroaenea*, *A. scotica*, *A. helvola*, *A. minutula*, *A. dorsata*, *A. subopaca*, *Bombus lucorum*, *B. hortorum*, *Lasioglossum pauxillum*, *L. morio*, *L. calceatum*, *L. rufitarse*, *Halictus tumulorum* and *Osmia bicolor*. The diversity of bee species was significantly fluctuating both by day within each apple orchard and when comparing different apple orchards. For example, in 2022, in one apple orchard, the Hill number ($^1D$) fluctuated between 1.00 and 10.32 in a six-day period. Among different apple orchards, the median values of this parameter varied as follows: in 2020, between 2.50 and 8.00; in 2021, between 3.02 and 7.88; in 2022, between 2.34 and 12.41.

Keywords: *Andrena haemorrhoa*, *Apis mellifera*, Hill number

## P338. The status of pollination services in the North China Plain, a case study in Quzhou

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Insects are globally in decline due to habitat loss and the usage of pesticides. Nuts, vegetables, and fruits are key components of healthy human diets, but growing these crops requires insect pollination. Here we assess whether insect-based pollination services are sufficient in Quzhou county in the North China Plain (NCP), a region with intensive agricultural land use and high pesticide use. Measurements were made at 24 sites across the county. At each site, we monitored insect abundance with pan traps and measured flower visitation and seed set in potted plants of buckwheat, *Fagopyrum esculentum*. Measurements were spread out over three measurement campaigns, one in the autumn of 2021 and two more in the spring and summer of 2022. Most flower visits were made by the syrphid flies *Eristalinus aeneus* and *Episyrphus balteatus,* and the common housefly, *Musca domestica*. Wild bees and honeybees made a few flower visits. *Halictidae* were abundant in pan traps indicating that wild bees were present but did not frequently visit buckwheat. During the first campaign, we confirmed pollinator contribution on the basis of a 14% difference in seed set between open pollinated flowers and bagged flowers. In the second campaign, we found a 6% difference in seed set between sites with and without beehives with *Apis mellifera*. No treatment differences were observed in the summer of 2022 when pollination success was low overall due to less suitable weather. Seed set with open pollination was positively correlated with crop diversity in the landscape around the sites.

Keywords: crop diversity, buckwheat, pollinator composition, seed set rate

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## P339. Interspecific competition among early flowering plants

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Entomophilous plants compete for pollinators, particularly in spring, when insects are scarce. Strong attraction to pollinators by one species (also called magnet species) can have a positive or negative impact on neighbouring plants. With respect to the former, the magnet species can attract pollinators that will also pollinate other, less attractive plants. With respect to the latter, the magnet species usurps the attention of a pollinator at the expense of other plants in the community. We investigated the competition for pollinators among two co-flowering plants, the white coloured snowdrop *(Galanthus nivalis)* and the two-leaf squill *(Scilla bifolia)* with blue flowers in a deciduous forest in Slovakia. We first tracked pollinating bees in the field and found that they strongly prefer *Scilla* (87% of visits) vs. *Galanthus*. In naive, inexperienced bumblebees, the same (90%) preference for the *Scilla* over the *Galanthus* was confirmed under laboratory conditions. Spectrometry failed to show the UV reflectance of *Scilla*. Finally, we experimentally removed Scilla in 4 of 8 plots (10 x 10m each) in the field and found that the probability of *Galanthus* seed production was significantly higher in plots where *Scilla* was removed. These results suggest that *Scilla* is a magnet species that outcompetes the more abundant *Galanthus*. The partial self-compatibility of *Galanthus* can be the result of the low competitive ability of this species compared to highly attractive *Scilla*. This work was supported by grant VEGA 1/0007/21.

**Keywords:** pollinators, *Galanthus nivalis*, *Scilla bifolia*, intraspecific competition

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## P341. The use of bumblebees as potential pollinating agents in vertical farming: drawbacks, feasibility and prospective research

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Food security across the globe has drastically been threatened by pervasive drivers of food insecurity, including climate change, scarcity of agricultural resources and human population growth. Vertical farming (VF) is an innovative plant production system that can potentially counteract these issues by improving food production without further aggravating land use. However, among other concerns, VF development is constrained by high labour costs. This study explores the feasibility of insect pollination in VF to potentially decrease labour costs and produce high-quality products. In this study, *Bombus terrestris*, was assessed as a potential pollinating agent in two different experiments. In the first, dwarf tomatoes placed in light chambers consisting of different light spectra were used to perform a choice test. The number of plant visitations was recorded to determine the response of bumblebees to artificial lighting and to establish which wavelengths were more conducive to maintaining pollinator activity. In the second experiment, the bumblebees were introduced within an active VF in an exclusion test using basil plants. Here, the objective was to determine if there was a difference in seed production (i.e., in the number and weight of seeds) between basil plants grown with and without the presence of bumblebees. The current findings suggest that bumblebees could be a helpful resource in VF. Given the paucity of research relating to this topic, the limitations, practical considerations and suggestions on how to ameliorate the methods and protocols for future research are also critically discussed.

**Keywords:** vertical farming, bumblebees, artificial light, pollination, food production

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## P342. Flower visitation through the lens: exploring bumblebees' behaviour with computer vision-based application

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To understand the processes behind pollinator declines, besides community monitoring, we also have to understand the behaviour of pollinators and the plant-pollinator interactions. In addition to traditional trapping and visual observation methods, developing fast and reliable automated methods which are capable of both monitoring species occurrences and flower visitation events would be highly advantageous. The buff-tailed bumblebee (*Bombus terrestris*) is common in Europe, and it is thus a good model species to examine whether flower visitation behaviour can be explored with computer vision-based tracking methods. The study was conducted in urban and seminatural areas in Terceira (Azores, Portugal) between July and August 2022. We recorded the bumblebees on pink-headed persicaria (*Persicaria capitata)* and red clover (*Trifolium pratense)* patches in five-minute-long slots. For the automated detection of bumblebees in the videos we created deep learning-based computer vision models (YOLOv5), trained with custom datasets. The similarity of the colour from a predefined 'optimal flower colour' was calculated for each pixel in the image. To estimate how much time bumblebees spent on flowers, and away from flowers, this similarity was recorded from areas in which the insects were present and compared between plant species. We found that bumblebees spent significantly more time on red clover than on pink-headed persicaria, which suggests that the optimum duration of stay on larger-headed *Trifolium* may be longer than that on the smaller-headed *Persicaria*. Our results justify the application of computer vision-based tracking in studying insect behaviour and highlight them as a potentially trusted tool in pollinator research.

Keywords: pollinators, behavioural ecology, flower visitation, insect tracking, deep learning, artificial intelligence

## P344. EAG test on *Apis mellifera* as a future prospect of application for *Varroa destructor* control

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In the honeybees, olfactory communication is crucial for many coordinated functions inside and outside the honeybee colony. Honeybees have a strong olfactory learning ability that takes place through olfactory receptor neurons, localized on antennae, able to change odour cues into chemical signals. In the frame of sustainable treatments to protect bee colonies, natural products, such as plant extracts and essential oils (EOs), due to their lower environmental impact, were used to control the mite *Varroa destructor*. In order to start to explore the effects of such substances on honeybee, the antennal sensitivity of *Apis mellifera ligustica* to EOs was assessed using the electroantennographic (EAG) technique, used to measure response of an isolated antenna to volatile compounds. For the EAG tests, only foragers of *A. m. ligustica* were used. The stimuli were provided by eleven EOs (lavender, arabian frankincense, indian frankincense, bergamot, citronella, lemon balm, carnation, thyme, oregano, geranium and cajeput) dissolved in mineral oil to obtain five concentrations (0.001-10 µg / µL). The insect head was placed between two glass capillary electrodes, filled with a 0.1 M KCl conductive solution. The mean EAG responses from the right and left antennae were compared with Student's *t test* ($P < 0.05$). The two most active oils were found to be geranium and lavender. The volatile compounds present in all EOs elicited EAG responses. The mean EAG amplitudes evoked by each dose of the EOs in right antennae were not significantly different ($p > 0.05$) from those of left ones.

**Keywords:** essential oils, active compounds, dose-response curves, antennae

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## P346. Including honeybees in the All-Ireland Pollinator Plan

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Irish pollinators are in decline. Approximately, one-third of the 101 Irish wild bee species are threatened with extinction. The reasons for this are multiple, but loss of habitats that provides nesting sites and food sources, coupled with increased agricultural intensification are two of the main drivers for pollinator decline in Ireland. To do something positive about this, the All-Ireland Pollinator Plan was initiated in 2015 – 2020. The plan was entirely voluntary. To see the outputs of the first phase of the plan visit >> www.pollinators.ie <<. Building on the success of the first plan, the current plan, running from 2021-2025, is an ambitious five-year roadmap that aims to produce a cohesive landscape level strategy for pollinator conservation. The plan has the key objectives of making farmland, public and private land pollinator friendly and conserving rare pollinators. In addition, there is a standalone All-

Ireland Honeybee Strategy. Honeybees are different to other pollinators in the plan. They are predominantly farmed, represented by beekeeping organisations and overseen by legislation. The aim is not therefore conservation of honeybees per se, aside from the Irish black bee, nor economic development of the sector. Rather, the overarching aim of this strategy is to include honeybees as part of a cohesive pollinator message: healthy honeybees, healthy pollinators. Approaches include all-Ireland disease management, raising awareness of native bee subspecies and other wild pollinators, collecting data on hive density and facilitating discussion amongst beekeeping organisations.

**Keywords:** pollinators, beekeepers, *Apis*, conservation

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## P347. *Nosema ceranae* spores and chronic bee paralysis virus decrease newly emerged Carniolan honey bee longevity

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It can highlight parasites and viruses among the known stressors that harm bees worldwide. The microsporidian *Nosema ceranae* (NOS) and chronic bee paralysis virus (CBPV) are highly prevalent in European honey bees. Thus, the goal was to evaluate the effects of the NOS and/or CBPV inoculation on the biological effects (longevity) of *Apis mellifera carnica* in laboratory conditions. The newly emerged bees were collected from three colonies and placed in cages. Caged bees were kept at laboratory conditions (28 ± 1°C temperature, 70 ± 5% humidity) and divided into three experimental treatment groups and untreated control (12 bees in five replicates). The CBPV inoculum was delivered *per os* (2 µL containing $3.1 \times 10^{10}$ copies of CBPV/honey bee), and NOS (100.000 spores/honey bee) was offered in syrup solution. Bees also received a treatment combination (CBPV + NOS) - virus inoculum *per os* and NOS in syrup solution. The untreated control bees (CTL) were offered sugar syrup in water (1:1). The mortality was recorded until the last bee died, and the statistical analysis was performed by GraphPad Prism 9.5.1. After thirty days of feeding experiment, the Log-rank (Mantel-Cox) test demonstrated that decreased bee's longevity ($p<0.05$) was shown in bees exposed to NOS (12 days), CBPV (14 days), and CBPV + NOS (12 days) when compared to CTL (16 days). The results indicate that the NOS and CBPV exposure reduce *A. mellifera carnica* longevity. Further studies will be conducted using other parameters to evaluate the sublethal effects on adult bees.

**Keywords:** biological parameter, honey bees, microsporidian, virus

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## P348. Assessment of post-fire vegetation recovery after forest wildfires: how honey bees and other pollinators are affected. The case of Voria Evia

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The forest wildfires associated with the climate crisis seem to be one of the most widespread extreme natural phenomena and affect many areas of the world. According to the National Observatory of Athens, the total area devastated during the wildfires of 2021 in North Evia is estimated at 46,582 hectares. Monitoring Post-Fire Vegetation Recovery (PVR) is vital, as it provides valuable information on analyzing the ecosystem resilience, on determining the dynamics of the landscape and the purposes of forest management. The scope of the present research is to study the post-fire vegetation recovery at Pappades forest in Northern Evia, after the wildfires of 2021. Within this forest, four plots of 0,5 ha each were selected. In each plot, monthly optical observations are made, in order to record the vegetation. Moreover, insect biodiversity is observed with pan traps, sweeping net and optical observation. Detailed results and extended discussion will be given during the presentation.

**Keywords:** forest wildfires, climate change, post-fire vegetation recovery, pollination biodiversity, *Apis mellifera*

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## P349. Monitoring the effects of plant protection on pollinating insects in apple orchard – case study from SE Poland

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The study was conducted in southeastern Poland in two apple (*Malus domestica* Borkh) orchards managed conventionally and an organic apple orchard. In each of the three locations, colonies of honey bees (*Apis mellifera*), bumble bees (*Bombus terrestris*) and nesting construction with red mason bees (*Osmia bicornis*) cocoons and nesting material were placed. In order to assess residues of plant protection products, 75 samples from honey bee nests, 45 samples from bumble bee nests and 9 samples from red mason bee nests were collected. Samples were taken at the end of flowering of apple orchards. We used a gas chromatograph (GC) and liquid chromatograph (LC) equipped with dual mass detectors (GC-MS/MS and LC-MS/MS) to detect residues of pesticides. Of the total number of samples tested, in only eight samples no residues of plant protection products were identified. Overall 50 residues of plant protection products or their metabolites were detected. In accordance with Regulation (EU) 396/2005 of the European Parliament and of the Council in 45% of the total number of samples maximum residue levels of pesticides (MRLs) were found to be exceeded. Among the biologically active substances of plant protection products and their metabolites, 11 substances which use is not permitted in the European Union were detected.

This study was possible through implementation of the research project DHR.bz.070.2.2022, task 6.5 entitled: “Monitoring the effects of plant protection on pollinating insects “financed by the Ministry of Agriculture and Rural Development, Poland.

**Keywords:** bee, pesticides, orchard, chemical pest control, bee products

# Session 13:

## Toxicology and pesticide resistance

## P048. ABCH2 transporter in the first line of defense protects malaria vectors from pyrethroids

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Contact insecticides are primarily used for the control of *Anopheles* malaria vectors. These chemicals penetrate mosquito legs and other appendages, the first barrier before reaching their neuronal targets. An ATP-Binding Cassette transporter from the H family (ABCH2) is highly expressed in *Anopheles coluzzii* legs, and further induced upon insecticide exposure. RNAi-mediated silencing of the ABCH2 caused a dramatic increase in deltamethrin mortality compared to control mosquitoes, coincident with a significant increase in $^{14}$C-deltamethrin penetration. RT-qPCR analysis and immunolocalization revealed that ABCH2 is mainly localized in legs and head appendages, and more specifically, the apical part of appendage epidermis, underneath the cuticle. To unravel the molecular mechanism underlying pyrethroid toxicity upon ABCH2 silencing, two hypotheses were investigated: An indirect role, based on the orthology with other insect ABCH transporters involved in lipid transport which could increase cuticle thickness, slowing down the penetration rate of deltamethrin; or the direct pumping of deltamethrin out of the organism. The content of leg cuticular hydrocarbons (CHC) was not affected by ABCH2 RNAi, indicating that the potential role of this transporter in pyrethroid toxicity is not associated with the transport of CHCs. Homology-based modeling suggested that the ABCH2 half-transporter adopts a physiological homodimeric state, in line with its ability to hydrolyze ATP *in vitro* when expressed in Sf9. Docking analysis revealed a deltamethrin pocket on the homodimeric transporter. Furthermore, deltamethrin-induced ATP hydrolysis in ABCH2-expressing membranes, further supports that deltamethrin is indeed an ABCH2 substrate. Overall, our findings pinpoint ABCH2 as a key regulator in deltamethrin toxicity.

Keywords: *Anopheles coluzzii*, legs, appendages, ABCH transporter, pyrethroid toxicity, deltamethrin

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## P049. Influence of a neonicotinoid on *Trissolcus japonicus* behavioral responses to cues from *Halyomorpha halys*

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*Trissolcus japonicus* (Hymenoptera: Scelionidae) is a key parasitoid of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), in its native area, East Asia. Additionally, it is an important biocontrol agent of *H. halys* in the areas invaded by this pest. Here, we tested the non-target effects of a sublethal dose of Acetamiprid, a neonicotinoid commonly used against *H. halys*, on *T. japonicus*. First, we evaluated parasitoid ability to exploit cues from two stinkbugs, the target species *H. halys* and the non-target predator, *Arma custos* (Hemiptera: Pentatomidae). Then, we tested the effects of the neonicotinoid on female parasitoid behavior. We conducted open arena behavioral assays with treated versus untreated females to evaluate their responses to kairomones left by either pentatomid species. Additionally, we investigated the behavior of naïve vs. experienced (i.e., with oviposition experience) parasitoid females and tested the effects of insecticide exposure on their learning ability. The parasitoid successfully exploited cues from both species, showing different responses in naïve vs. experienced females and a preference towards *H. halys*. Exposure to low doses of the neonicotinoid insecticide altered the foraging duration, although no direct effect on learning was revealed. Our results suggest that parasitoid efficacy in the field could be impaired by exposure to even low doses of a common neonicotinoid insecticide, further supporting the need to develop sustainable products against *H. halys*, especially in those areas where biocontrol with *T. japonicus* is in place.

Keywords: egg parasitoid, associative learning, host selection behaviour, acetamiprid, sublethal effects

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## P050. Investigating putative synergistic interactions in spider mite resistance to abamectin via engineered *Drosophila* strains

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We investigate the putative synergistic interaction of target site mutations and enhanced detoxification in abamectin resistance manifested in strains of the spider mite *Tetranychus urticae*, a major pest threatening crop production. While previous results focusing on pyrethroid resistance in insects have indicated synergistic action of target-site mutations with P450s overexpression to induce “super-resistant” phenotypes, a similar mechanism for other insecticide classes like avermectins, which are routinely used as acaricides, has been postulated but not functionally validated yet. Our strategy involves a combination of standard genetic transformation and CRISPR/Cas9, to generate *Drosophila* lines expressing insecticide metabolizing cytochrome P450 enzymes in a genetic background featuring engineered mutations known to confer target-site resistance. Our previous work has already demonstrated, in a *Drosophila* system, low to moderate abamectin resistance induced by mutation I321T in the glutamate-gated chloride channel, as well as the role of a specific P450, CYP392A16, in enhanced abamectin metabolism. Here, we combine both components in the same genetic background and investigate the role of each one as well as their putative combined action in abamectin resistance, within a framework devoid of any confounding genetic effects.

Keywords: spider mite, abamectin, insecticide resistance, CRISPR/Cas9, P450 expression, *Drosophila*

## P052. The impact of cyanotoxin anatoxin-a on mosquito and dragonfly larvae: a serious ecological threat to aquatic ecosystems

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Toxic cyanobacterial blooms are a widespread phenomenon throughout the world and their frequency is increasing with global climate change. High concentration of cyanotoxins threaten aquatic ecosystem health. Little is known, however, on their impact on the physiology and behavior of aquatic insects like mosquitoes and dragonflies, which are key species in aquatic systems. In this study, we examined the impact of anatoxin-a, a neurotoxic cyanotoxin, on the physiology (growth and metabolic rates) of dragonfly larvae (*Sympetrum meridional*) and mosquito larvae (*Aedes aegypti*) at realistic (sublethal) field doses. We also examined the toxin impact on a crucial behavioral trait of mosquito larvae (learning ability) that drive the prey-predator relationship. Our results showed that anatoxin-a depressed the growth of mosquito larvae, but it had no impact on the growth of dragonfly larvae. Higher metabolic rate was also observed in mosquito larvae exposed to anatoxin-a suggesting enhanced physiological activity to tolerate the stress and/or detoxify the body. However, the toxin did not influence the metabolic rates of dragonfly larvae. Learning ability decreased in larvae exposed to anatoxin-a. The negative impacts of anatoxin-a on the development and learning of mosquito larvae can generate serious ecological consequences in aquatic ecosystems. However, dragonflies may be more tolerant to this toxin, providing resilience to the ecosystem. Besides, the potential bioaccumulation of anatoxin-a into the adult stage of mosquitoes and dragonflies can allow the transfer of anatoxin-a from aquatic to terrestrial ecosystem, thereby threatening the entire food web.

Keywords: cyanotoxins, Odonata, Diptera, cyanobacteria, aquatic insects, Culicidae

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## P053. Determination of the therapeutic potential of two volatile organic compounds as a treatment against fipronil poisoning in bees

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Fipronil is a non-competitive antagonist of γ-aminobutyric acid receptors (GABA-R) which inhibits the flow of chloride ions in the nerve cells, leading to the bee death. We analyzed the global Volatile Organic Compounds (VOCs) produced by bees chronically exposed to low doses of fipronil (0.5 and 1 µg/L) and characterize the bee metabolic responses to this insecticide. This high-throughput approach, called volatolomics, has highlighted two VOCs (1-octen-3-ol and 2,6-dimethylcyclohexanol) that could act as modulators of the GABA-R. When their bound to the receptor, they can extent the opening time of thee

chloride channels (by allosteric effect). We assumed that these VOCs, produced by bees, could act as modulators of the GABA-R to counteract the effect of fipronil. The toxicity of these VOCs was tested on emerging bees studied in pain-type cages for concentrations ranging from 0 to 3.4 µg/L. Bees survival and the sucrose solution consumption was recorded during 21 days of chronic exposure, no dose-response effect could be observed. 1-octen-3-ol and 2,6-dimethylcyclohexanol show a moderate toxicity in bees. After the preliminary VOCs-fipronil co-exposure experiment, a trend seems to show a potential beneficial effect on bee health. Optimization steps are still needed at this stage to draw definitive conclusions. The studies on key genes (oxidative stress, detoxication pathways, GABA metabolism) and metabolic markers (using volatolomics) will give clues to the relevance of these two compounds as a potential therapeutic tool.

**Keywords:** GABA receptor, volatile compounds, pesticide poisoning, honeybee, therapeutic tool

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## P054. Comparative biochemical characterisation and inhibitory profiling of cattle tick, human, bovine and mosquito Flavin Adenine Dinucleotide sub-domains.

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Southern cattle tick, *Rhipicephalus microplus (Rm)*, is a significant ectoparasite of tropical/sub-tropical regions, causing health detriments and severe economic losses in the cattle industry. Resistance to common acaricides via mechanisms such as increased rates of cytochrome P450 (CYP) detoxification are a growing concern. CYPs are also essential for hormone biosynthesis, requiring a single redox partner cytochrome P450 oxidoreductase (POR) facilitating electron supply from nicotinamide adenine dinucleotide phosphate (NADPH). Inhibition of POR function would affect all CYP-catalysed reactions, resulting in multi-system failure. We report the expression and biochemical characterisation of flavin adenine dinucleotide (FAD) binding domains of RmPOR and domestic cow, *Bos taurus (Bt),* in terms of kinetic parameters for NADPH and inhibitory activity of adenine nucleotide analogues, to determine differences between host/parasite POR isoforms for exploitation. Malarial mosquito, *Anopheles gambiae (An)*, and *Homo sapiens (H)* were included to extend the study value. Purified FAD domains displayed characteristic flavin spectrum (absorbance peaks at ~379 nm/ ~454 nm). Michaelis-Menten constants and turnover, $K_{cat}$, for NADPH were calculated as 35.98, 62.57, 66.2 and 110.18 µM plus 0.1, 0.62, 0.96 and 1.71 $sec^{-1}$ respectively for RmFAD, BtFAD, AnFAD and HFAD. Half-maximal Inhibitory Concentration ($IC_{50}$) values for 2'-AMP/$NADP^+$ were 3.5 mM/90.4 µM for RM FAD; 4.7 mM/119.6 µM for BtFAD; 3.58 mM/149.7 µM for AnFAD and 14.38 mM/209.5 µM for HFAD. Comparison of kinetic/inhibitory profiles across all four species identified differences between

host-parasite pairings, supporting the potential of POR as a target for rational design of safer and more potent insecticides/acaricides against CYP-mediated resistance populations.

Keywords: acaricide, resistance, metabolism, cytochrome P450, P450 oxidoreductase, *Rhipicephalus microplus*

## P055. **Insecticide resistance status and functional characterization of pyrethroid resistance in olive fruit fly Bactrocera oleae**

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The olive fruit fly, *Bactrocera oleae*, is the most devastating pest of olive orchards in the Mediterranean region, and its control is based mainly on chemical insecticides. The resistance status of registered insecticides is monitored, in order to identify emerging resistance cases. Extensive use of pyrethroid insecticides has led to resistance evolution, in most populations from Greece, especially Crete, with resistance levels reaching up to 132-folds. The resistance phenotype was substantially suppressed by the PBO synergist, suggesting the involvement of detoxification enzymes. Comparative transcriptomic analysis between pyrethroid resistant and susceptible populations, using both whole bodies and Malpighian tubules, revealed several differentially transcribed genes, with P450s being among the most highly over-expressed detoxification genes, in resistant populations. Four P450 genes (*Cyp6A61*, *Cyp6G6*, *Cyp4P6* and *Cyp6G28*), which showed increased transcription levels in resistant populations, were recombinantly expressed and *in vitro* metabolism assays revealed that CYP6A61 is capable of metabolizing alpha-cypermethrin, while CYP6G6*,* CYP4P6 and CYP6G28 are capable of metabolizing deltamethrin. *In vivo* silencing of *CYP6G6*, *via* RNAi, led to a small but significant increase in deltamethrin toxicity. Monitoring of resistance and elucidation of underlying mechanisms are crucial for developing effective strategies for olive fly control.

**Keywords:** olive fruit fly, monitoring, pyrethroid resistance, transcriptomics, cytochrome P450 localisation

## P056. Tracking the diamide-resistance dynamics of Korean field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae), and reconsideration of its resistance diagnostic marker

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Beet armyworm, *Spodoptera exigua*, is one of the most destructive lepidopteran pests worldwide, including Korea. Diamides have been widely used to control *S. exigua*. Bioassay-based diamide resistance was tested in field populations of *S. exigua* in the years from 2014 to 2021. When 3rd instar larvae were treated with recommended label rates, 100% mortality was observed in all tested field populations in 2014. However, three years later, in 2017, the mortality rate was less than 10%, depending on the region. In addition, mortality varied from region to region and year to year (2017- 2021). In 2021, for some populations, the mortality was 0%. Among them, only an I4790M mutation in ryanodine receptors was detected. However, in some cases, there was no correlation between bioassay-based resistance ratio and mutation frequency. Therefore, we hypothesized that other mechanisms might be involved in diamide resistance. Indeed, the presence of the I4790M mutation was confirmed in the sample before diamide launch. As a result, I4790M was observed in samples collected before diamide has been commercialized in Korea. Therefore, I4790M may not be a suitable diagnostic marker for diamide resistance in *S. exigua*. Alternatively, we tried to find new resistance-markers. After assembling the Korean reference genome data set (ASM1567961v1), we identified novel diamide resistance related mutations and/or InDels. Additionally, some cytochrome P450 genes were highly expressed in diamide resistant strains. These results will be discussed.

**Keywords:** *Spodoptera exigua*, genome assembly, Ryanodine receptor, Insecticide resistance, Integrated pest management

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## P057. Utilising a field survey of acaricide resistance to create a genetic database for the discovery of genetic markers

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Tetranychus urticae, the two-spotted spider mite (TSSM) is a generalist plant pest with worldwide distribution, known for its ability to quickly develop resistance to any acaricide to date. The variety of acaricide materials licenced in Israel is limited and many have lost their effectiveness. My lab, in collaboration with the extension services at the Israeli ministry of Agriculture, have set up a testing system in which TSSM samples are shipped to the lab on a daily basis from crop fields all over Israel. The samples are tested with 13 acaricides with different active ingredients from 8 different acaricide classes. Our aim is

to deliver a report to the grower of the mortality ratios from each individual acaricide and from each class of acaricides in order to enable them to then apply the ones most likely to effectively eliminate the TSSM outbreak in their field. In addition, each field sample is kept for DNA and RNA extraction and sequencing in order for us to identify SNP markers for the corresponding acaricides' resistances. This data will be used to create a comprehensive database of genetic resistance markers in TSSM. The database will have widespread applicability in development of faster, PCR-based, resistance tests which will enable smart and effective acaricide control of TSSM in Israel with potential worldwide implications.

Keywords: spider mite, acaricide resistance, SNP markers, transcriptome

## P058. Monitoring population dynamics and damage levels of olive oil fruit fly (*Bactrocera oleae*) in relation to the implementation of the bait sprays program in two regions of Heraklion, Crete

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The olive fruit fly *Bactrocera oleae* (Diptera: Tephrididae) is the main pest of olive-growing regions causing both quantitative and qualitative damage. In Greece, the control of *B. oleae* is mainly based on ground bait sprays under a large-scale control system implemented by the Ministry of Rural Development and Food. The aim of this study was to monitor the populations of *B. oleae* and the level of fruit damage in two adjacent regions in Heraklion Crete (Episkopi & Sgourokefali) in relation to the governmental bait sprays program. Thus, a McPhail glass trap network was established along with olive fruit sampling from June to October 2022. Three locations were monitored in each region with three distinct orchards in each location to increase accuracy level (3 orchards x 3 locations: 9 traps per region). The active ingredient applied in both regions was spinosad (Success 0.24CB). In parallel, the efficacy of spinosad bait sprays was evaluated under laboratory conditions on adults emerging from regionally collected infestations. The *B. oleae* population exhibited uniform fluctuations in the two regions. After each regional application the population levels were decreasing suggesting good control of the pest by the bait sprays. Regional damage levels never exceeded 4% of infested fruits per sample (n=150 fruits x 9 locations per region) throughout the season which is in agreement with indications from the trap network. Under laboratory conditions, populations collected from Episkopi and surrounding areas exhibited high mortality to spinosad bait (77- 100%) justifying the satisfactory control levels observed in the field.

Keywords: bait sprays, efficacy, fruit damage, monitoring, McPhail, spinosad, Greece

## P059. Evaluation of insecticide resistance in Western flower thrip, *Frankliniella occidentalis* on pepper in Korea

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Among the registered insecticides, 10 different insecticides belonging to 8 chemical classes (Acrinathrin 5.7% SC, Acetamiprid 5% SL, Dinotefuran 10% SL, Spinetoram 5% WG, Abamectin 1.8% EC, Emamectin benzoate 2.15% EC, Chlorfenapyr 5% EC, Cyclaniliprole 4.5% EC, Fluxametamide 9% EC and Flometoquin 10% SC) were investigated for insecticide resistance in western flower thrip (*Frankliniella occidentalis*) populations. The tested populations were sampled from pepper in three regions of Gyeonsangbuk-do (Andong, Bonghwa and Yeongyang), Korea. A susceptible population cultured in laboratory was also included in the study to enable comparison of resistance, and insecticide responses based on resistance ratios which were calculated using $LC_{50}$ values. The resistance ratios differed according to region and insecticides. Among the tested insecticides, treatments with cyclaniliprole showed the highest resistance ratio (11020 in the Andong population, but with lower resistance ratios of 40.5 and 246.2 in Bonghwa and Yeongyang region, respectively). Also, relatively high resistance to acrinathrin was recorded in all the three regions, with resistance ratios ranging from 394 to 5313. Resistance ratios in treatments with acetamiprid, dinotefuran, abamectin, emamectin benzoate and chlorfenapyr ranged from 1.87 (abamectin on Andong population) to 144.1 (chlorfenapyr on Yeongyang population). Treatments with flometoquin and fluxametamide showed low resistance ratios of 0.4-0.65 and 0.5-1.95, respectively. Notably, a large variation in resistance to spinetoram was observed among regions, with lowest resistance ratios (0.07-0.1) in Bonghwa and Andong, but high resistance ratio of 112 in the Yeongyang region.

Keywords: *Frankliniella occidentalis*, resistance, susceptibility, insecticide, hot pepper

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## P060. Detection of extensive multiple insecticide resistance and likelihood of control failure in populations of tomato borer *Tuta absoluta* from Greece

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*Tuta absoluta* control relies extensively on the use of chemical insecticides. Proactive resistance management is based on early detection of resistance cases thus hereby we present a compressive resistance monitoring study on five *T. absoluta* populations collected in 2021 from Greece. Using the IRAC 022 method the following insecticides were tested and the $LC_{50}$s (in mg $L^{-1}$) were estimated (presented in brackets): cyantraniliprole (8.4–98), chlorantraniliprole (37–2277), indoxacarb (36–3462), spinosad (1.7–

98), spinetoram (3.2–65), emamectin benzoate (0.89–7.4) and abamectin (0.33–2.1). Comparing results to the susceptible reference strain, high resistance levels were detected for indoxacarb, chlorantraniliprole and cyantraniliprole up to 2,997- 11,985- 376-fold respectively. Moderate to high resistance was detected for spinosad, spinetoram and emamectin benzoate (up to 75-, 88- and 74-fold respectively). Low resistance levels were detected only for abamectin (up to 6-fold). Multiple resistance (RR >30-fold) was detected in four out of five populations to four or more insecticides tested that belong to at least three different chemical classes. The potential likelihood of control failure (LCF) was detected based on the comparison of the $LC_{80}$ to the recommended label rate. The most striking case was that of the insecticide chlorantraniliprole where LCF was detected in all five populations, while a population from Ierapetra exhibited LCF in five out of seven tested insecticides making conventional control of the particular population extremely difficult. Such information, at a regional scale, is of critical importance for the design of rational pest management schemes case by case.

**Keywords:** *Tuta absoluta*, insecticide resistance, tomato pinworm, multiple resistance

## P061. Evaluation of the effect of conventional and biological insecticides against the tomato borer, *Tuta absoluta* with a novel bioassay method

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Tomato borer *Tuta absoluta*, is mainly controlled using insecticide sprays and the accurate evaluation of the efficacy levels is of critical importance for the design of any rational IPM scheme. Hereby, the efficacy of two insecticides (azadirachtin, spirotetramat) and two biocontrol agents (*Phthorimaea operculella Granulovirus* (PhopGV, Baculoviridae) and *Bacillus thuringiensis* Aizawai (Bt)) was investigated on 2nd instar larvae (L2). A new bioassay method, based on the IRAC 022 protocol, allowing prolong exposure intervals up to pest pupation (12 Days After Treatment, DAT) and adult emergence (27 DAT) was used on a susceptible reference strain and a wild *T. absoluta* population. Each experiment was replicated twice allowing method validation. No differences were observed between the replications in all cases suggesting that the novel bioassay protocol is robust and reliable. The estimated $LC_{50}$ values indicated marginal differences between the two populations tested for the aforementioned insecticides (Resistant ratio (RR) ≤ 4). In relation to the efficacy levels exhibited by the tested insecticides, the control failure likelihood was estimated by comparing respective recommended label rates (RLR) with $LC_{80}$ values. Azadirachtin and spirotetramat exhibited 10-fold and 50-fold lower $LC_{80}$ values compared to the respective RLR (azadirachtin RLR: $39 mgL^{-1}$, spirotetramat RLR:$100mgL^{-1}$) suggesting substantial levels of control. In contrast the $LC_{80}$ values for PhopGV (min $LC_{80}$: $3.8 \times 10^{10}$ $obsL^{-1}$ at 27 DAT, wild population). and BT (min $LC_{80}$: 409 $MIUL^{-1}$ 27 DAT, susceptible strain) were always found higher than the respective RLRs (PhopGV: $2x10^{10}$ $obsL^{-1}$, BT: 75 $MIUL^{-1}$) indicating potential cases of pest control failure.

**Keywords:** *Tuta absoluta*, azadirachtin, spirotetramat, *Bacillus thuringiensis*, *Phthorimaea operculella Granulovirus*, integrated pest management

### P062. Impact of toxic substance exposure on life history and reproduction of black scavenger flies (Diptera: Sepsidae)

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The use of pesticides has increased globally over the years to adversely affect the well-being of ecosystems and organisms. Neonicotinoids are one such widely used type of insecticide toxic to non-target organisms. While most studies have tested effects on pollinators, both sub-lethal and lethal effects on the development, survival, or physiology of non-target insects have been reported. We experimentally quantified the effects of larval (1-2 instar) exposure to imidacloprid (neonicotinoid) on the life history of four non-target black scavenger fly species (Diptera: Sepsidae) that are beneficial as decomposers of vertebrate dung. With higher concentration of imidacloprid in dung we found increases in larval to adult mortality, prolonging of development and decreases in final body size. Adult flies were sex-specifically adversely affected in all reproductive traits measured, notably days to egg laying, egg hatching success, and the number of offspring. However, the effects varied amongst the fly species being merely subtle in some. Our study demonstrates subtle to strong lethal and sub-lethal effects of imidacloprid on life history traits after exposure of juveniles, as well as subsequent carry-over effects in surviving adults of four *Sepsis* species. This study projects future research directions, and currently we are investigating transgenerational effects of imidacloprid in adult *Sepsis* flies.

**Keywords:** neonicotinoid, non-target insects, black scavenger flies, life-history traits, sub-lethal effects

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### P063. Resistance to pyrethroids of the predatory mite *Amblyseius andersoni* (Acari: Phytoseiidae)

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*Amblyseius andersoni* (Chant) is a generalist predatory mite from European orchards and vineyards, where it is considered a key natural enemy of spider mites. Its conservation is fundamental for successful pest management strategies. Pesticide use, particularly pyrethroids, threatens the persistence of the population of this predatory mite in the agroecosystems. Pyrethroids are currently extensively used to control primary fruit and grape pests. We conducted an experiment to evaluate the effect of pyrethroids on different *A. andersoni* strains. These were collected in commercial fruit orchards and vineyards in North-Eastern Italy. Lethal effects on survival and sub-lethal effects on fecundity have been assessed under laboratory conditions. Results showed that the investigated strains were characterized by a different susceptibility to a pyrethroid insecticide. Comparing dose-response curves obtained by different strains highlights that some of them showed resistance to pyrethroids insecticides with a high resistance factor. The implications for Integrated Pest Management programs in perennial cropping systems of pesticide resistance in *A. andersoni* are discussed.

**Keywords:** predatory mites, pyrethroids, resistance, Integrated Pest Management

## P064. Pesticide use and operator safety perceptions of the Greek public

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The public's perception of pesticide use is a driver of their attitudes, which in turn affect the agricultural sector. Future policy can be shaped by understanding the factors that influence their views. A survey was conducted in spring 2021 using an online questionnaire completed by 1,846 participants. The questionnaire included demographic questions and five-point Likert scale questions. Principal components analysis was used to summarise the research variables, followed by binomial logistic regression on the research question "whether using pesticides as directed can contribute to user safety". Those who have a positive perception of the contribution of pesticides (OR = 8.218), who obtain information about pesticides from specialised information sources (OR = 2.119), who express greater confidence in the certification of plant foods (OR = 1.596) are more likely to respond positively to the research question. The opposite is true for those who have greater confidence in the safety of plant foods (OR=0.671). Specific consumption habits of plant foods, general sources of information on pesticides, perceived threat of pesticide residues in plant foods and demographic parameters such as population, age, education and gender were not significant predictors of public opinion on the research question. Targeted information on the correct use of pesticides and on food safety in general should be provided through mainstream information sources (television, radio, print and electronic press) and, in particular, through social networks aimed at the general public.

**Keywords:** *pesticides*, public opinion, attitudes, perceptions, information sources

## P065. Predicting Greek public opinion on the use of pesticides and consumers' safety

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Consumer perceptions of food safety in relation to pesticide use are a determinant of their behaviour and have implications for the primary sector. Future interventions can be better targeted by understanding the factors that influence their views. An online questionnaire survey was conducted in spring 2021 and completed by 1,846 participants. The questionnaire included demographic questions and five-point Likert scale questions. Principal components analysis was applied to summarize the data and determine unmeasured latent variables. Binomial logistic regression was used to identify parameters that might predict user perceptions of the research question 'whether the use of plant protection products according to instructions can contribute to consumer protection'. According to the results, those who have a positive perception of the contribution of pesticides (OR = 9.406), who express greater confidence in the certification of plant foods (OR = 1.788) and who obtain information about pesticides from specialised sources (OR = 1.735) are more likely to respond positively to the research question. Conversely, those who express a high perceived threat of pesticide residues in plant foods (OR = 0.704), who obtain information about pesticides from general information sources (OR = 0.726) and who are male (OR = 0.653) are more likely to answer the research question negatively. Based on these findings, we consider that there is a need to provide targeted information on the correct use of pesticides and food safety through general information sources (TV, radio, print and electronic press) and especially through social media channels aimed at the general public.

Keywords: Pesticides, food safety, consumers, public opinion, perceptions, information sources

## P066. Evaluation of the larvicidal activity of insecticides on olive fruit fly *Bactrocera oleae* (Diptera: Tephrididae)

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Olive fruit fly *Bactrocera oleae* is the most damaging pest of olives. It’s management is mainly based on two approaches: a) regionally applied bait sprays for adult control and b) canopy cover sprays targeting mainly the larvae infesting the olive fruits. Although substantial information is available on bait sprays, no information can be found on the efficacy of canopy cover sprays. Hereby, the larvicidal activity of six insecticides, namely acetamiprid, azadirachtin, cyantraniliprole, deltamethrin, lambda-cyhalothrin and spirotetramat, was investigated using a dipping bioassay of olive fruits. Briefly, infested olives were immersed for a few seconds in dilution at the recommended label rate simulating cover spray application. The vitality status of the larvae was evaluated only at pupa formation and adult emergence thus avoiding destructive olive fruit examination that could be detrimental for insect development. From 2020 to 2022, thirteen populations of *B. oleae* were collected from Greek olive groves and the effect of the insecticide treatment was evaluated. The lowest average number of pupae formed (Univariate analysis, ANOVA). was systematically detected for cyantraniliprole treatment followed by spirotetramat and acetamiprid and azadirachtin in all three years of testing pyrethroid insecticides deltamethrin and lambda-cyhalothrin were not significantly different compared to untreated control. Regarding the number of emerging adults the lowest values were detected for spirotetramat and cyantraniliprole (near-zero emerging insects) followed by azadirachtin. Acetamiprid indicated intermediate numbers of emerging adults while pyrethroids were similar or marginally different to control. This information is essential for rationally implementing larvicide control based on canopy cover sprays.

Keywords: *Bactrocera oleae*, insecticides, cover sprays, larvae

## P067. Monitoring the efficacy of baited insecticide applications for the olive fruit fly *Bactrocera oleae*: a 5-year survey

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The evaluation of insecticide efficacy is a key parameter for control of adults of olive fruit fly, *Bactrocera oleae* (Diptera: Tephrididae), where large scale applications of baited insecticides are carried out as part of the regional pest management schemes under the auspices of the Hellenic Ministry of Rural Development and Food. The number of effective compounds for *B. oleae* control is increasingly limited due to the EU Green Deal policy and the development of resistance to insecticides influencing the efficacy of treatments and crop protection levels. The effectiveness was evaluated against compounds used for *B. oleae* control in Greece: α-cypermethrin, beta-cyfluthrin, λ-cyhalothrin, thiacloprid, deltamethrin and spinosad with small lab-scale bait applications on immersed olive branches at the recommended label rates (RLRs). The survey was conducted against 74 populations of *B. oleae* collected from Greek olive orchards between 2018 and 2022. The average mortality for the pyrethroids α-cypermethrin, λ-cyhalothrin, deltamethrin was found below 20% in all cases while mortality for beta cyfluthrin ranging between 10-80%. Spinosad exhibited mortality >90% in all cases up to 2019. In 2020, the first case exhibiting mortality <80% to spinosad was recorded. Thereafter, in 2021 and 2022 additional cases were reported exhibiting mortalities as low as 68.6% and 45.9% respectively. Median mortality to spinosad is exhibiting a gradual decline (from 94% in 2018 to 83% in 2022) indicating a negative trend with the time on the efficacy levels of the insecticide. Nonetheless, spinosad remains the most effective amongst the registered insecticides.

Keywords: *Bactrocera oleae*, pyrethroids, spinosad, bait applications

## P068. Pediculicide sales during the period 2015-2022 in Turkey

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Increasing prevalence of head louse infestations over the years causes an increase in treatment-related costs and the development of resistance to pediculicides. The aim of this study was to evaluate the sales of pediculicides during a period of eight years (2015-2022), the trends for pediculicides with different active ingredients used, the possible development of resistance of local lice to the ingredients as well as to explore the effect of Covid-19 pandemic on head louse infestations. Data regarding pediculicides which were sold with prescription and with the permission of the Ministry of Health of the Republic of Turkey, were formally requested from the Turkish Pharmaceutical Index Dataview, the Intercontinental Medical Statistics database and the relevant pharmaceutical companies. In 2015, a total of 1,832,006 units of pesticide-based pediculicides were sold, when their consumption has fallen more than half to 792,988 units in 2021, while during the first nine months of 2022, 366,956 units were sold. The consumption of physically acting products containing dimethicone has increased from 620,840 units in 2015 to 1,340,003 in 2021, while 1,190,530 units were sold during the first nine months of 2022. During 2015-2022 neither a significant reduction nor an increase in total pediculicide sales could be observed. The decrease in pesticide-based products and the increase in dimethicone-based ones shows that local lice developed resistance to the former ones. Though in countries such as Argentina and Israel the pediculicide sales dropped significantly during the Covid-19 pandemic in 2020, this was not the case in our study.

**Keywords:** head lice, *Pediculus humanus capitis*, pediculicides, resistance, Covid-19

# Session 14:

## Biological Control and Biopesticides

## P069. Life table analysis and development of *Delphastus pallidus* LeConte (Insecta: Coleoptera: Coccinellidae), under different constant temperatures

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*Delphastus pallidus* LeConte is a small whitefly-specific predatory ladybird beetle belonging to the coccinellid tribe Serangiini. In a recent survey to assess the host range of *D. pallidus*, it was found feeding on silverleaf whitefly, *Bemisia tabaci* Gennadius (also known as B biotype/MEAM1); solanum or pepper whitefly, *Aleurotrachelus trachoides* Back; bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi; and cloudy-winged whitefly, *Dialeurodes citrifolii* Morgan. Survey results indicated that *D. pallidus* could potentially be used for controlling multiple whitefly pests of horticultural importance in the region. This poster will demonstrate its life table and development on different constant temperatures.

Keywords: predatory beetle, whitefly, temperature

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## P070. *Typhlodromus recki* as a potential biological control agent of key tomato pests

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*Typhlodromus (Anthoseius) recki* is a phytoseiid predator commonly found on Solanaceous plants throughout the Mediterranean basin. In the present study the potential efficiency of an indigenous population of the mite against the tomato russet mite *Aculops lycopersici*, the whitefly *Bemisia tabaci*, the two-spotted spider mite *Tetranychus urticae* and the western flower thrips *Frankliniella occidentalis* was assessed under laboratory conditions. Life-history traits such as juvenile development and survival, prey consumption and oviposition rate of the predator on tomato leaves were recorded. The highest prey consumption rates and daily oviposition were recorded on *A. lycopersici* whereas thrips larvae were proven an unsuitable prey. For all prey species tested the provision of *Typha angustifolia* pollen as supplementary food, resulted in substantial increase in both the survival and oviposition of the predator. These initial results will be followed by small-scale experiments on tomato plants focusing on recording the population dynamics of the predator and the most suitable prey species tested, the tomato russet mite.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 2496).

Keywords: *Aculops lycopersici*, *Bemisia tabaci*, *Tetranychus urticae*, tomato, *Typhlodromus (Anthoseius) recki*, *Frankliniella occidentalis*

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## P071. *Amblyseius andersoni* as a biological control agent of the tomato russet *mite Aculops lycopersici*

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*Amblyseius andersoni* is a phytoseiid predator with well-documented potential as a biological control agent of spider mites. In the present study, the efficiency of an indigenous population of the predator collected from tomato plants, against the tomato russet mite *Aculops lycopersici* was evaluated under laboratory conditions. Daily prey consumption and oviposition of adult females, as well as development rate and survival of juvenile mites were recorded on tomato leaf arenas. Daily prey consumption ranged between 25 and 55 prey individuals whereas the provision of *Typha angustifolia* pollen as supplementary food, slightly increased the oviposition of the predator. These preliminary results of the possible role of *A. andersoni* as biological control agent of *A. lycopersici* on tomato is under evaluation by small-scale experiments under semi-field conditions.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers” (Project Number: 2496).

Keywords: *Aculops lycopersici*, *Amblyseius andersoni*, biological control, tomato

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## P072. Effects of temperature and relative humidity on development and reproduction in *Feltiella acarisuga* (Vallot) (Diptera: Cecidomyiidae)

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The predatory gall midge *Feltiella acarisuga* (Vallot) (Diptera: Cecidomyiidae) is a biological control agent for twospotted spider mites on greenhouse vegetable crops. Effects of temperature and relative humidity (RH) on development of immatures, reproduction, and prey capture were determined in order to confirm the suitability of *F. acarisuga* for use in greenhouses. Developmental time ranged from 10 days at 27°C to 34 days at 15°C. At 20°C, developmental time was significantly shorter at 96% RH than at 80% RH. There was very poor survival of immatures at 67% RH and none at 39%. Lifespan of adult females decreased with increasing temperature, but temperature had no significant effect on number of eggs laid. At 20°C, lifespan was longer at 84 and 96% RH than at 64 or 36% RH. We conclude that *F. acarisuga* will complete its life cycle and reproduce under conditions typically found in vegetable greenhouses in northern temperate climates. However, extended periods of low RH (<60% RH) could reduce reproduction and survivorship sufficiently to impair the predator’s action against spider mite populations.

Keywords: *Feltiella acarisuga*, *Tetranychus urticae*, biological control, temperature, relative humidity, development, fecundity, survival

## P073. Searching and foraging behaviour of the predators *Macrolophus pygmaeus* (Hemiptera: Miridae) and *Nesidiocoris tenuis* (Hemiptera: Miridae) on *Tuta absoluta* (Lepidoptera: Gelechiidae) eggs

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*Tuta absoluta* is one of the most serious insect pests of tomato crops. Among its natural enemies, the omnivorous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* are the most commonly successful biological agents. In this study, the prey consumption of both predators on *T. absoluta* eggs offered on leaflets in Petri dishes or on young tomato plants in cages was investigated. In first case, 8 eggs of *T. absoluta* were allocated on four tomato leaflets as follows: a) 2 eggs in each leaflet, b) 4 eggs on each of two opposite leaflets, c) 8 eggs on a single leaflet. In the second case, a single *M. pygmaeus* or *N. tenuis* female was released in a cage where four plants were placed, on one of which 30 eggs were previously laid by *T. absoluta* females. *M. pygmaeus* consumed an almost equal percentage of the prey items among the prey spatial arrangements, while *N. tenuis* was more effective when the eggs are dispersed in all leaflets, i.e 2 eggs. In the second experiment, no significant differences were recorded in the egg consumption between the two predators on the tomato plants. However, the consumption rates were found much lower compared than other studies where 30 eggs of *T.absoluta* were placed on a single tomato plant in a cage. Conclusively, this study showed that complexity in spatial arrangement of *T. absoluta* eggs has an important impact in the foraging behavior of both predators and should further be considered in prey consumption studies of these predators.

**Keywords:** *Tuta absoluta*, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*, predation, prey distribution

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## P074. Assessment of predation behaviour of *Dicyphus cerastii* (Hemiptera: Miridae)

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The zoophytophagous mirids are polyphagous predators occurring naturally in many crops. Their high plasticity on their feeding strategies allows them to survive when prey is scarce, and consequently, allows for population upkeep. *Dicyphus cerastii* is among such species. It is a Palearctic species that is a potential candidate for conservation biological control, mainly in protected tomato crops. We aim at evaluating: the preference of *D. cerastii* among four prey pest species: nymphs of the aphid *Myzus persicae*, of the whitefly *Bemisia tabaci* and of the mealybug *Planococcus citri,* and eggs of the South American leafminer, *Tuta absoluta;* the diel pattern of predation, using *Ephestia kuehniella* eggs, under light and dark conditions, and comparing with that of *Nesidiocoris tenuis,* a mirid widely commercialized as a biological control agent; the aptitude for scavenging, using *M. persicae* as prey and tallying the amount of live and dead consumed. *Dicyphus cerastii* and *N. tenuis* were both active predators during day and night. *D. cerastii* was a more active predator during the day than *N. tenuis* during day and night. *D. cerastii* preyed on more dead aphids, showing preference to scavenging, and presented preference for *M. persicae, B. tabaci* and *T. absoluta* with similar consumption, and significantly lower consumption on *P. citri*. The defensive secretions of *P. citri* seemed to be effective at deterring *D. cerastii* from preying this pest.

**Keywords:** diel pattern, necrophagy, prey preference, aphids, whiteflies, *Tuta absoluta*

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## P075. Exploring the searching ability of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae) on different *Tuta absoluta* (Lepidoptera: Gelechiidae) egg densities and oviposition patterns

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The searching behavior of *Macrolophus pygmaeus* (*Mp*) and *Nesidiocoris tenuis* (*Nt*) on eggs of *T. absoluta* was investigated when eggs were offered at various densities (3, 5, 8 or 10), and following different distribution patterns on a leaflet in a Petri dish: a) The linear pattern b) grouped c) marginal, placing the eggs close to the leaflet margin and d) the natural pattern i.e. that *T. absoluta* females follow to lay the respective number of eggs on a leaflet. After 15′, 30′, 60′, 120′ and 180′ the number of *T. absoluta* consumed eggs was recorded. Our results revealed that in case that 3 eggs/leaflet positioned linearly or in group, *Mp* consumed more eggs than *Nt* at 15′. When 5 *T. absoluta* eggs/leaflet were positioned in group or in margin, *Mp* consumed more eggs in 15′ and 30′ than *Nt*. In case of 8 eggs/leaflet, higher number of eggs were consumed by *Mp* than *Nt* in 15′. In addition, when 10 eggs were offered, *Mp* consumed more eggs in 30′ and 60′ in all patterns except of the natural one. In conclusion, both predators showed a similar prey searching efficiency in the case that eggs were offered following the natural pattern. However, in most other cases *M. pygmaeus* was able to locate and consume the eggs of *T. absoluta* faster than *N. tenuis.* These results indicate that differences exist in the searching behavior between *M. pygmaeus* and *N. tenuis* which may have an impact in their comparative efficacy in biological control.

**Keywords:** predator, searching behavior, prey density, consumption rate, tomato leafminer

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## P076. Different densities, different predator. Evaluation of four aphidophagous predators in sweet pepper crops

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Aphids are one of the most common and problematic pests in horticultural ecosystems. Nowadays, its biological control in greenhouse crops relies mainly on periodical releases of parasitoids and the gall midge *Aphidoletes aphydimiza* (Rondani) (Diptera: Cecidomyiidae). However, these biocontrol agents do not always succeed, as they cannot survive or establish in the crop when aphids are scarce. The purpose of our study was to screen four different aphidophagous predators that might have potential for preventive aphid control in greenhouses: the ladybeetles (Coleoptera: Coccinellidae) *Scymnus interruptus* (Goeze), *Hippodamia variegata* (Goeze) and *Propylea quatuordecimpunctata* L., and the brown lacewing *Micromus variegatus* (Fabricius) (Neuroptera: Hemerobiidae). We evaluated their performance on two different aphid densities: low and high, and recorded their population densities. Trials were conducted under greenhouse conditions with sweet pepper plants infested with the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Our results showed that with low aphid densities *M. variegatus* was the most effective in suppressing aphid populations, and remained present in the host plant by the end of the 4-week-experiment. On the other hand, with high aphid densities *P. quatuordecimpunctata* was the most efficient species for aphid control. Our future studies will focus on the establishment of aphid predator populations in absence of pest aphids.

**Keywords:** Hemerobiidae, Coccinellidae, *Myzus persicae,* biological control

### P077. Characterization of an orange-nymph mutant in *Orius laevigatus* (Hemiptera: Anthocoridae)

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*Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) is a key predator in biological control programs in protected crops, used to control mainly thrips and other small pests. In 2021, a nymph was found in a mixture of wild populations, which did not present a normal yellowish coloration, but orange. This mutation showed a distinct orange color in the different nymphs stages. From that nymph, a laboratory strain carrying this body color mutation *ambar* was established. Genetic analysis determined that the mutation (*ambar*) was controlled by a single autosomal recessive allele. Some biological characteristics of the orange strain were compared with a normal population. Longevity, fecundity and fertility were similar in both populations, but body size, starvation tolerance and predation capacity were inferior in the orange strain. The utility of the orange mutant could be used as a visible marker for biological and ecological studies, such as to compare establishment and biocontrol services of different strains of this predator carrying or not the *ambar* mutation.

**Keywords:** *Orius laevigatus*, biological control, mutation, body color, recessive allele

## P078. Prey preference and intraguild interactions between *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) and *Macrolophus pygmaeus* (Hemiptera: Miridae) toward *Tuta absoluta* (Lepidoptera: Gelechiidae) biological control

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*Macrolophus pygmaeus* and *Trichogramma achaeae* are widely used in integrated pest management, including against the South American leafminer *Tuta absoluta*, a major pest of tomato crops in Europe. In this study we evaluate the potential of the natural enemies against *T. absoluta*. We assessed the voracity and prey preference of *M. pygmaeus* for *T. absoluta* eggs unparasitized or parasitized by *T. achaeae* and the effect of intra-guild interactions between the natural enemies on the number of *T. absoluta* eggs consumed and/or parasitized. Lastly, we evaluated, under field conditions, the impact of biotic interactions between *M. pygmaeus* and *T. achaeae* on *T. absoluta*. *Macrolophus pygmaeus* consumed more unparasitized than parasitized eggs. Under mixed diets, voracity decreases as the proportion of parasitized eggs increases and Manly indices reveal preference for unparasitized eggs. Conspecific interactions between *M. pygmaeus*, contrarily *T. achaeae*, reveal antagonistic interactions. For heterospecific interactions, the number of eggs of *T. absoluta* consumed by *M. pygmaeus* and parasitized by *T. achaeae* are smaller than predicted for additive and non-interactive scenario, and this indicate possible antagonistic interaction between biocontrol agents. Under field conditions, a slightly higher success rate was obtained when both biocontrol agents were used simultaneously, that is, adding *T. achaeae* may significantly increase the level of control of *T. absoluta* over what could be attained when only *M. pygmaeus* is present on tomato crops in greenhouses.

Keywords: augmentative biological control, *Tuta absoluta*, *Trichogramma achaeae*, *Macrolophus pygmaeus*, prey preference, intraguild interactions

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## P079. Screening for predators of *Aphis nerii* and *Myzus persicae* on *Mandevilla*

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*Mandevilla* (=*Dipladenia*) plants (Apocynaceae) are increasing in popularity in the greenhouse ornamental plant industry. These plants produce toxic cardenolides that, through the food chain, can affect natural enemies compromising biological control strategies. We conducted experiments to identify promising natural enemies able to suppress populations of two main aphid pests of *Mandevilla*: *Aphis nerii* (Boyer de Fonscolombe) and *Myzus persicae* (Sulzer). We tested four aphid predator species in semi-field conditions to evaluate their potential to control aphid populations. *Micromus variegatus* (Fabricius) and *Propylea quatordecimpunctata* (Linnaeus) reduced both aphid populations compared to the control. *Scymnus interruptus* (Goeze) showed contradictory results between *A. nerii* and *M. persicae. Aphidoletes aphidimyza* (Rondani) didn't show an effect on pest suppression. In addition, a laboratory experiment was carried out to clarify the effects of prey on the brown lacewings *Micromus variegatus.* On *Mandevilla*, a lower fecundity of *M. variegatus* females was observed on *A. nerii* than on *M. persicae*. These effects can be related to *A. nerii*'s ability to sequester toxic cardenolide produced by *Mandevilla*. Information obtained here will be used for future research on biological control strategies in this ornamental plant.

Keywords: *Mandevilla*, aphids, *Aphis nerii*, *Myzus persicae*, biological control, predator

## P080. Evaluation of the parasitoid *Ooencyrtus mirus* (Hymenoptera: Encyrtidae) as a biological control agent for *Halyomorpha halys* (Hemiptera: Pentatomidae): oviposition preference and development time on fresh and frozen eggs of different age

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The egg parasitoid *Ooencyrtus mirus* (Hymenoptera: Encyrtidae) is a potential biological control agent for the invasive pest *Halyomorpha halys* (Hemiptera: Pentatomidae). In this study, we conducted both no-choice and two-choice tests in all possible combinations to assess the oviposition preference of *O. mirus* towards eggs of *H. halys* of different status (fresh vs frozen) and age (1-day-old vs 3-days-old). Additionally, we monitored the development time of *O. mirus* larvae within the different treatments of *H. halys* eggs. Our results showed that in no-choice tests, *O. mirus* readily oviposited in both fresh and frozen *H. halys* eggs with no statistically significant difference (47.7 ± 3.4 and 47.2 ± 3.4, respectively), but showed a preference in 3-days-old eggs compared to 1-day-old ones (58.2 ± 3.3 and 36.7 ± 2.9, respectively). In two-choice tests, *O. mirus* showed 1.4 times greater preference for ovipositing on fresh eggs over frozen and 2.2 times greater preference for 3-days-old eggs over 1-day-old eggs. Furthermore, we observed that the development time of *O. mirus* was significantly shorter in fresh *H. halys* eggs than in frozen ones (15.6 ± 0.2 and 16.5 ± 0.2 days, respectively). Our study provides valuable information regarding the potential of *O. mirus* as a biological control agent for *H. halys*. The high acceptance and successful development of *O. mirus* within *H. halys* eggs suggest that this parasitoid could be an effective natural enemy against this invasive pest.

Keywords: *Ooencyrtus mirus*, fresh eggs, two-choice test, development time, *Halyomorpha halys*

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## P081. The effects of antimicrobials treatments on egg-infested olives targeting the primary endosymbiont of the olive fly

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*Bactrocera oleae* (Diptera, Tephritidae) is one of the main pests of olive orchards across the world. Its control has been traditionally conducted through synthetic insecticides that now have been revoked. For this reason, there is a growing demand to reduce chemical use due to environmental, human health and non-target organisms' concerns. In the recent years, researchers have proposed the manipulation of symbiotic bacteria inhabiting insect pests, as a new sustainable strategy, allowing insecticide-free control. In this case study, we tried to disturb the strict association between the olive fly and its primary endosymbiont "*Candidatus* Erwinia dacicola", attempting to disrupt the vertical transmission. Commercial products with putative antimicrobial activity were used for treating olives infested with eggs. Specifically, a zinc-copper-citric acid biocomplex showed a significant reduction of the symbiotic bacterial load in larvae as well as a reduction in the pupation rate of this pest. However, further aspects should be investigated, such as the timing for product application concerning the development of the wound in the olive, the addition of adjuvants that may increase the capillary capacity of the product and the used concentration. Overall, these results present future perspectives on the possibility of controlling the olive fly by targeting the eggs inside the fruit in a sustainable manner. These studies may then be useful for other closely related pests that harbor symbiont bacteria.

**Keywords:** *Bactrocera oleae*, primary endosymbiont, symbiosis disruption, vertical transmission

## P082. Interspecific competition between egg parasitoids of *Megacopta cribraria* (Hemiptera: Plataspidae): seasonal egg parasitism rates and host limitation

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The kudzu bug, *Megacopta cribraria* (Fabricius), was detected in the state of Georgia in 2009 but quickly invaded all the southeastern states in a few years and became a severe economic pest of soybeans due to the lack of natural enemies in the United States of America. Two exotic egg parasitic wasps, *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae) and *Ooencyrtus nezarae* (Ishii) (Hymenoptera: Encyrtidae), were discovered in soybean and kudzu fields in the state of Alabama after being infested 3 and 6 years, respectively. To understand host-parasitic wasps' population dynamics and parasitoids' interactions, a field survey investigated the seasonal egg-parasitism rates and host egg emergency dynamics on soybean (*Glycine max*) plants over five soybean seasons (2013-2017). The egg parasitism rate by the specialist parasitoid, *P. saccharalis,* ranged from 45% to 98% from 2013- 2015 but declined significantly from 45% to 11% from 2016 to 2017, and peaked in June and August.  This reduction was clearly due to the occurrence of the generalist parasitoid, *O. nezarae*. This generalist parasitic wasp competed for host eggs and super-parasitized the eggs parasitized by P. saccharalis. The parasitism rate by *O. nezarae* ranged from 15-90% in 2016-2017 and peaked in July, with a superparasitism rate of approximately 25%. These results indicate that the two parasitoids' interspecific interaction influences host egg masses' parasitism.

**Keywords:** Invasive insect, biological control, natural enemies interaction

## P083. *Psyttalia lounsburyi* (Silvestri) and *Psyttalia ponerophaga* (Silvestri) (Hymenoptera: Braconidae) as potential biological control agents of *Bactrocera o*leae (Rossi) (Diptera: Tephritidae) in Greece

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The existing indigenous natural enemies in Greece have proven insufficient in suppressing *Bactrocera oleae* populations. To address this issue, a classical biological control program was initiated in 2021 in Greece, introducing populations of *Psyttalia ponerophaga* and two strains of *Psyttalia lounsburyi* (from Kenya and South Africa) from the USDA-ARS European Biological Control Laboratory in Montpellier, France. These parasitoids are specialized in controlling *B. oleae* and were established in separate laboratory colonies at IMBB-FORTH, Greece, where they are reared on *Ceratitis capitata* (Weidemann) larvae fed on an artificial diet. Under semi-field conditions in Chania, Crete, the potential of the aforementioned parasitoids to control olive fly was evaluated in 2021 using field cages. The results showed that *P. ponerophaga* had a significantly higher percentage of parasitism (25%) compared to *P. lounsburyi* (Kenya and South Africa) (8.8% and 6.8%, respectively). Moreover, a hybrid strain resulted from the admixture of the two parental strains of *P. lounsburyi* (originated from Kenya and South Africa) was also evaluated using field cages, in September and October 2022. The percentage of parasitism ranged from 33.3% to 76.5% (mean 52.1%) and 24% to 55.5% (mean 35.9%) in September and October, respectively. These results are encouraging and suggest that the use of these specialized species of the genus *Psyttalia*, with low non-target risk, makes them a promising option for classical biological control programs in controlling *B. oleae* in Greece.

Keywords: *Psyttalia lounsburyi*, *Psyttalia ponerophaga,* olive fruit, *Bactrocera oleae*

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## P084. Using antimicrobial peptides as bifunctional molecules to control the bacterium ('*Candidatus* Liberibacter asiaticus') causing citrus greening disease and its vector the Asian citrus psyllid (*Diaphorina citri*)

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Citrus greening disease (Huanglongbing, HLB) is caused by a phloem-limited bacterium ('*Candidatus* Liberibacter asiaticus', CLas) transmitted by a single insect, the Asian citrus psyllid (ACP, *Diaphorina citri*). The ACP was first detected in Florida in 1998 and HLB was subsequently identified in Florida in 2005. HLB has caused a greater than 70% reduction in Florida commercial citrus production with most

of the processing plants closing and many producers going out of business. A biologically-based solution to HLB was explored by testing two antimicrobial peptides (AMPs), trypsin modulating oostatic factor from mosquitos (TMOF), and a truncated version of Oncocin, an antimicrobial peptide from *Oncopeltus fasciatus* (milkweed bug) for their effects on CLas and ACPs. Using a detached leaf assay, both AMPs showed activity against the CLas and the psyllid with an ~80% drop in leaf CLas titer and up to 80% mortality of CLas+ ACP fed for 7 days on leaves. Subsequently, transgenic citrus plants were developed to express these AMPs and the highest expressing lines demonstrated the greatest delay in ACP nymphal instar development, up to 60% reduction in nymphal emergence, and a 10X higher mortality rate as compared to the controls. The surprising mortality inducing effect on the ACP was hypothesized to be a response to their effect on obligate endosymbiotic bacteria within the psyllid and this is being evaluated further. These results have stimulated further studies to determine if AMPs can be used to prevent or alleviate HLB symptoms in commercial citrus production.

**Keywords:** citrus, citrus greening, Huanglongbing, HLB, antimicrobial peptides, psyllid

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## P085. Egg parasitism of stink bugs (Hemiptera Pentatomidae) by Scelionidae and other parasitoid wasps (Hymenoptera) in Greece

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Since the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera Pentatomidae), was first reported in Europe, great attention has been given to the evaluation of Biological Control Agents (BCAs) to be used to control the pest population, with special regard to egg parasitoids. An intensive campaign of Heteroptera egg collection has been applied in the newly expanded areas of *H. halys*, greatly improving knowledge of the biodiversity of egg parasitoid wasps (Hymenoptera) of stink bugs. In recent years, the community of pentatomid egg parasitoids has been investigated also in Greece. A two-year survey was conducted in six localities in Greece (Dion, Episkopi, Meliki, Nea Efessos, Stavros, Thermi). In total, 374 naturally laid egg masses (9,950 eggs) of *Apodiphus amygdali*, *Dolycoris baccarum*, *H. halys*, *Nezara viridula* and other unidentified species (Hemiptera Pentatomidae) were collected. Among them, a total of 29 egg masses were found to be parasitized by Encyrtidae, Eupelmidae and Scelionidae (Hymenoptera). *Trissolcus saakovi* and *Trissolcus* sp1 emerged from eggs of *A. amygdali*. *Ooencyrtus telenomicida*, *Telenomus* sp1 and *Tr. semistriatus* emerged from eggs of *D. baccarum*. *Anastatus bifasciatus*, *O. telenomicida*, *Telenomus* sp1, and *Trissolcus* sp2 emerged from eggs of *H. halys*. *Ooencyrtus telenomicida*, and *Tr. basalis* emerged from eggs of *N. viridula*. *Telenomus* sp1 and *Tr. basalis* emerged also from other unidentified species. The results obtained contribute to providing more knowledge to the community of bug egg parasitoids, enriching it with species reported here for the first time in Greece.

**Keywords:** Scelionidae, *Anastatus bifasciatus*, *Ooencyrtus telenomicida*, egg masses, first record

# P086. Synopsis on the complex of egg parasitoids of Heteroptera (Hemiptera) in two areas in Europe

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The suborder Heteroptera (Hemiptera) includes some important agricultural crop pests. Chemical control of these bugs is not always effective or applicable in all agricultural environments, and egg parasitoids play an important role as biological control agents (BCAs), even within classical biological control programs. The use of exotic species requires special care in assessing their impact on indigenous biocenosis, therefore a large monitoring program has been carried out in France and Italy, where the exotic *Trissolcus mitsukurii* and *T. japonicus* were released, respectively, as BCAs of *Halyomorpha halys*. Eggs of Heteroptera species were collected in the field since 2020 in France and 2016 in Italy. Data on habitat, host plant and bug species were recorded for all eggs. All the egg parasitoids emerged in laboratory were identified at species level. Overall, in the two countries eggs of seventeen Pentatomidae species, two Acanthosomatidae species, plus unidentified species of Coreidae, Reduviidae and Scutelleridae were collected. Overall, the parasitoid species emerged included: *Anastatus bifasciatus*, *A. catalonicus*, *A. gansuensis*, *A. japonicus* (Hymenoptera: Eupelmidae), *Hadronotus bosellii*, *H. leptocorisae*, *H. muscaeformis*, *Telenomus heydeni*, *Te. turesis*, *Trissolcus basalis*, *T. belenus*, *T. cantus*, *T. colemani*, *T. cultratus*, *T. elasmuchae*, *T. japonicus*, *T. kozlovi, T. manteroi*, *T. mitsukurii*, *T. semistriatus*, *T. tumidus*, *T. viktorovi* (Hym. Scelionidae), *Ooencyrtus telenomicida* (Hym. Encyrtidae), and the hyperparasitoid *Acroclisoides sinicus* (Hym. Pteromalidae). Knowledge on the community of egg parasitoids of indigenous Heteroptera represents the essential background for the evaluation of the biocenotic effects of any classical biological control strategy.

**Keywords:** BCAs, France, Italy, *Trissolcus japonicus*, *Trissolcus mitsukurii*

## P102. Do spray nozzles of foliar application affect the performance of *Beauveria bassiana*?

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Entomopathogenic fungi are a sustainable alternative for crop protection but elements of spray equipment for its application could affect them. The aim of this study was to evaluate the effect of nozzle type and working pressure on *Beauveria bassiana* Vuillemin conidia viability and their infectivity over *Ceratitis capitata* Wiedemann. For that, different nozzles were tested: ATR-brown, ATR-orange (hollow cone, extra-fine droplets class), XR110-yellow (Flat, fine droplets class), TVI-orange and TVI-yellow (hollow cone, coarse and extra-coarse droplets class, respectively) at 1 MPa. ATR-brown was also tested at 1.5 MPa and 2 MPa. The viability was assessed through the conidia germination percentage of mixture having passed through each nozzle. To assess infectivity, medfly adults placed in Petri dishes were sprayed and mortality percentage was evaluated. Also, quantitative, and qualitative characterization of the spray deposition with each nozzle was performed. Results showed that ATR-brown at 2 MPa, and XR110-yellow and ATR-orange at 1 MPa significantly reduced the germination percentage respect to control, but all of them reached 90%. XR110-yellow nozzle produced the highest mortality percentage of *Ceratitis capitata* with non-significant differences with the two TVI, and ATR-orange, that were the nozzles that produced higher deposition and/or larger droplets. Significantly lower mortality percentage was found with ATR-brown independently of the pressure, which were the nozzles that produced the lowest deposition and droplet size. Individuals sprayed with ATR-brown at 1 MPa, presented the significantly lowest fungal out-growth percentage. In conclusion, nozzles affect efficacy of *B. bassiana* against *C. capitata* and it is important to consider.

**Keywords:** entomopathogenic fungi, pressure, flow rate, biopesticides, spray application technology

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## P103. Exploring the transmission of viral infections in the agricultural pest *Ceratitis capitata*

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The Mediterranean fruit fly (medfly) *Ceratitis capitata* is a polyphagous species causing economic losses in agriculture worldwide. The most widespread strategy for medfly control is the sterile insect technique (SIT), which depends on the mass-rearing and release of sterile males. In this context, the presence of pathogens infecting medfly represents a great risk to the efficacy of mass rearing and the subsequent application of the SIT. Specifically, up to 13 RNA viruses have been described in medfly, although no correlation with lethal symptoms has been observed for any of those viruses. Nevertheless, recent studies indicate that an infection with Ceratitis capitata nora virus causes detrimental effects in medfly biology, suggesting that covert viruses could influence medfly ecology, the production of medflies in mass-rearing facilities, and the efficacy of the SIT. To determine how viral infections can be established and maintained in medfly populations, we explored the transmission routes of several RNA viruses. First, we assessed the viral abundance in medfly developmental stages and adult tissues using quantitative

PCR, and we visualized RNA viruses in adult tissues using fluorescence in situ hybridization. Overall, the viral abundance in different developmental stages and tissues was specific for each virus species, suggesting that each virus may have a preferred route of transmission. To confirm this hypothesis, we explored the viral transmission by analyzing the viral abundance in sterilized and non-sterilized eggs, and through crossing experiments. Results confirmed that the main transmission route varied between virus species, although vertical and horizontal transmission can simultaneously occur.

Keywords: RNA virus, covert infection, viral transmission, Tephritidae

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## P104. Application of molecular communications for the development of an entomopathogenic nematode-plant growth promoting bacterial bioagent for plant health

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This contribution presents an innovative project that has just commenced at the South East Technological University (SETU) in Ireland, aiming towards the development of a novel bioagent for sustainable plant health, assisted by computational synthetic biology for molecular communications. Molecular communications characterise and develop communication systems from biological components, such as those proposed in this project. The bioagent will be a combination of (1) entomopathogenic nematodes, which are well established and environment friendly biological insecticides, and (2) endophytic bacteria which normally live inside plants, and/or in close proximity and association with plant roots in the soil. Once they colonise the plant, they cause plant growth promotion reducing the need for application of environmentally harmful conventional mineral fertilisers. In addition, they enhance plant immunity against plant pathogens. Previous work in enviroCORE at SETU revealed that these groups of organisms interact well, so there is good potential for research to explore this interaction further. The proposed project will therefore build on previous such findings from enviroCORE and utilise expertise from the Walton Institute, in the form of molecular communication analysis. This will help better elucidate and understand the association between these groups of organisms, with the main aim to use the new knowledge towards developing the novel plant health bioagent.

Keywords: entomopathogenic nematodes, plant growth promoting bacteria, molecular communications, computational synthetic biology, plant health

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## P105. Towards a holistic and sustainable control strategy against the invasive pest *Drosophila suzukii*

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Invasive insect species pose an increasing threat to biodiversity and cause extensive damage in agricultural production. The most common control method against insect pests still relies on synthetic insecticides, but the wide use of broad-spectrum insecticides comes along with negative consequences on health and the environment. As a consequence, growers are confronted with an increasing number of bans restricting the types of chemical insecticides that can be applied. Innovative and sustainable strategies are therefore urgently needed to control the spread of insect pests. Here, we are using the invasive insect pest *Drosophila suzukii* as a model host for the development of biological practices based on entomopathogenic microorganisms. *D. suzukii* infests ripening fruits and causes extensive damage to crops worldwide. We isolated microbes including viruses of moribund *D. suzukii* larvae and performed infection studies with the strains to test their potential insecticidal activity. The studies were complemented with the examination of the host response upon infection using in vivo and in vitro models. One promising candidate as control agent is the La Jolla virus (LJV), a positive sense RNA virus belonging to the Iflaviridae. Further strategies will be presented. Our work provides new tools for the development of biological control agents that protect crops against *D. suzukii*.

**Keywords:** spotted wing drosophila, entomopathogens, biological control, insect virus

### P107. Combination of botanical insecticide and entomopathogenic fungus as a better control agent against *Riptortus pedestris* (Hemiptera: Alydidae)

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*Riptortus pedestris* (Fabricius) is a major soybean pest that causes injury by piercing and sucking the pods and seeds. Growers usually rely on synthetic chemical insecticides to control *R. pedestris*, but alternative control agents are needed for environment-friendly management of the pest. As an effort to develop better microbial pesticide using an entomopathogenic fungus (EPF) of *R. pedestris*, *Beauveria bassiana* ARP14, we evaluated the synergistic effect of a botanical insecticide (BI) containing azadirachtin on EPF in a series of bioassays using method of topical application against eggs and second instars of *R. pedestris*. At ten days, the hatching rates for the BI and the BI/EPF mixture were all lower (33%) than those for EPF (73%) or no treatment control (100%). Similarly, in the assay of second instars in the absence of food sources, the $LT_{50}$ values for BI and the BI/EPF mixture were almost 1.8 times lower than that for singly applied EPF. More interestingly, when food was provided for the second instars, faster mortality was observed after becoming the third instars in the treatment of BI/EPF mixture compared to BI or EPF applied singly. Furthermore, mycosis rate was 3.5 times higher in maximum when BI/EPF mixture was applied compared to single application of EPF. These results suggest that there is a synergistic effect of BI containing azadirachtin on the EPF against second instars of *R. pedestris*. Thus, the BI/EPF mixture can be a potential control agent to replace synthetic chemical insecticides used against *R. pedestris*.

**Keywords:** *Beauveria bassiana*, azadirachtin, synergism, microbials, mycosis

## P108. Biological control of *Halyomorpha halys* (Hemiptera: Pentatomidae) on kiwi trees *Actinidia deliciosa* var. Hayward using a formulation with *Beauveria bassiana* (Hypocreales: Cordycipitaceae)

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Entomopathogenic fungi (EPF) consist of a wide range of fungi that can be used as pest control agents, endophytes, and plant growth promoters. In this work, the action of a commercial formulation with *Beauveria bassiana* (Hypocreales: Cordycipitaceae) in the biological control on the new sucking pest in Greece *Halyomorpha halys* (Hemiptera: Pentatomidae) on kiwifruit cultivation, was evaluated in field conditions. The experiment was carried out in an experimental field of commercial kiwifruit *Actinidia deliciosa* var. "Hayward" in Arta, Greece. The application of the formulation was performed in 3 different ways in the kiwi crop: (S) Trunk Spray, (R) Root Injection and (I) Trunk Inoculation. No formulation treatment was used as a positive control and biostimulant treatment enclosed with humic and fulvic acids was used as a negative control. During the 127-day experiment, weekly measurements of the *H. halys* population in sticky traps were determined. The insect sucks the nutrients from the plants, and for this reason, the total chlorophyll content (TCHL) in leaves of the treatments was recorded on a weekly basis and at the end of the experiment the number of fruit infestations was estimated. It was shown that the entomopathogenic fungus *B. bassiana* can be used for biological control of *H. halys* in kiwifruit culture even with a single application. This result is quite encouraging for the Mediterranean areas affected by the new exotic pest.

Keywords: *Halyomorpha halys, Beauveria bassiana*, *Actinidia deliciosa*

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## P109. Mycovirus-related differential response of a *Beauveria bassiana* strain to abiotic and biotic stresses

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Entomopathogenic mitosporic ascomycetes (EMA) can have mycoviral infections that either have not a detectable effect on the infected fungal strain or cause dramatic changes in their fungal hosts such as irregular growth, abnormal pigmentation, altered sexual reproduction or modification of the virulence of the infected strain. Nonetheless, there is scarce information, if any, on the possible adaptative effect of these viral infections on the strain response to key environmental stresses. In the present work, we investigated whether *Beauveria bassiana* (Bals.) Vuill., wild-type strain EABb 01/126-Su infected with a double strain RNA virus (dsRNA) mycovirus and this same mycovirus-free strain varied their response to temperature, water activity and ultraviolet radiation (UV-B), as well as their *in vitro* antagonistic activity against *Trichoderma* spp. Interestingly, the presence of the mycovirus led to broadening temperature range for growth and germination, and improved drought stress and UV-B stress responses of the fungal strain. *Additionally, the mycovirus-free strain was antagonized by T. harzianum, while the wild strain grew radially without inhibition.* These results indicate the response of EMA to abiotic and biotic stresses can be modulated by dsRNA mycoviruses, which can have a key influence on selecting environmentally competent EMA strains for mycoinsecticide development.

Keywords: *Beauveria bassiana*, mycovirus, temperature, water activity, UV-B radiation, antagonism

### P110. Unraveling the cause of aphid mortality in melon plants endophytically colonized by entomopathogenic fungi

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Entomopathogenic fungi have been successfully used for aphid control within microbial pest control by direct spraying on crops. In addition, new ecological roles of these fungi as plant endophytes have recently been described. In the present work, the lethal and sublethal effects of three entomopathogenic fungal strains on the cotton aphid, *Aphis gossypii*, were investigated; the insects encountered the inoculum while feeding on leaves of endophytically colonised melon plants using three different methods of fungal application. The effect of endophytic colonisation of the plant on aphids was evaluated over time: 2, 7, 14, 21 and 28 days (S1, S2, S3, S4, and S5 respectively) after fungus application. Although the percentage of endophytic colonisation in plants decreases with each sample time, the results showed constant aphid mortality rates, between 44 and 80% for *M. brunneum* endophytically colonised plants, and between 52 and 84% for *B. bassiana* endophytically colonised plants over time. However, fungal outgrowth from the cadavers decreased from the first sampling (2 days) to the last sampling (28 days) dates. These results suggest that endophytic colonization by entomopathogenic ascomycetes could cause both direct mortality by targeting by contact the aphid specimens in the plant and by activating the induced resistance genes expression in melon plants, therefore activating their defenses against this aphid species.

Keywords: *Aphis gossypii, Beauveria bassiana, Metarhizium brunneum, Cucumis melo*, induced resistance, tritrophic interactions

## P111. Clues from cues: inferring *Anagonia lasiophthalma* safety from a simple experimental design

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Environmental risk assessment is a critical step on the implementation of a classical biological control programme against insect pests. Testing the host range of a biological control agent is crucial prior to its release outside its native range. *Anagonia lasiophthalma* (Diptera: Tachinidae) is a larval natural enemy of the *Eucalyptus* snout beetle *Gonipterus platensis* (Coleoptera: Curculionidae), both native to Australia, which has been studied for its safety for release. Results of those tests indicate that the species has a narrow host-range that might be limited to *G. platensis* in the introduced area (Portugal). Despite that, traditional quarantine host-range testing in larval parasitoids has several limitations. As such, an additional precaution against non-target effects from an exotic biological control agent is the understanding of its biology and the mechanisms it relies on for host selection. In dipterans, such mechanisms of host selection are related to their oviposition strategy and depend on physical stimuli, such as host size or movement, and on chemical cues, both from the host and the host plant, and cues derived from the interaction between the host and the host plant, such as host-induced plant volatiles or frass. Here, we present the results from a simple experimental setup designed to test how chemical cues emitted by eucalypt (host plant) and *G. platensis* larvae (host) may influence *A. lasiophthalma* foraging behaviour, and how these results aid the assurance of this natural enemy's environmental safety as a biological control agent.

Keywords: *Anagonia lasiophthalma*, *Gonipterus platensis*, larval, eucalyptus, biological control

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## P112. Evaluation of induced effects of two novel photoreactive substances against the whitefly *Bemisia tabaci*

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*Bemisia tabaci* is a devastating crop pest that has developed resistance on the majority of insecticides, worldwide. Looking for alternative insecticidal methods, like pest photo-inactivation, the activity of two photosensitizers, (*Z*)-*N'*-(4-chlorophenylcarbamoyloxy)-3-nitrobenzimidamide (**1**) and 6-nitro-quinazolin-4(*3H*)-one (**2**), belonging to different chemical classes of compounds has been evaluated. Novel compound **1** is an amidoxime carbamoylester which posses a light sensitive N-O bond able to homolyse and produce radicals that possibly destroy biomolecules. Compound **2**, under photodynamic mode of action was found to create powerful singlet oxygen. The toxicological bioassays followed for these two compounds were based on IRAC methods 015 and 016 in order to test their possible lethal effects on *B. tabaci* adults and nymphs (L2 instar), respectively. Compounds were dissolved in dimethylformamide (DMF) and an emulsifier (EM). Aqueous solutions at 400 mgL$^{-1}$ were used, in

aforementioned experimental protocols for nymphs and adults. Insects were allowed to feed on treated plant tissues with or without 24h exposure under UVA irradiation (365 nm) for photosensitizer activation. The mortality of adults by compound **1**, was 25% under UVA irradiation, whereas it was significantly lower (0,2%) when UVA irradiation was absent. In contrast, UVA irradiation had no significant effects on nymph mortality. Compound **2** exhibited marginal mortality on nymphal stage (17%) with no effect of UVA irradiation exposure, while zero mortality on adults was observed in both cases. These basic experiments are providing encouraging results for the development of novels pesticides that could by activated via selective UV irradiation exposure (i.e., under controlled greenhouse environments).

Keywords: *Bemisia tabaci*, amidoxime esters, UVA irradiation, quinazolinones

## P113. *Allium sativum* essential oil-based insecticides: target and non-target toxicity

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The ecotoxicological, environmental and social consequences of the widespread and often indiscriminate use of synthetic insecticides in agriculture have led researchers to find more sustainable alternatives to conventional insecticides. Among the alternative solutions, botanical extracts, and in particular essential oils (EOs), are receiving significant attention from researchers and consumers. EOs, due to their wide distribution across the globe, relative affordability, rapid degradation, and proven biocidal activity, seem to be the ideal candidates for the development of innovative and environmentally sustainable insecticide formulations. We report the results of two different types of *Allium sativum* EO-based insecticide formulations namely nanoemulsion (NE) and emulsifiable concentrates (EC) for the control of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) and *Planococcus citri* Risso (Hemiptera: Pseudococcidae). Side effects towards two non-target organisms, specifically *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) and *Apis mellifera* L. (Hymenoptera: Apidae) were also assessed.

The developed EO-based insecticide formulations (NE=15% and EC=50% a.i., respectively) had particle sizes in the nanometric range with different dimensions depending on the production method.

The toxicity against the two key pests showed, for both formulations, a dose-dependent pattern that fit the Probit model. The low negative effects towards the two non-target organisms suggest a potential use of these formulations in organic and/or IPM programs.

Keywords: botanicals, nano-insecticide, *Planococcus citri, Spodoptera littoralis*

## P114. Intra and interspecific competition between *Trissolcus mitsukurii* and *Trissolcus japonicus*, parasitoids of the brown marmorated stink bug

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The brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), native to east Asia, is an invasive pest of agricultural crops in southern Europe and north America. *Trissolcus japonicus* (Ashmead) and *T. mitsukurii* (Ashmead) (Hymenoptera, Scelionidae) are the main parasitoids of *H. halys* in its native area. Adventive populations of both parasitoids have been found in Italy since 2016 and, starting from 2020, classical biological control programs against the invasive stink bug are carried out in northern Italy using *T. japonicus*. Previous studies on interspecific competition performed in petri dishes showed that both *T. japonicus* and *T. mitsukurii* were able to parasitize an eggmass already parasitized by the other species and the order of oviposition was crucial for the parasitization success of each species. In this study, we investigated both interspecific and intraspecific competition in a more complex environment by supplying each parasitoid species with *H. halys* egg masses previously parasitized by the other/same species. Egg acceptance, oviposition activity and parasitism efficiency were recorded. A Y- tube olfactometer was used to assess whether the parasitoid presence on the egg mass or the footprints left on the egg mass surface at different time intervals from the oviposition by one parasitoids species discouraged the other species or a conspecific in approaching the eggs. The results will be presented and discussed.

Keywords: competition, parasitoids, *T. japonicus*, *T. mitsukurii*, Y-tube, behaviour

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## P115. Bioprospecting the environmental microbiome of Crete towards the development of novel biopesticides

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Increased emergence and spread of resistance against current insecticides amongst a wide range of both disease vector and agricultural pest insects has rendered ineffective many traditional forms of chemical control. This trend poses serious threats to public health, food security and economies relying on agriculture. Furthermore, the negative impact of many insecticides on human and animal health, and the environment, has resulted in new regulations favouring the development of 'green' biopesticides within the European Union. Prospective biopesticides include entomopathogenic bacteria

and fungi as living organisms, alongside their inactivated forms and derived secondary metabolites. We have extensively surveyed a range of diverse Cretan natural habitats to establish a library of over 1500 microorganism isolates. Bioassays against larval stages of the mosquito species *Culex pipiens molestus* and *Aedes albopictus*, as well as the model agricultural pest *Tenebrio molitor*, has yielded a collection of microbes with entomopathogenic activities that appear to rely on different modes of action and to meet basic criteria for biopesticide development. Our ongoing studies are focusing on the spectrum of activity, mode of action and other characteristics of promising candidates to evaluate their suitability for biopesticide development and commercialization and use within integrated vector and pest management programmes.

**Keywords:** biodiversity, biopesticides, microorganisms, mosquito

## P116. A comparison of commercialized and wild populations of housefly parasitoids using molecular and behavioural approaches

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*Spalangia cameroni* and *Muscidifurax raptor* are pupal parasitoids of *Musca domestica* commonly used worldwide for its biocontrol. However, in Italy there is a lack of knowledge on the molecular characterization and distribution of natural populations of these parasitoids and dairy farms mostly rely on commercial populations for biocontrol purposes. This study aims to investigate the distribution and the behavioural and molecular characterization of these parasitoids in Emilia Romagna region (Northern Italy) at population level, and to compare parasitization rates and performances between commercialized and wild populations. Dairy farms where parasitoids releases had never been previously conducted were monitored every two weeks between May and September 2021-2022 to collect housefly pupae. For each parasitoid species iso- female lines have been established, starting both from parasitoids emerged from sampled pupae and from parasitoids purchased from insects farms. Subsamples of each population were used for molecular identification and mitochondrial cytochrome c oxidase subunit I gene (COI) was analyzed in both species. In addition, single mated females (n=30) for each species and population were provided with 15 housefly pupae each, and their parasitization activities were recorded for 3 hours. Results on parasitization efficiency (number of parasitized pupae and time required for the parasitization), rate of housefly emergence, parasitoid sex ratio and DNA barcoding characterization will be presented and discussed.

**Keywords:** parasitoids, houseflies, DNA barcoding, biocontrol, behavior

## P117. Antimicrobial properties of the chitosan from different developmental stages of the bioconverter insect *Hermetia illucens*

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Chitin and its deacetylated derivative, chitosan, have various applications in biomedical and pharmaceutical fields. The market needs to have these two biopolymers readily available has led to the search for alternative sources to crustaceans, the commercial source. The bioconverter diptera *Hermetia illucens*, is one of the most exploited, thanks to the possibility of recovering waste materials from its breeding. Chitin can be extracted from different developmental stages and biomasses of *H. illucens* (larvae, pupal exuviae and dead adults) and converted into chitosan. Chitosan has some important properties such as biocompatibility, biodegradability, non-toxicity, antioxidant, humectant and antimicrobial activity. This chitosan quality makes it particularly versatile for pharmaceutical and medical applications, as well as innovative pesticide and insecticide. Some pathogens have acquired new mechanisms of drug resistance, leading to antimicrobial resistance, that makes the human body progressively weaker to fight and deal with common infections. New antibacterial molecules are needed to tackle this problem. Among them, natural ones can be a safe alternative solution. After protonation in acid conditions, chitosan can inhibit the proliferation of many bacteria, fungi and yeasts. The chitosan antimicrobial activity depends on its chemical-physical characteristics, mainly molecular weight and degree of deacetylation, and on some specific experimental conditions, such as temperature and pH. The evaluation of chitosan antimicrobial activity was carried out through agar diffusion test and microdilution assay. Bleached and unbleached chitosan from larvae, pupal exuviae and dead adults of *H. illucens* induced the formation of inhibition zones. This important property was also confirmed by microdilution assay.

**Keywords:** chitosan, antimicrobicity, black soldier fly

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## P118. Soil treatment with Botanigard®WP22 (*Beauveria bassiana* GHA): *ON and OFF-season biocontrol tool of Ceratitis capitata*

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Control of fruit flies (Diptera: Tephritidae) is mainly based on insecticide treatments targeting the adults. Targeting the soil-dwelling stages by soil treatment with an entomopathogenic fungus is a strategy to consider. Botanigard®WP22 is a commercially mycoinsecticide based on the *Beauveria bassiana* for use as a spray. The objective of this study was to examine the efficacy of Botanigard®WP22 as soil treatment in orchards, targeting the soil-living stages of *Ceratitis capitate.*

Two sets of experiments were carried out on late L3 larvae. The first consisted in testing a high dose of $10^7$ conidia/g of soil of Botanigard®WP22 in apple orchards in Italy. The second was carried out to test in the laboratory doses ($10^5$, $10^6$ and $10^7$ conidia/g of soil) of Botanigard®WP22 and temperature (10, 15, 20, 25°C) effects. The fungus was able to maintain itself in the soils of apple orchards reducing significantly the emergence of flies for at least one year. Laboratory experiments demonstrated i/ that Botanigard WP22 soil treatments significantly reduced emergence and increased mortality of emerged

adults of *C. capitata* whatever the dose and temperature tested. *C. capitata* mortality was positively correlated with the dose of Botanigard®WP22 and that 2/ mortality was negatively correlated with the temperature, which demonstrated that it can provide an OFF-season control of *C. capitata*. At low temperature, the fungus remained active while the insect developed slowly or did not.
This biocontrol strategy could be suitable to target the first and the latest generations of *C. capitata* produced in spring and in autumn.

**Keywords:** biological control, Mediterranean fruit fly, *Beauveria*

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## P119. Toxicity of eight essential oils to *Trialeurodes vaporariorum*

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The resistance of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), caused by the overuse of synthetic chemicals and the associated side effects on the environment and non-target organisms, urgently promotes other efficient, environmentally friendly and sustainable strategies for its control in the Integrated Pest Management framework. Plant essential oils (EOs), having a safer toxicological profile, can fully or partially replace synthetic insecticides playing thus an important role as an alternative control tool for agricultural pests. On the other hand, incorporating EOs into controlled-release nanoformulations could help overcome practical application problems (e.g., phytotoxicity, stability and degradation processes) and potentially develop efficient bioinsecticides. In laboratory conditions, we evaluated the baseline toxicity of eight nanoformulated EOs on fourth-instar whitefly nymphs, following topical contact exposure. The results of the bioassays showed that all tested EOs caused significant mortality on *T. vaporariorum* fourth-instar nymphs, with 10-75 fold lower $LC_{50s}$ (median Lethal Concentrations) compared to the maximum applied concentration. Rosemary EO was the most toxic compound (1.728 mg a.i./l), followed by peppermint, garlic, lavender and fennel EOs (2.039, 2.358, 2.680 and 3.185 mg a.i./l, respectively), while the least toxicity to fourth-instar whitefly nymphs was estimated for artemisia, anise and sage EOs (5.252, 8.781 and 14.978 mg a.i./l, respectively). However, the potential application of these new-generation bioinsecticides relies on their safety towards beneficial arthropods, besides their efficacy against *T. vaporariorum*. Therefore, further research should focus on the risk assessment of EOs on biological control agents used for the management of the greenhouse whitefly.

**Keywords:** biopecticide, essential oils, greenhouse whitefly, nanotechnology, toxicology

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## P120. Effective irradiation and dormancy based mass rearing protocol for propagation of biological control of insect pests

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In Southeast Asia, food supply is commonly based on smallholder structures, especially for important staple foods such as cereals. Commercial biological control agents (BCA) are becoming increasingly important in modern, sustainable agriculture in SE Asia including Bangladesh. Mass production as well as quality of parasitoidsis the key factor for success of implementation of biological control. Our findings showed that the techniques of irradiation and dormancy in insect host may prove as potential for enhancing mass production of parasites.Results showed that the use of irradiated *Galleria mellonella* and *Plodia interpunctella* larvae at the dose of 150 Gy proved useful for enhancing the parasitism and adult emergence of *Harbobracon hebetor* due to the absence of negative repercussions on parasitoid development. Overall, parasitism was increased as the host larvae was irradiated with higher doses, while significantly higher parasitism was recorded at 150 and 300 Gy compared to lower doses. Moreover, *H. hebetor* adult progeny production from dormant host larvae was higher than the number of progeny produced on non-dormant host larvae. The implementation of these findings would be helpful for improving the mass production of parasitoids and the effectiveness of releases of biocontrol agents for the control of insect pests.

Keywords: mass rearing,biological control, parasitoids, Lepidoptera, irradiation

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## P121. Application as culture substrate for chitinase-producing microorganisms using chitin shells obtained from Korean cicada (*Cryptotympana atrata*) sheaths

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In this study, chitin shells obtained from Korean cicada (*Cryptotympana atrata*) sheaths was used as a culture substrate for chitinase-producing microorganisms. In the first step as washing cicada sheath, it was carried out by two methods, ethanol washing and water washing. After washing of ethanol and water, the dry weight and yield of cicada shells were investigated. In the case of ethanol washing, the dry yield was 71.58%, and in the case of water washing, it was 73.8%. Colloidal chitin was prepared from washed cicada sheaths to use as a chitinase-producing microbial culture substrate. The activity of chitin-degrading enzymes was investigated by date during the cultivation of chitinase-producing microorganisms. The production of chitin oligosaccharides from colloidal chitin of cicada shells was investigated using activated carbon column in response to the crude enzyme of chitinase-producing microorganisms.

Keywords: *Cryptotympana atrata*, colloidal chitin, chitinase-producing microbial, chitinase activity, chitin oligosaccharides

## P122. Modification of aphid probing behavior by plant flavonoids quercetin and rutin

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Rutin and its aglycone quercetin occur in the fruits, leaves, seeds, and grains of many plant species and are involved in plant-herbivore interactions. Due to flavonoid nutritional importance, breeding attempts, conventional and involving genetic engineering, have been made to increase flavonoid levels in plants. It is often expected that the elevated levels of secondary plant compounds in plant tissues may protect these plants against pathogens and herbivores. We studied the effect of the exogenous application of rutin and quercetin on the probing behavior (= stylet penetration activities in plant tissues) of *Acyrthosiphon pisum* on *Pisum sativum, Myzus persicae* on *Brassica rapa* ssp. *pekinensis*, and *Rhopalosiphum padi* on *Avena sativa* using the electrical penetration graph technique (EPG = electropenetrography). The response of aphids to quercetin and rutin and the potency of the effect depended on aphid species, the flavonol, and the flavonol concentration. Quercetin promoted probing activities of *A. pisum* within non-phloem and phloem tissues, which was demonstrated in the longer duration of probes and a trend toward a longer duration of sap ingestion, respectively. *M. persicae* reached phloem in a shorter time on quercetin-treated *B. rapa* than on the control. Rutin caused a delay in reaching sieve elements by *A. pisum* and deterred probing activities of *M. persicae* within non-phloem tissues. Probing activities of *R. padi* were not affected by quercetin or rutin. The potency of behavioral effects increased as the applied concentrations of flavonols increased. The prospects of using quercetin and rutin in plant protection are discussed.

Keywords: flavonoids, pea aphid, bird cherry-oat aphid, peach-potato aphid, stylet penetration, antifeedants

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## P123. Attracting performance of the pheromone neryl (S)-2-methylbutanoate and kairomones towards the western flower thrips (*Frankliniella occidentalis*)

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Understanding olfaction in insects allows more specific alternative methods of pest control. We combined here analytical chemistry and behavioral assay to evaluate the responses of the western flower thrips (*Frankliniella occidentalis*), a major agricultural pest, to estimated gas phase concentrations of the aggregation pheromone (neryl (S)-2-methylbutanoate) and known kairomones such as methyl isonicotinate, verbenone and p-anisaldehyde. The behavioral responses were evaluated in a Y-olfactometer where insects were exposed to air transporting the olfactory stimuli released from filter papers in wash-bottles. The gas phase concentrations of these compounds were deduced from the release rates measured in dynamic headspace cells. The compounds were collected from the headspace with dried SPE cartridges and analyzed with a triple quadrupole GC-MS/MS. We show that the proportion of thrips attracted increased as a function of the dose loaded on the filter papers, except for verbenone that produced bell-shape results. Although the pheromone produced the highest attraction rate, it was closely followed by methyl isonicotinate and p-anisaldehyde. A completely different picture is obtained when the release rate is considered. The pheromone evaporated at a rate at least one order of magnitude lower than the other compounds did (pheromone < p-anisladehyde < verbenone = methyl isonicotinate). This means that the pheromone produced a gas-phase concentration at least one order of magnitude smaller than methyl isonicotinate and p-anisaldehyde did. Thus, the pheromone performed far better than the other compounds to attract thrips. The performance of cellulose, rubber septum and bentonite to release attractants was evaluated too.

**Keywords:** chemical ecology, olfactometry, release rate, thrips

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## P124. Effects of natural insecticides on the green peach aphid *Myzus persicae* (Sulzer) and its natural enemies *Propylea quatuordecimpunctata* (L.) and *Aphidius colemani* Viereck

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Natural insecticides are frequently advocated as environmentally safer alternatives to synthetic insecticides. The efficacy and selectivity of these plant protection products, however, are often only partially supported by empirical evidence. In this study, we tested the effectiveness of four natural pesticides belonging to different categories against the green peach aphid *Myzus persicae* (Sulzer), as well as their selectivity on two of its natural enemies, the ladybird *Propylea quatuordecimpunctata* (L.) and the parasitoid *Aphidius colemani* (Dalman). The tested products (used both in direct and residual exposure assays) were white thyme essential oil (EO), a commercial product based on sweet orange EO, crude garlic extract and Marseille soap. Pyrethrum based commercial insecticide was included as a positive control and deionized water was used as negative control. Tested products were scarcely effective against aphids if compared with pyrethrum, and no difference among treatments was detected on parasitoid emergence from sprayed aphid mummies. All products were in general less detrimental to ladybird larvae than pyrethrum. Therefore, if applied against other pests than aphids, they have a lower chance of disrupting the aphid biocontrol agents in real-world scenarios. Marseille soap and sweet orange EO had insecticidal activity when directly sprayed on ladybird larvae, and thyme oil caused extensive phytotoxic effects on the cabbage leaves used as a substrate in the experiments, possibly indirectly leading to mortality in ladybird adults via biofumigation. Our findings underline the necessity for case-by-case evaluations of natural insecticides, considering both efficacy and side effects, all while avoiding broad generalizations.

**Keywords:** botanical insecticides, selectivity, essential oils, ladybird beetles, parasitoids, integrated pest management

## P125. Identification and functional characterization of *Toxoneuron nigriceps* ovarian proteins involved in the early suppression of host immune response

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*Toxoneuron nigriceps* (Hymenoptera, Braconidae) is an endophagous parasitoid of the larval stages of *Heliothis virescens* (Lepidoptera, Noctuidae), the tobacco budworm. During oviposition, T. nigriceps injects into the host body the egg, the venom, the calyx fluid, which contains a Polydnavirus (*T. nigriceps* BracoVirus: *Tn*BV), and the Ovarian Proteins (OPs). OPs, originating from the ovarian calyx cells, are involved in the induction of precocious symptoms in the host immune system alteration. Indeed, although viral gene expression in the host reaches detectable levels within a few hours, the host metabolism and immune system are disrupted shortly after parasitization. To functionally characterize the OPs, we carried out two approaches. Firstly, incubating hemocytes with OPs *in toto*, detecting several alterations on host cells Then, we evaluated the effect of HPLC fractions deriving from *in toto* OPs. Among the 28 analyzed fractions, two fractions caused a reduction in hemocyte viability and were tested to detect changes in hemocyte morphology and functionality. In accordance with previous results, obtained with *in toto* OPs, the two fractions caused severe oxidative stress, actin cytoskeleton disruption, loss of hemocyte encapsulation ability and high mortality rate. Moreover, a transcriptome and proteomic approach was applied to identify all OPs proteins and particularly the proteins of the two fractions. The eight detected proteins might be involved in the observed host hemocyte changes. Our findings will contribute to a better understanding of the ovarian components and their role in parasitic wasp strategy to escape the host immune responses.

**Keywords:** maternal factors, host-parasitoid interaction, proteomic and transcriptomic approach

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## P126. The long and challenging road to capitalize on plant-based extracts use against agricultural crop pests in Senegal

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Reducing pesticide use for plant health has become a goal shared by CILSS and ECOWAS countries and a major issue in public policies due to their negative footprint on the environment, human health, and food security in a context of augmentation of phytosanitary risks linked to climate change. In Senegal, a low-income country, where available chemical pesticides are of uncertain origin and quality, the use of unproven quality homemade plant extracts preparations tends to be normalized.

Surveys conducted in the regions of Fatick, Tamba, and Thies in 2021 and 2022 have shown that chemical pesticides are considered as effective by 97% of respondents. Less than 10% of them use natural products. Regarding organic horticulture, in the vegetable crops and fruits production area in Thiès, 98% of interviewed people were not convinced of the efficacy of homemade plant extracts preparations to control pests, while country and trans-border expertise have capitalized a set of plant species among which *Azadirachta indica*, *Cassia senegalensis*, *Eucalyptus* sp., *Allium cepa*, *Allium sativum*, and *Capsicum annuum*. They invest in the production of biopesticides or source from local input suppliers without enough knowledge on pests, biopesticide preparation techniques and compliance of commercial biopesticide formulations with regulatory requirements. The adoption of the use of plant extracts against crop pest in Senegal where the chemical pesticides use, trade, and availability have reduced traditional local plant protection knowledge transmission in farmers communities, finding alternatives is challenging for producers engaged in organic and those engaged in agroecology.

Keywords: plant extracts, pest, agricultural crops, senegal

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## P127. Volatile organic compounds – their repellent activity and effects on survival and reproduction of *Tenebrio molitor* pest

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Soil pollution, accumulation of harmful substances or undirected impact on the environment are just some of the consequences of modern agriculture. To prevent or reduce these adverse effects scientists have been conducting intensive research for many years on the development of compounds that would be an alternative to classic insecticides. This approach uses, for example, compounds of natural origin, like secondary metabolites of plants, due to their broad biological activity. They can cause, for example, the reduction of food intake, the inhibition of growth or reproductive processes of insects or nematodes pests.

In research, we focused on four secondary metabolites of plants classified as volatile organic compounds (VOCs). (*E*)-2-decenal, furfural, 2-undecanone and (*E,E*)-2-4-decadienal were used in concentration $10^{-5}$ M. Their activity towards survival and reproductive processes, including the number of laid eggs and their structure as well as hatchability of larvae of *Tenebrio molitor beetle* were tested after insects' injection. In turn, the repellent properties of tested compounds were analyzed in 'choice test'. Our studies indicate that compounds possess a repellent effect, as insects avoid choosing surfaces or food on which they were applicated. Also, the survival rate was reduced. Tested substances impact on reproduction processes by reducing the number of laid eggs and limiting the number of hatched larvae.

Taking into account the obtained results, economy and the possibility of using plant residues like citrus peels, leaves, stems or corn cobs to obtain them, it might be stated that VOCs can be considered as sustainable natural plant protection products.

**Keywords:** insects, plant secondary metabolites, reproduction, survival, bioinsecticides

# Session 15:

## Integrated Pest Management

## P231. Evaluating the use of X-ray and temperature for the sterilizing of *Drosophila suzukii* for a Sterile Insect Technique program

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*Drosophila suzukii* (*D. suszukii*) is an invasive species in the northern and southern hemisphere, which causes damage by infesting soft-shelled fruit. With high tolerance to different environments, a wide host plant range, and a short generation time it can be devastating to some crops. Traditionally control has depended on chemical insecticides which are not very selective and therefore pose a risk for the biodiversity. In addition, *D. suzukii* lays its eggs inside the shell of the fruit which makes the larva protected from insecticides. Sterile insect technique could be a sustainable alternative to controlling the pest. Our research explores optimizing SIT for *D. suzukii* by using X-ray, and a novel approach by using temperature. Both methods are more sustainable then chemical and nuclear radiation. Also, they do not conflict with the EU's policy against genetically modifies organisms. In our research, we explore the optimum radiation dose for sterility, and how these doses effect the fitness of the flies. All x-ray doses cause different degrees of sterility, with a very slight effect on the health of the flies. We also explore using temperature as a sterilization method and how the flies are affected by being raised on different temperatures. Depending on the time spent on 30 C° different levels of sterility is achieved. A combination of both methods is done for the highest efficiency. With our results we have taken the first step into establishing an X-ray induced SIT program for *D. suzukii* control.

Keywords: Sterile Insect Technique, *Drosophila suzukii*, pest control

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## P232. Rapid expansion and impact of a new invasive insect in Douglas-fir stands in France and Wallonia (Belgium): the Douglas-fir needle midge (*Contarinia pseudotsugae*)

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A Douglas-fir needle midge was first detected in Wallonia (Belgium) in 2015 and then in northern France in early 2016. In 2019 it was morphologically and genetically identified as *Contarinia pseudotsugae*, a pest native to North America and observed very occasionally in the Netherlands and Germany also in 2016. Cooperation between the Forest Health Department (DSF), the Walloon Forest Health Observatory (OWSF), and the research institutes INRAE and CRA-W has led to progress in the knowledge of the insect.

Joint monitoring has been set up with the support of the forest correspondents-observers of the DSF network and OWSF since 2019. The impact of the insect was measured over 4 years through a network of 116 plots in France and in Wallonia (1740 seedlings and 1160 adult trees). An impact on growth was observed during the installation phase of the insect but without mortality in the plots. The monitoring of its spread in France shows a range expansion of approximately 30 to 35 kms per year. In late 2022, the discovery of several pioneer populations beyond the expected expansion front raises questions about their connection with the initial introduction. Preliminary genetic studies (barcode) have been performed on specimens from Wallonia and different regions of France. The first analyzes suggest that individuals from northern France could be the result of natural or accidental introductions from Wallonia, while other new introductions could explain the colonization of localities in central and southern France.

**Keyword:** invasive pest, Douglas-fir, Douglas-fir needle midge, impacts, barcoding

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## P233. ResBerry: Resilient organic berry cropping systems through enhanced biodiversity and innovative management strategies

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Agricultural land is mostly laid out in monocultures although the ecological optimum, supporting ecosystem services, looks different. Consumer demand for organically produced food is increasing, including berries. Since organically produced berries are often negatively affected by various pests and diseases, there is an urgent need for robust and resilient cultivation systems.

ResBerry is an EU-project funded under Core Organic Cofund. Its main objective is to provide the necessary knowledge and demonstrate the effectiveness of enhanced above- and belowground biodiversity in organic berry orchards to increase their resilience against major pests and diseases. ResBerry addresses the following fields:

a) Identifiying suitable habitats and management strategies for natural enemies in organic berry orchards for preventive pest control, including companion plants (flower strips, trap plants, cover crops) to enhance ecosystem services. Movement of natural enemies and pests between crops and companion plants will be assessed.
b) Analyzing the soil microbial community in organic berry orchards and the impact of companion plants. Measures to favour beneficial soil microorganisms to protect against soil-borne pathogens and for higher general resilience are tested.

c) Raising awareness for innovative, little implemented curative pest control strategies, such as entomopathogenic nematodes against *Drosophila suzukii* and entomovectoring of microbiological products against *Botrytis cinerea*.
d) Evaluation of the measures' impact on yield and nutritional quality of berries.
e) Dissemination of the results to diverse audiences.

The multidisciplinary approach develops systemic solutions for organic farmers to reduce the dependency on fertilizers and plant protection products while increasing economic sustainability and restoring biodiversity.

**Keywords:** pest control, habitat management, functional biodiversity, soil microbial community, organic farming, companion plants

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## P234. Biopesticide efficacy trials against the southern green stink bug *Nezara viridula*

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The southern green stink bug, *Nezara viridula* (Hemiptera: Pentatomidae) is a widespread polyphagous pest of high economic importance. Farmers heavily rely on synthetic pesticides to control this insect, namely on pyrethroids, which are being phased out in the EU and are on the candidates for substitution list. In an effort to develop alternative, low-risk solutions in managing pest populations, the efficacy of three naturally sourced biopesticides, PREV-AM (orange oil 6% w/w), Spinosad and Pyrethrin was assessed against N. viridula 3rd and 4th nymphal instars. The contact toxicity of these biosolutions was tested using a potter spray tower by applying the products at the recommended label rates. Mortality was evaluated at 0h, 1h, 18h, 24h and 48h post-treatment. When sprayed at an equivalent of 0.5L/100L (0.5L of product in 100L of water), PREV-AM consistently caused the highest mortality rates, with 71% average mortality. When applied at 0.08L/100L, Spinosad showed 54% mortality, followed by Pyrethrin with 40% at 0.08L/100L. PREV-AM also exhibited the highest speed of kill, as mortality peaked as early as 0h and 1h. In contrast, Spinosad and Pyrethrin acted at significantly slower speeds, as insect mortality only peaked at 48h and 24h after treatment, respectively. Our findings suggest that the integration of one or more of these biosolutions in an IPM strategy may yield satisfactory results in the control of N. viridula populations. In this study, the practical implications of including PREV-AM in an IPM strategy of N. viridula was also investigated and discussed.

**Keywords:** stink bugs, bioinsecticide, PREV-AM, spinosad, pyrethrin

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## P235. Protecting and enhancing ecosystem services and plant defenses in tomato crop

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Tomato is an important crop in Greece and in the Mediterranean countries. We aim to develop an agroecological approach that aims at the protection (conservation) and the enhancement of functional biodiversity, aboveground and belowground, to increase and exploit the ecosystem services related to plant protection, plant nutrition and pollination, particularly in water-limited environments, where smallholder farmers of Mediterranean Basin live. Furthermore, we seek to enhance plant defenses against pests using natural compounds and further integrate this with the use of natural antagonists (beneficial insects and entomopathogens). In the University farm of AUTH we set up three different plots of open tomato crop each with a different companion plant *Phacelia tanacetifolia*, *Fagopyrum esculentum* and *Lobularia maritima*. In addition, in selected plots we released beneficial predatory arthropods and applied sodium alginate (as a plant growth promotor and inducer of plant defenses). Therefore, we set up a multifactorial experiment testing the simultaneous influence of all these treatments on plant development, flowers and yield, the abundance of important pests such as mites, *Tuta absoluta*, *Bemisia tabaci* and their natural enemies as well as key diseases. We also conducted trials with entomopathogenic nematodes against tomato pests in greenhouse conditions. We discuss our results in the context of an agroecological approach that seeks to maintain satisfactory yield stability, while consistently reducing external inputs (fertilizers, pesticides, herbicides, water).

**Keywords:** functional biodiversity, agroecology, IPM, biological control, pollination, plant defense

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## P236. Effects of climatic variables on olive infestation by *Bactrocera oleae*

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The impact of climate change on insect populations can significantly affect future pest management strategies. Therefore, effective IPM approaches should implement successful forecasting models and appropriate digital tools for monitoring insect populations and limiting crop damage. In this study, we analyzed *Bactrocera oleae* (Diptera: Tephritidae) infestation over seven years (2015-2021) across 85 olive farms in Umbria, Italy. We evaluated the relationships between infestation indexes and a set of agroclimatic variables for modelling purposes. The analysis was based on weather data, morphometric and environmental data, and on data obtained from infestation surveys. *Bactrocera oleae* infestation was negatively affected by altitude and spring mean daily temperatures, while it was positively influenced by winter mean daily temperatures and cumulative degree days. An increase in altitude and cumulative degree days delayed the occurrence of attacks in the July-August period. Conversely, high winter and spring temperatures had a positive effect on anticipating the attacks. Infestation occurring in September-October was negatively affected by increasing altitude and high spring temperatures, but positively by high winter temperatures and the average temperatures prior to the monitoring day. Furthermore, precipitation delayed *B. oleae* attacks, while high temperatures before the monitoring day anticipated the attacks. Our data could be helpful for developing predictive models and increasing the reliability of decision support systems in olive orchards.

**Keywords:** Tephritidae, pest forecasting, integrated pest management, decision support systems, olive fruit fly

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## P237. Investigating the role of wheat variety on the bird cherry-oat aphid performance to improve BYDV management in cereals

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Transmission of barley yellow dwarf virus (BYDV) is economically the most damaging outcome from aphid infestations in autumn sown cereal crops. The bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) is one of the most important vectors of BYDV in the UK. This work investigates the performance of the bird cherry-oat aphid based on fitness parameters (intrinsic rate of increase, growth rate and fecundity) on different winter wheat varieties. Old genotypes associated with aphid outbreaks, such as Maris Huntsman, and modern wheat varieties, such as the BYDV resistant RGT Wolverine were included. This knowledge is essential to develop robust IPM strategies in the field such as the use of trap crops and efficient monitoring tools. Our results so far showed the following trend in the ranking of wheat cultivars for susceptibility: Wolverine (modern) was the least susceptible, Gleam (modern) and Skyscraper (modern) were moderately susceptible, Flanders (old) and Maris Huntsman (old) appeared to be more susceptible than the modern varieties, although no significant differences were found. Less susceptible wheat varieties will usually reduce aphid weight gain, extend developing time of nymphs, which therefore increases the risk of predation and parasitism, decrease fecundity, and increase mortality. With these results, we aim to identify suitable wheat varieties to be used as trap crops as part of the integrated pest management of BYDV in cereals.

**Keywords:** intrinsic rate of increase, mean relative growth rate, integrated pest management, trap crops

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## P238. Evaluation of hydrosol toxic effects on *Tuta absoluta, Aphis gossypii* and *Tetranychus urticae* and their predator *Macrolophus pygmaeus*

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Hydrosols (or hydrolates) are by-products of essential oil's distillation process and may have insecticidal properties. In this work, the toxic effect of cultivated peppermint (*Mentha piperita*), lemon verbena (*Aloysia triphylla*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and chamomile (*Matricaria chamomilla*) hydrosols was assessed on eggs of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), adult individuals of *Aphis gossypii* Glover (Hemiptera: Aphididae) and adult females of *Tetranychus urticae* Koch (Acari: Tetranychidae). Their side-effects on the 5th instar nymphs of the predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) were examined, too. The main component of the peppermint, lemon verbena, oregano, rosemary and chamomile hydrosols was menthol, geranial, thymol, verbenone and bisabolol oxide A, respectively. Hydrosols were applied by the dipping method for both the tomato leaflet and the target individual. The results showed that the peppermint hydrosol caused a high mortality of *T. absoluta* eggs (82%) followed by lemon verbena (46%) and oregano (42%). In the case of *T. urticae* oregano hydrosol caused a very high mortality (96%), followed by peppermint (84%) and chamomile (66%). Rosemary hydrosol caused significant mortality on *A. gossypii* (72%) followed by peppermint (64%). In contrast, mortality caused on *M. pygmaeus* was kept at low levels. It was highest for lemon verbena followed by oregano (40% and 30%, respectively). Conclusively, hydrosols of aromatic plants may have a high potential in the control of serious insect and mite pests and future studies should explore this further.

**Keywords:** hydrosols, aromatic plants, pests, biological agent, mortality

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## P239. Exclusion netting – a non-insecticidal tool to manage huanglongbing for replanted citrus orchards?

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The citrus industry in Florida has been devastated by huanglongbing, a fatal citrus disease vectored by Diaphorina citri. Current management relies on protecting trees from psyllids with frequent insecticide applications, an unsustainable practice that has led to insecticide resistance in D. citri and increased costs of orchard management. Citrus growers are exploring options such as mesh exclusion covers over trees, reflective ground cover, and red kaolin clay particle film sprays, although the effects of these treatments have never been fully evaluated. We tested the efficacy of these treatments in reducing D. citri densities on young citrus trees against a grower standard control (monthly insecticide application) as well as their effects on other citrus pests and plant pathogen incidence. Over a two-year period, the exclusion mesh successfully excluded ACP, with no trees developing huanglongbing. Trees in this treatment also showed the lowest rate of citrus leafminer infestation and citrus canker incidence. However, trees with exclusion mesh were still susceptible to spider mites, sooty mould, and greasy spot, suggesting that trade-offs exist with this tool. While these covers appear to be a promising tool for

protecting young citrus trees from citrus greening, citrus growers need to monitor for other pests and pathogens that can thrive within the closed mesh environment created by the covers.

**Keywords:** citrus, huanglongbing, integrated pest management

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## P240. Landscape level pest management strategies in tomato and potato production systems influence insect resistance evolution by *Phthorimaea* spp

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Insecticide resistance is only one of the possible reasons for the variable results obtained with chemical control of pests under field conditions. Potato tuber moth (PTM), *Phthorimaea operculella* (Gelechiidae), is an important pest of potato and *Phthorimaea absoluta* (Gelechiidae) an important pest of tomato in the world. However, both these pests occur on both these crops. The potato and tomato industries in South Africa are heavily reliant on insecticides for pest control. Control failure of PTM has been reported by several farmers in South Africa. The susceptibility of PTM, sampled from four localities in the main potato producing areas in the country was assessed for azinphos-methyl (organophospate), lambda-cyhalothrin (pyrethroid), lufenuron (benzoylureus) and indoxacarb (oxadiazine). Results indicated that control failure with azinphos-methyl, lambda-cyhalothrin and lufenuron can be expected, but that indoxacarb should provide effective control. To mitigate control failures, spray programs were adapted to include active ingredients such as the diamides, chlorantraniliprole and flubendiamide, pyridalyl dichloropropene-derivative as well as the spinosyns, spinosad and spinetoram. These active ingredients are, however, also intensively applied on tomato for control of *P. absoluta.* The polyphagy of *P. operculella* and *P. absoluta* on solanceous crops and application of the same insecticides on potato and tomato for control of these pests increases the risk for insecticide resistance evolution by both pests. Integrated pest management strategies that consider both pest species, both crops and different control options on a matrix basis is important for sustainable *Phthorimaea* spp. management on solanaceous crops in South Africa.

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## P241. Insect artificial rearing may select for negative to SIT adult behavior. The case of olive fly

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Area-wide releases of mass-reared sterile insects (SIT) have been developed for “clean” control of insect pest populations. With certain insect pest species, this methodology proved to be field effective. Those species were easy to rear artificially and of relatively simple field behavior. In other cases, no practical field control could be achieved. For a considerable period, following the development of SIT, the field behavior of mass-reared insects received reduced attention, in spite of negative signs, as compared to the emphasis on mass-rearing, sterilization and lab quality. Of course, the sexual behavior of sterile insects in the field should be similar to that of their wild partners for successful cross-matings and control. The case of the olive fruit fly *Bactrocera oleae* (Rossi)(Diptera: Tephritidae) is discussed, e.g.

mating time asynchrony between artificially-reared and wild flies in a caged olive tree in natural photoperiod. The olive fly is an insect of immense socio-economic importance with its larva feeding exclusively in the olive fruit, a fruit of unique chemical synthesis, and its adults exhibiting an elaborate field sexual biology.

**Keywords:** *Bactrocera oleae*, olive fruit fly, mass-rearing, quality

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## P242. Comparative effect of six kind of traps on *Bactrocera oleae* and the beneficial insects

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Within the framework of FruitFlyNet-ii project "Commercialization of an Automated Monitoring and Control System against the Olive and Med Fruit Flies of the Mediterranean Region", a study was carried out to compare the effect of different Traps on the olive fruit fly (OFF) captures and the beneficial insects, between 29 August and 10 October 2022, in 6.250 Ha of non-irrigated Olive Groves- in Hassbaya in South Lebanon. McPhail with Diammonium Phosphate 4%, Yellow and Green Panels, White and Yellow Delta Trap with Yellow or White Panels with Ammonium Bicarbonate (10g.), were installed under latin square Design at 50m distance, with permutation weekly. Results showed that the green Trap was not attractive at all and the yellow sticky Trap was the most attractive for the Olive Fruit Fly (OFF) and for the majority of the beneficial insects. Only one of the OFF parasitoids was observed, *Eupelmus urozonus* (Eupelmidae), from 5 till 19 September, and represented 7% of all parasitoids catched. Many other Parasitoïds were captured belonging to Pteromalidae (10%), Encyrtidae (12%), Ichneumonidae (4%) and Braconidae (66%) families and in particularly, *Cotesia congregata*. Predators such as *Chrysopa* sp., *Leuchocrysa americana* (Chrysopidae 28% of the total Predators), *Auplopus* sp. (Pompilidae 58%), *Eupeodes americanus* and *E. corollae*, *Chrysotoxum cautum*, *Toxomerus occidentalis* (Syrphidae 5%) and species belonging to Coccinellidae family (10%) were also catched. Many Pollinators, Honey bees (Apididae 21% of the total Pollinators), *Hylaeus* sp. (Colletidae 46%), *Halictus* sp. (Halictidae 16%), *Megachile* sp. (Megachilidae 9%), and *Andrena labiata* (Andrenidae 7%) were also captured.

**Keywords:** olive fruit fly, *Eupelmus urozonus,* beneficial insects, sticky trap, delta trap, mcphail trap

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## P243. Diamondback moth (*Plutella xylostella*) - a significant pest of oilseed rape in the southeast area of Romania

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Diamondback moth (*Plutella xylostella*) is a major pest of oilseed rape (OSR) worldwide. This paper presents a three-year study concerning the effectiveness of the OSR seed treatment with the cyantraniliprole active ingredient in controlling the diamondback moth (DBM) larva attack in autumn and a four years monitoring of the DBM fly dynamics using pheromone sticky traps. The experience and DBM monitoring were carried out at the experimental field from the National Agricultural Research and Development Institute Fundulea in southeast Romania. Between 2019 and 2022, the average annual temperature was higher than long-term averages, while rainfall amount during the autumn was lower. Data from the field assessment reveal that higher attacks of the DBM larva at OSR plants occurred in the last month of autumn. In 2019, on 20 November, in the untreated variant, the attack degree was 3.04%, while in the variant with treated seeds was 2.72%. A higher pest attack from this experience was observed on 11 November 2020, when the DBM larva attack degree was 16.26 % in the untreated variant and 11.24% in the variant with treated seeds. In the autumn of 2021, the DBM larva attack at OSR plants was low. Results of the monitoring of DBM adult fly dynamics reveal an unusually higher activity of this pest during November 2019, 2020 and 2022, and December 2022. This is the first mention from the Romanian literature concerning higher DMB larva attacks at OSR plants in autumn and high moths' activity during November and December.

**Keywords:** oilseed rape, diamondback moth, control, monitoring

## P244. Mass rearing the Mediterranean fruit fly in Greece: Innovations in larval diet formulations and economic aspects

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The Tephritidae family of Diptera includes many agricultural pests of economic importance. Different factors facilitate the invasion and establishment of these pests in Europe. Considering the new trends in plant protection, and the public perception that leads to the reduction in the use of chemical pesticides, chemical-free control methodologies are urgently required. New tools are being developed and classical ones gain reviving interest. Among tephritids, the Mediterranean fruit fly, *Ceratitis capitata* is a major agricultural pest. Due to its extended geographic range and significance, it has been a model for the development and application of the Sterile Insect Technique (SIT), an environment-friendly pest control method relying on the production and release of sterilized males into the wild. In this respect, the HORIZON 2021 'REACT' project is developing an emergency response strategy against invasive fruit flies, with SIT as a key element, and *C. capitata* as a model for the development of such responses in a

European setup. The success of SIT depends on the efficiency of the insects' rearing. A diet must be a) efficient, in terms of sterile males' production and quality, b) of affordable cost and labor, and c) based on ingredients of long-term availability. The purpose of this study is to incorporate alternative protein sources in the larval diet, aiming either to reduce the cost or increase its productivity.
According to our experiments, *Enterobacter sp.* or insect-derived protein can support efficient rearing of *C. capitata* for release purposes. The economic aspects of such diets are also discussed.

Acknowledgment: Within the framework of the projects: HORIZON-CL6-2021-FARM2FORK-01-04 'REACT' Rapid elimination of invasive insect agricultural pest outbreaks by tackling them with Sterile Insect Technique programs; IAEA- Programme of Coordinated Research Activities "Identification and Characterization of Temperature Sensitive Lethal Genes and Response to Thermal Shock of SIT Target Species"(IAEA-CRP Contract No: 23372)

Keywords: *Ceratitis capitata, medfly, Enterobacter*, mass rearing, larval diet, SIT, Mediterranean fruit fly

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## P245. Two-year monitoring of economically important pests from Fam. Gelechiidae and Tortricidae (Lepidoptera) on stone fruit in North Macedonia

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*Anarsia lineatella* Zeller (fam. Gelechiidae, Lepidoptera), *Grapholita funebrana* Treitschke and *Grapholita molesta* Busck (fam. Tortricidae, Lepidoptera) are economically significant pests on the stone fruit in North Macedonia. Monitoring of this pests has been established in 2021 and 2022 in order to identify the most critical period of their appearance in two regions in the country (v. Chelopek, Northeast and v. Sirkovo, Vardar region), in orchards with mixed stone fruits. This gives new insights of the flight period and biological development of these pests in the specific climatic and geographical conditions.
*A. lineatella* in 2021 had total flight period of 206 days (30.3-21.10) in Chelopek and 214 days (2.4-1.11) in Sirkovo versus 202 days (18.04-5.11) in Chelopek and 215 days (27.04-27.11) in Sirkovo in 2022. *G. funebrana* in 2021 had total flight period of 188 days (30.3-3.10) in Chelopek and 208 days (31.3-24.10) in Sirkovo versus 209 days (20.03-15.10) in Chelopek and 229 days (31.03-14.11) in Sirkovo in 2022. *G. molesta* in 2021 had total flight period of 178 days (9.4-3.10) in Chelopek and 222 days (29.3-5.11) in Sirkovo versus 201 days (20.03-7.10) in Chelopek and 258 days (2.3-14.11) in Sirkovo in 2022.
Concerning the locations there are statistically significant differences for *G. molesta* and *A. lineatella* ($p=<.0001$) but no statistically significant differences for *G. funebrana.*
Continuous monitoring of the pests will help in establishing Integral Pest Management in stone fruit orchards in order to improve food safety standards and to keep environment from insecticide pollution.

Keywords: Gelechiidae, Tortricide, Lepidoptera, pest insects, stone fruits, North Macedonia

## P246. Assessing the ecological interactions between invasive and endemic species of gall midge pests of canola

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The swede midge (*Contarinia nasturtii*), an invasive pest of brassicaceous crops, was first discovered in Ontario, Canada in 2001 and until recently was thought to be the only gall midge pest of canola (*Brassica napus* L. and *B. rapa* L.) in Canada. However, the canola flower midge (*Contarinia brassicola* Sinclair)[2], an apparently endemic, newly described species, has since been found to be established throughout the canola-growing regions of the Prairies. Following its discovery in western Canada, monitoring for *C. brassicola* presence and injury symptoms was initiated in Ontario. Although the canola flower galls characteristic of *C. brassicola* infestation have not been observed in Ontario, *C. brassicola* males were captured in relatively high numbers on pheromone-baited sticky traps at canola fields throughout the province. Additionally, *C. brassicola* adults were observed emerging from *C. nasturtii*-infested canola plants. *C. nasturtii* is a devastating pest and has resulted in a 53% decline in canola acreage in Ontario since 2011. Because *C. brassicola* is also present in Ontario, some of the damage attributed to *C. nasturtii* could in fact be the result of its congener. However, management recommendations for *C. nasturtii* may not be effective for *C. brassicola* if there are physiological or behavioural differences between the two species. Studies are underway to determine the host-plant interactions and population dynamics of both species, and the resulting implications for their management in canola.

Keywords: Cecidomyiidae, *Contarinia nasturtii*, *Contarinia brassicola*, Brassicaceae, canola, *Brassica napus*

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## P247. Biological control effect of “Natural Enemy in First” method in hot pepper

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For the past 10 years, we have developed a simple and reliable plants habitat system with Biological control agents (BCAs), named the “Natural Enemy in First” (NEF) method, which we introduce here. The NEF method is a low-cost, highly efficient control system that does not require pest monitoring; involves managing habitats inoculated with BCAs and food insects with the main crops; and encourages the development of populations of BCAs, such as *Aphidius* sp., *Orius* sp., and *Cyrtopeltis tenuis*, before pest populations emerge. To test our method, a “self-production model” was established and distributed to growers to manage BCAs and their habitats in pepper. In the NEF treatment group, about 180 kg of dried peppers were harvested, 2.06 times more than in the control group. We confirmed that retention rates for natural enemies in NEF treatments were more than three times higher than in conventional natural enemy treatments.

Keywords: biological control agents, natural enemy in first, pepper, self-production model

## P248. Evaluation of the predatory mite *Blattisocius tarsalis* (Mesostigmata: Blattisociidae) and the egg parasitoid *Trichogramma euproctidis* (Calcidoidea:Trichogrammatidae) for the control of *Tecia solanivora* (Lepidoptera:Gelechiidae) under semi storage conditions.

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*Tecia solanivora* is a quarantine pest in Europe. First identified in Guatemala in the 1970s, it progressively spread through Central and South America, reaching the Canary Islands in 1999 and mainland Spain in 2015, which meant its entry into Europe continent. The pest has generated unaffordable economic losses, both in the field and in storage, where losses can reach 100% of the stored production. In the absence of authorized chemical treatments, the use of an egg-predator mite, *Blattisocius tarsalis*, and an egg parasitoid *Trichogramma euproctidis* are being analyzed for possible application in storage conditions. In previous laboratory trials, the potential of both natural enemies was analyzed with positive results for non-refrigerated storage chambers, typically between 15-20 ºC. In the present work we compared the efficacy of both natural enemies under semi-storage conditions at 17ºC. Our results showed that while *B. tarsalis* and *T. euproctidis* significantly reduced the number of surviving individuals of *T. solanivora* compared to the control (28.00 ± 13.65, 99.50 ± 23. 73 respectively, against 250.50 ± 8.70), only the predatory mite allowed to obtain a significant percentage of healthy tubers (71.66 ± 3.33 %) compared to the control treatment (17.50 ± 5.95 %), for this reason, *T. euproctidis* was discarded and in future works *B. tarsalis* will be tested under real conditions.

**Keywords:** warehouse, biological control, parasitic wasp, predator mite, postharvest, potato tubers

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## P249. Conservation biological control potential in sugar beet fields in Denmark

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Aphids are a critical pest in sugar beet production across Europe and North America. *Aphis fabae* and *Myzus persicae* are two species which pose an economic threat to the crop. *A. fabae* causes direct damage by covering the plant in dense colonies, while *M. persicae* is a vector for Beet Virus Yellows (BYV), which can be devastating to the crop. Starting in the 1980s, the aphids were effectively controlled with a neonicotinoid seed coating, which was banned in the European Union in 2019. Because of this, very little has been researched into other forms of pest control including IPM and biological pest control. This project aims to study the potential of perennial flowering margins (FAB margins) to improve pest control, as well as identifying key natural enemies using gut content PCR of field collected arthropods. Preliminary results from the first field season show that the FAB margins promote the presence of natural enemies in the adjacent sugar beet field while decreasing the rate of aphid colonization. The natural enemies include Coccinellidae, Cantharidae and Chrysopidae. A high proportion of the arthropods collected had *M. persicae* and/or *A. fabae* DNA present in their gut, suggesting a high degree of predation in the field.

**Keywords:** aphids, natural enemies, integrated pest management

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### P250. Strip cropping: Improving biodiversity and crop resilience in organic farming

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Strip cropping, a type of intercropping, increases the diversity of crop species in the field. Crop diversification in the agroecosystem can potentially increase biodiversity of arthropods, and microorganisms above- and belowground, improve crop yields, and reduce pest and pathogen attacks. We here present a project that investigates the potential benefits of strip cropping under Danish conditions, by 1) design and implementation of optimal strip cropping fields, 2) functional and biological diversity of arthropods, with focus on pests and predators, 3) microbial diversity and pathogen damage, 4) agronomical aspects, such as crop rotation, tillage, weed control, and yield effects, and 5) development of practical implementation, and sustainability analyses. The strip crop fields were established in 2021, in rows with two levels of crop diversity: 3 m rows with wheat, sugar beet, barley, faba bean, rye, oat, pea, and quinoa, and 6 m rows with wheat, sugar beet, barley, and faba bean, in a block design of four repetitions. The arthropod assessments, with focus on arthropod diversity and aphid infestations in faba bean and barley, were conducted by visual observations and the use of trap methods (pitfall traps and pantraps). Preliminary data indicate an effect of row width, by a lower aphid infestation and an earlier presence of predators in 3 m strips compared to 6 m strips. This project contributes with practical as well as scientific knowledge for implementation of strip cropping to support biodiversity and agroecosystem resilience.

**Keywords**: biodiversity, intercropping, aphids, predators, faba bean, barley

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### P251. Sentinel prey in oilseed rape fields in Estonia indicate high predation rate regardless of the field bordering semi-natural habitat type

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Oilseed rape in crop of considerable economic value and agricultural importance and it's frequently affected by several insect pests. *Carabidae* and *Staphylinidae* are amongst the most abundant generalist predators on oilseed rape fields and both their abundance and variability is affected by landscape structure, but measuring the abundance of generalist predators does not truly reflect the level of biological control they provide in oilseed rape fields.

We measured predation rate using sentinel pray (*Calliphora* sp larvae) and assessed the abundance of ground dwelling predatory arthropods using pitfall traps on 18 conventional winter oilseed rape fields bordering conterminously with grassy margin, hedgerow or arable field in 2014. Sentinel pray were placed at equal pace at four distances up to 75 meters from the field edge and predation rate was measured after 24 (sampling 1) and after 8 hours (sampling 2). Pitfall traps were active for 7 days covering the period of sentinel pray assessments. The predation rate exceeded 98% regardless of the field bordering habitat type, distance from the field edge or exposure time. Although the abundance of both *Carabidae* and *Staphylinidae* were lower on the fields bordered by hedgerow, the same level of predation was found, indicating that the suffient level of natural enemies were present.

Our study revealed very high pest management potential due to high abundance of *Carabidae* and *Staphylinidae* in all conventional oilseed rape fields. It is likely that the weather conditions were exceptionally favorable and under less favorable conditions the predation rate could be significantly lower.

**Keywords:** sentinels, field margins, ground beetles, rove beetles

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## P252. Attract-and-kill method for *Drosophila suzukii* control in grapes and cherries in Greece

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The two spotted wing *Drosophila suzukii* has invaded Greece 10 years ago, causing in some cases severe infestations in soft and stone fruits. Controlling *D. suzukii* with insecticides is difficult because repeated operations are required increasing thus production costs, they disrupt integrated pest management (IPM) programs and increase the risk of pesticide residues (infestations close to the harvest season). An alternative strategy to reduce insecticide input is the attract-and-kill (A&K) method wherein pesticides are applied to a specific point source along a lure. We tested this strategy in grapes and cherries in Northern Greece. The lure formulation used consisted of olfactory attractants and feeding stimulants specific to *D. suzukii* and pink coloring for visual attraction. The lure was formulated in a SPLAT medium (Specialized Pheromone and Lure Application Technology, ISCA Technologies, Inc.) and the insecticide spinosad (0.5%ww). In grapes in the treatment plots SPLAT+ Spinosad was applied and in the control plots there was no intervention, whereas in cherries in the treatment plots SPLAT+ Spinosad was applied and in control plots only SPLAT. The population of *D. suzukii* in all experimental plots was monitored with simple traps bearing apple cider until harvest. In both trials both *Drosophila* spp and *D. suzukii* catches in traps of the treatment experimental blocks were significantly reduced. The efficacy of the method and its adoption in IPM programs is discussed.

**Keywords:** *Drosophila suzukii*, lure, SPLAT, Spinosad, Integrated Pest Management

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## P253. Distribution of the olive fruit fly off, *Bactrocera oleae*

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Within the framework of FruitFlyNet-ii project “Commercialization of an Automated Monitoring and Control System against the Olive and Med Fruit Flies of the Mediterranean Region”, a study was carried out to evaluate the distribution of the olive fruit fly (OFF), on yellow sticky traps. This experiment followed From 22 August to 21 November 2022, in 2Ha (142m×142m) of non-irrigated olives groves cultivar, in Hassbaya in South Lebanon, on yellow sticky traps with or without ammonium bicarbonate (8 with salt, $T^+$ , and 37 without salt, T), installed at a distance of 20m between traps without salt (T), and 30m between $T^{+,}$ and T. The observations were made weekly for the adult captures, and biweekly for the rate of the fruit infestation, RFI (25 fruits/ tree-Trap), in relation with the fruit phenological stage (BBCH), and the climatic data. Results showed that from 15 September, the fruits turned to the purple colour (BBCH: 80-81%) and became 90% from 10 October, the beginning of the fruit harvesting. The total number of the adults was inversely proportional to the temperatures. $T^+$ were more attractive for the fly adults than the T traps. The mean adults captured were on 21 November, 12.9 and 100 by trap, respectively. Male density was higher than that female at the beginning. Thereafter, female increased progressively to reach equal densities, on 31 October. The RFI was in the $T^+$ zones trap less than that registered in T zones trap: 0 to 0.5% and 0.3 to 5.3%, respectively.

**Keywords:** olive fruit fly, yellow sticky trap, diammonium bicarbonate, bbch, rate of the Fruit Infestation

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## P254. Sterile Insect Technique to control pentatomid pest species: irradiation screening on *Halyomorpha halys* and *Bagrada hilaris*

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The painted bug, *Bagrada hilaris* (Burmeister) and the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) are two extremely polyphagous invasive species. The feeding activities of these two species cause severe damage to many economically important crops worldwide. *Bagrada hilaris* is native to Africa and Asia, but it has extended its distribution to the southern part of the United States, Chile, Central Asia, Africa and two European islands. *Halyomorpha halys* is currently considered amongst the most harmful agricultural pests in Europe, United States and Chile.
Among the various control methods, the possibility of using the Sterile Insect Technique (SIT) in combination with classical biological control was tested. Indeed, this technique could be used to control *Bagrada hilaris* on the island of Pantelleria, where the geographical-ecological conditions are favourable. On the other hand, sterile *Halyomorpha halys* are being evaluated for eradication programmes in New Zealand or other jurisdictions, as well as possible application in integrated pest management strategies.
The main objective of this work was to perform an irradiation screening to evaluate the suitability of a SIT program in the two species. Two different methods were used for irradiation: *Bagrada hilaris* individuals were irradiated with gamma rays, while *Halyomorpha halys* males were irradiated with high-energy X-rays. For both protocols, insects were irradiated at different doses, and then physiological and behavioural bioassays were performed. Parameters such as longevity, fecundity and fertility were evaluated.
The results obtained are encouraging for the feasibility of the SIT to manage these two pentatomid pest species.

**Keywords:** sterile insect technique, irradiation, sterility, biological control, insect pest, pentatomids

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## P256. Side-effects of two strobilurin fungicides on *Orius laevigatus* (Hemiptera: Anthocoridae), predator of the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae)

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*Orius laevigatus* is successfully used as predator of the western flower thrips *Frankliniella occidentalis* in various protected horticultural and ornamental crops. To evaluate the side-effects of pesticides used in these kind of crops on this predator is, therefore, of great interest to make compatible the combined use of both methods in Integrated Pest Management. Thus, in the present work, two strobilurin fungicides (kresoxim-methyl and trifloxystrobin) were tested against the adults of *O. laevigatus*. A negative (water) and a positive control (chlorpyrifos) were also used. Adult mortality was evaluated by contact on fresh residue. To that end, the compounds were applied at the maximum field rate on glass substrate by using a Potter Tower. Mortality was recorded after 24, 48 and 72 hours of exposure. Additionally, the sublethal effects after 72 h of exposure were evaluated, when possible (only in the case of trifloxystrobin). To do this, pairs of surviving adults were used and the necessary data to obtain the parameters of the life tables were collected. Abbott's correction of natural mortality after 72 h of exposure was of 100% in the case of chlorpyrifos, while kresoxim-methyl was classified moderately harmful and trifloxystrobin, harmless (category 3 and 1, according with the International Organisation for Biological Control, respectively). Trifloxistrobin negatively affected female fecundity, fertility and longevity, and significantly increased the percentage of mortality of eggs and nymphs. Besides, the $r_m$ value recorded for the predator was of 0.0746±0.01, significantly lower than the water treated control (0.1483±0.005).

Keywords: biological control; kresoxim-methyl; trifloxystrobin; biological parameters; intrinsic rate of increase

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## P257. Side-effects of three fungicides on *Anthocoris nemoralis* (Hemiptera: Anthocoridae), predator of *Cacopsylla pyri* (Hemiptera: Psyllidae)

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Making compatible the combined use of registered pesticides and natural enemies is one of the key issues to achieve success in biological control programs. Currently, *Anthocoris nemoralis* is a predator efficiently used against *Cacopsylla pyri* in pear crops. In this context, to evaluate the side-effects of pesticides frequently used in this crop on this predators is of great importance. Thus, in the present work, the fungicides captan, tebuconazol and trifloxystrobin were tested against *A. nemoralis* adults. A negative (water) and a positive control (imidacloprid) were also used. The mortality of adult predators was evaluated by contact on fresh residue. To that end, the compounds were applied at the maximum field rate on glass substrate by using a Potter Tower. Mortality was recorded after 24, 48 and 72 h. Additionally, the sublethal effects after 72 h were evaluated. Moreover, biological traits of adults that survived 72 hours post fungicide treatment were followed during their life time to estimate sub-lethal effects on their life-table parameters. Abbott's correction for natural mortality after 72 h of exposure was of 100% in the case of imidacloprid. The three fungicides were classified harmless (category 1, according with the International Organisation for Biological Control). However, fungicides produced sublethal effects. Captan and tebuconazol negatively affected some biological parameters of the adults, but no significant differences of $r_m$ values were obtained with respect to the control (water). On the contrary, the $r_m$ value recorded for trifloxystrobin was of 0.0663±0.0052 which was significantly lower than the control (0.1453±0.0025).

**Keywords:** biological control, fungicides, captan, tebuconazole, trifloxystrobin, intrinsic rate of increase

## P258. Susceptibility of egg masses, nymphs and adults of *Halyomorpha halys* (Hemiptera: Pentatomidae) to three chitin synthesis inhibitors

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The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is a high-concern invasive species causing severe damage to fruit crops in many countries outside the native Asian regions. Management methods other than frequent sprays of broad-spectrum insecticides are needed to restore integrated pest management (IPM) practices in orchards. Chitin synthesis inhibitors are usually regarded as desirable options in IPM programs because of lower toxicity to beneficial insects and other non-target organisms compared to neurotoxic insecticides. In this study the activity of three chitin synthesis inhibitors (namely buprofezin, novaluron and triflumuron) was investigated on BMSB egg masses, 3rd instars and adults by means of laboratory bioassays. Novaluron caused significant mortality to early instars emerged from sprayed egg masses. Novaluron and to a lesser extent triflumuron exerted also a significant insecticidal activity on BMSB 3rd instars exposed to residues on potted peach plants. No significant differences were found between buprofezin and water control on egg masses or 3rd instars. When sprayed on BMSB adults, none of the chitin synthesis inhibitors affected survival, fecundity or egg hatching. The long-lasting activity of novaluron, triflumuron, and possibly other chitin synthesis inhibitors, could be exploited to reduce damage by BMSB nymphs without the need for frequent treatments, which is a major problem of neurotoxic insecticides. However, given the lack of effects on adults, chitin synthesis inhibitors might be considered for field applications only as a tool in a wider management strategy along with other methods aimed at preventing the invasion of crops by adult bugs.

**Keywords:** brown marmorated stink bug, invasive species, integrated pest management, buprofezin, novaluron, triflumuron

## P259. Understanding and managing Barley Yellow Dwarf Virus

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Aphids are economically important cereal pests that reduce grain quality and crop yield through direct feeding and vectoring Barley/Cereal Yellow Dwarf Virus (B/CYDV). Controlling aphids and therefore managing B/CYDV is increasingly difficult due to insecticide resistant aphid populations, climate change and fewer insecticide options. There is an urgent need for enhanced understanding and detection of B/CYDV to inform control decisions. Plant pathogens are constantly emerging and spreading and there are often limited (or none in case of B/CYDV) post diagnosis treatment options, making surveillance key to their control. Previous work indicated that MAV was the most predominant isolate of B/CYDV in Irish cereal crops. The MAV-strain of the disease was either the only serotype of the virus found or the most common strain. These identifications were ELISA based (not able to discriminate between BYDV-PAV and BYDV-PAS, and demonstrates cross reactivity between MAV & PAV). Advanced detection of B/CYDV is needed to determine if this observation still applies almost a decade later. A pilot sequencing survey of symptomatic barley plants from around Ireland has identified many BYDV species (with BYDV-GAV and BYDV-PAS being prominent) and co-infection frequent. These data were used to develop improved molecular surveillance tools for C/BYDV monitoring. The ability of *Sitobion avenae* and *Rhopalosiphum padi* to transmit the predominant Irish BYDV strain was then investigated, as well as the potential for Irish strains of BYDV to manipulate aphid behaviour.

**Keywords:** aphid, BYDV, virus, IPM

## P260. Influence of temperature on the toxicity of six chemical substances on a beneficial parasitoid

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The codling moth *Cydia pomonella* (L.) is one of the most important insect pests in apple orchards worldwide, causing serious yield losses. Insecticides are commonly used to manage this species but their intensive use, combined with the multivoltinism and the high fecundity of this pest, has led to the development of resistance to several chemical groups of insecticides. Moreover, some studies reported a decreased toxicity with increasing temperature for some commonly used insectides against this species. Several solutions were developed to ensure a more sustainable management of this pest. One of them is the use of a parasitoid, *Mastrus ridens* Horstmann, successfully introduced in several countries. Our study aimed to investigate cross effects between temperature and six chemical substances used in orchards - i.e., chlorantraniliprole, emamectin, spinosad, azadirachtin, copper and sulphur - on the mortality of the introduced parasitoid *M. ridens* in order to ensure a sustainable establishment of this species in a climate change context. Increasing temperatures increased significantly the toxicity of spinosad, chlorantraniliprole, copper and sulphur against *M. ridens*. This study provides essential insight to make recommendations for using these products in combination with *M. ridens* to control the codling moth in prevision of global warming.

**Keywords:** Integrated Pest Management, climate change, *M. ridens*, codling moth, chemical toxicity

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## P261. Assessing the impact of European hazelnut weevils and green shield bugs on hazelnut yield: insights for integrated pest management strategies

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The hazelnut weevil, *Curculio nucum* (Curculionidae), and the green shield bug (GSB), *Palomena prasina* (Pentatomidae), are the main indigenous pests of European hazelnut orchards (*Corylus avellana*). Nymphs and adults of GSB feed by using piercing-sucking mouthparts, while larvae and adults of *C. nucum* use their mandibles to feed or oviposit on nuts. Various defects can be induced, some of which are not exclusive to pests and therefore are not estimated. This study aimed at listing all symptoms due to pest attacks in relation with hazelnut growing. Insects were weekly collected in the field and isolated per species in net sleeve containing branches of Pauetet cultivar. Insects were kept in contact with nuts for one week, from nutlets to fully mature nuts. At harvest, results showed that during the period of kernel expansion -end of shell expansion, high rates of empty nuts were observed in net sleeves containing last instar of GSB or adults of *C. nucum.* The feeding behavior leads to kernel abortion. Then, when shell is fully lignified and kernel is fully developed, kernel defect was only observed in net sleeves containing N5 or pre-overwintering GSB adults. In contrast to *C. nucum*, the shell is not an impenetrable physical barrier against stylet penetration of GSB. To conclude, the results show that empty nut is one of the main defects caused by both pests, and therefore a clear net loss for growers. In the Pauetet cultivar, IPM strategies should focus on the kernel expansion time.

**Keywords**: stink bugs, pests, yield loss, pre-dispersal seed predators, empty nuts, aborted nuts, net sleeves

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## P262. Looking for the best trapping design for early detection of xylophagous invaders

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Globalization is resulting in an exponential increase in the introduction of non-native insects into other continents, of which a growing proportion are emerging species yet never found outside their native range and not included in the quarantine lists. Since they are unexpected, their detection at arrival at

ports of entry could not rely on specific attractants. The deployment of traps baited with broad-spectrum semiochemical lures could be one of such early detection tools. Owing to the conservation of pheromone structures observed at world level for a number of Cerambycid taxa, a blend mixing 8 of these pheromones has successfully been tested as a potential large scope, generic attractant. Trappings deployed during 2018-2021 in Eurasia, North America and Australia allowed to detect 376 species from 8 different subfamilies and 60 cerambycid tribes. Increasing to 10 the number of cerambycid pheromones in the blend allowed to detect additional species without altering the previous catches. The addition of doses of plant volatiles (ethanol, (-) α-pinene, α-copaene and quercivorol) largely enhanced the trapping scope, allowing to detect simultaneously cerambycid and scolytid invaders in several European ports-of-entry. However, no generic lures have yet been defined for buprestids whereas trap color appeared to play an attractive role especially for *Agrilus* species. Therefore, we tested during 2021-2022 the detection efficiency for the major xylophagous groups of 4 different colors (black, green, yellow, purple) of multifunnel traps baited with the 10-cerambycid pheromone blend and the plant volatiles shown above. The results will be presented during the session.

**Keywords**: xylophagous insects, invasion, early detection, multipheromone blend, plant volatiles, trap color

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## P263. Crop diversity and floral resources influence the abundance of pest and natural enemies in pear orchards

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Pears are one of the most important fruit crops in the European Union. Pest control in pear orchards relies on the use of broad-spectrum insecticides, which generally results in reduced natural enemy populations and pest outbreaks. Additionally, the current climate change scenario is expected to increase pest outbreaks and reduce biodiversity. In this context, the DREAM project in Spain is looking for farming alternatives to increase resilience by using varieties with different phenology, and by implementing cover crops to enhance natural enemy populations. A new pear orchard was set up to assess the effect of crop diversity (mono- or multi-varietal) and cover crop (presence-absence) on pests and natural enemies. These two factors were assayed in a complete factorial randomized block design with three repeats. Pear trees and the cover were sampled for arthropods using conventional methods along spring. The preliminary results show significant differences in the abundance of pear psyllid and aphids on pear trees among some of the pear varieties. Psyllids density was similar in the plots with and without cover; in contrast, the abundance of aphids on pear trees was higher in the presence of cover. No significant differences in the abundance of natural enemies were observed between mono- and multi-varietal plots, but the abundance of predatory mirids was higher in the presence of cover crops. The preliminary results of this project show that both crop diversity and increasing floral resources may have an impact on the population dynamics of pest and natural enemies in pear orchards.

**Keywords:** biological control, cover crops, predatory mirids, phloem feeding hemipterans, PRIMA, DREAM

## P264. Updated distribution and main host plants of *Eotetranychus lewisi* (McGregor, 1943) in mainland Portugal and Madeira Island

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The Lewis spider mite, *Eotetranychus lewisi*, can be found in south, central and north America, and although it has a strong preference for poinsettia, *Euphorbia pulcherrima*, it can also attack several economically important agricultural crops. Considering its polyphagy and damages to citrus, strawberry, grape vine and peach in the American Continent, *E. lewisi* is classified as a regulated quarantine pest within the European Union. Nevertheless, the Lewis spider mite has been present for more than 30 years in Madeira Island (Portugal) without information on its distribution or damages to crops, which requires clarification with specific surveys. In 2019, E. lewisi was also found in two localities in western Algarve (Portimão and Loulé county), southern Portugal, representing the first established population in outdoor conditions in continental Europe. An ongoing scientific project is assessing the Lewis spider mite populations in Madeira and Algarve, including distribution and main host plants. Surveys conducted in 2022 confirmed the mite to be widespread in Madeira and in the Algarve, where it was found in an eastern location close to the Spanish border. Maps with the current distribution of the Lewis mite will be presented. Field samplings confirmed the high preference of *E. lewisi* for *E. pulcherrima*, with castor bean *(Ricinus communis)* also found to be a host in Madeira. Ongoing samplings and trials will allow to elucidate the mite´s pest status in Europe. Funding was provided by the project EXPL/ASP-AGR/0082/2021, financed by the Portuguese Foundation for Science and Technology (FCT).

**Keywords:** Lewis spider mite, invasive, distribution, *Euphorbia pulcherrima*, Algarve

## P265. Phenology and pest status of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) on walnut, *Juglans regia* L. in field and post-harvest conditions in Italy

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The carob moth, *Ectomyelois* (*Apomyelois*) *ceratoniae* (Lepidoptera: Pyralidae) is a widely distributed pest capable to damage fruits of different crops. Recently, damages of *E. ceratoniae* were documented also for the English walnut, *Juglans regia* L. in northern Italy, particularly in the post-harvest period. We presented phenological observations on the carob moth from studies with pheromone traps and fruit sampling in-season and post-harvest, conducted in northern Italy in summer 2021 – spring 2023, together with laboratory experiments aimed at the evaluation of life history parameters on different diets. Larvae were rarely found in walnuts during the growing season, and this indicates that it may not be suitable for the carob moth as a host in this period. Adults were however regularly captured in walnut orchards, from the beginning of May to the end of October, showing three/four generations in field. Male captures on pheromone traps peaked in the last part of the season, close to walnut harvesting. Hereafter, *E. ceratoniae* larval infestation consistently increased during the post-harvest period, where the development of one complete generation may occur based on the presence of new specimens found in stored walnuts. However, laboratory experiments showed a general high mortality and longer developmental time of insects reared on walnut as the only diet than on an artificial diet. This research highlighted the role of *E. ceratoniae* as a food storage pest of walnut in northern Italy, providing details about insect phenology and pest status in field and walnut storage.

**Keywords:** emerging pest, carob moth, English walnut, field, post-harvest, Integrated Pest Management

## P266. Current developments in the control of insect vectors in sugar beet

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The peach potato aphid (PPA - *Myzus persicae*) is an important pest in sugar beet cultivation as a vector of three yellowing viruses – Beet Chlorosis Virus (BChV), Beet mild yellowing virus (BMYV) and beet yellows virus (BYV). Yield losses of these virus can be up to 50%. Control of the viruses had relied on the chemical control of the aphid through neonicotinoid seed treatment in the last 2 decades. Since the ban of neonicotinoids in 2018, yellowing viruses have caused economic losses in major sugar beet markets such as France and England. Besides the loss of chemical control options, global warming has given rise to new pests in sugar beet such as the beet planthopper *Pentastiridius leporinus*. It is an important vector of the proteobacterium Candidatus *Arsenophonus phytopathogenicus* , the main causal agent of Syndrome Basses Richesses (SBR). The diseases has spread drastically to over 50 000 ha sugar beet acreage since 2017 in Germany and Switzerland. In this talk we will give an overview of the current challenges of managing insect vectors in sugar beet and provide an outlook on the development of new management approaches.

**Keywords:** aphids, planthoppers, sugar beet, control

## P267. Seed treatment induces antibiotic resistance to *Rhopalosiphum padi* in Barley

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Cereal aphid infestations cause widespread damage through nutrient removal, honeydew secretion attracting saprophytic fungi, and virus transmission. The Bird cherry-oat aphid (*Rhopalosiphum padi*) is a major vector for Barley Yellow Dwarf Virus (BYDV) causing up to 80% yield loss. Whilst BYDV tolerant varieties are important in reducing yield losses, farmers have limited options for aphid control as part of integrated pest management strategy. Here we investigated whether a new seed treatment could be used to control *R. padi* and BYDV in Barley *(Hordeum vulgare*), using non-viruliferous (-BYDV) and viruliferous (+BYDV) *R. padi.* Plant responses to-BYDV and +BYDV aphid feeding were investigated using chlorophyll fluorescence kinetics of the OJIP transient. Disease progression was determined through visual disease assessment and qRT-PCR. In +BYDV plants, seed treatment increased absorption per active reaction centre, reduced BYDV disease symptom scores and viral gene expression. In whole plant pre-alighting choice experiments, only alate-BYDV aphids preferred untreated barley. In post-alighting experiments, apterous-BYDV and +BYDV aphids had reduced survival and fecundity on seed treated barley. Studies on aphid feeding behaviour using Electrical Penetration Graph showed +BYDV aphids spent less time feeding, had fewer sustained phloem feeding events and more penetration difficulties on treated barley. This work showed that the novel seed treatment by Syngenta reduced *R. padi* feeding, reproduction, and BYDV symptoms presenting an effective aphid and BYDV control method for barley crops. Transcriptomics and hormone analysis are in progress to further elucidate the underlying mechanisms of this induced resistance.

**Keywords:** aphid, antibiosis, barley, Barley Yellow Dwarf Virus, electrophysiology, transcriptomics

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## P268. Evaluation of the side effects of the biopesticide Clavitus® 13SL on natural enemies

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Within the frame of IPM practices, beneficial organisms can be used in combination with conventional or biological insecticidal formulations, provided they exhibit a selective profile to beneficials. Therefore, the side-effects of a novel biological pesticide, Clavitus®13SL, on Hemiptera predators (*Orius laevigatus*, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*) and predatory mites (*Amblyseius swirskii*, *Amblyseius montdorensis*, *Phytoseiulus persimilis*) were estimated. Hemiptera predator assays were conducted with three different approaches: a) residual toxicity, b) direct spray contact and c) topical application at commercial rates (1-1.9lt/100lt). The methods of direct spray contact and residual toxicity was followed for predatory mites, using the Potter spray tower apparatus at a range of five doses. Fifteen to twenty adults were exposed to each dose with three replications per rate respectively. The percentage of mortality was estimated in all experiments at 24 and 72 hours after treatment and was corrected using Schneider-Orelli's formula. Mortality levels (%) were found below 38%, 15% and 5% for M. *pygmaeus, N. tenuis* and *O. laevigatus* respectively. The $LC_{75}$ for the predatory mites was estimated at 1,558, 2,498 and 2,586 ppm for *A. swirskii*, *A. montdorensis*, *P. persimilis* respectively, close to the range of the recommended rates (RLR range 1304- 2480 ppm), while the residual toxicity was below 6% in all cases. Clavitus®13SL exhibited high compatibility with Hemiptera predators. For the predatory mites it was characterized as moderately toxic (<75%) under laboratory conditions thus, in follow up studies, the exact toxicity of Clavitus®13SL for these bifacials will be accurately estimated *in planta*.

**Keywords:** Hemiptera insect predators, predatory mites, IPM, toxicity, non-target effects

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## P269. Pest abundance in oilseed rape fields can be controlled by parasitoids and spatiotemporal distancing from previous year fields

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Pest abundance has increased, as the agricultural landscape is becoming more homogeneous and crop diversity is diminished. Using non-chemical methods and implementing integrated pest management (IPM) strategies for pest control can effectively control agricultural pests while maintaining conservation biocontrol. When implementing IPM practices, it is important to understand how spatiotemporal location and adjacent habitats influence crop colonization and damage severity. Oilseed rape (*Brassica napus* L.) is attacked by multiple insect pests and two of the major ones are the pollen beetle (*Brassicogethes aeneus* Fabricius) and cabbage seedpod weevil (*Ceutorhynchus obstrictus* Marsham). Our studies on both pests have shown that using spatiotemporal field separation, where the previous year's oilseed rape fields are kept at least 500 meters apart from the currently managed fields, reduces pest abundance and infestation in the present year while maintaining at least a 31% parasitism rate. Seminatural habitats adjacent to crops and the distance from its can also affect both *B. aeneus* and *C. obstrictus* while maintaining their high parasitism rate. Hymenopteran parasitoids can potentially control the pest abundance but only if the agricultural practices support their presence. The use of spatiotemporal distancing from the previous year's same crop should be investigated as a promising approach to integrated pest management strategy and agricultural producers should work towards bringing biodiversity back to the agricultural landscape to preserve and enhance natural enemies of pests and magnify their contribution to pest control.

**Keywords:** *Brassicogethes aeneus*, *Ceutorhynchus obstrictus*, biocontrol, field margins, parasitism, pest control

## P270. Pesticide resistance in natural populations of two predators, key biocontrol agents in horticultural crops

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Integrated pest management often combines the application of biological control with conventional methods such as chemical pesticides. The aim of this study is to make key predators for biocontrol better suited to agrosystems, by increasing their pesticide resistance. The tolerance against insecticides commonly used against major pests of greenhouse vegetable crops was evaluated in one wild population of the predator *Amblyseius swirskii* Athias Henriot (Acari: Phytoseiidae) and in four wild populations of the predator *Nesidiocoris tenuis* Reuter *(Heteroptera: Miridae)*, in order to assess standing genetic variability. Furthermore, *A. swirskii* mites were artificially selected, to increase their pesticide resistance. Wild *A. swirskii* mites showed increased tolerance, to spinosad and abamectin, when compared to their commercial counterparts. In addition, variation in the tolerance of wild populations of *N. tenuis* to abamectin and flupyradifuron was found as well. Resistance to these insecticides may have appeared in wild populations of the predator species due to the long-term use of these active ingredients in agriculture. Artificial selection with abamectin and spinosad of a wild population of *A. swirskii* did not result in an increase of its resistance to these pesticides. However, the wild *A. swirskii* population is more suitable for combination with these insecticides in IPM strategies compared to the commercial strain because, at the recommended field doses of abamectin and spinosad, the wild population suffers less than 50% mortality. The above findings suggest that insecticide resistance can be found in natural populations of biocontrol agents that can be successfully combined with chemical control in IPM strategies.

Keywords: phytoseiid, mirid, genetic variation, IPM, artificial selection

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## P271. Agroecology-inspired Strategies and Tools to Enhance Resilience and ecosystem services in tomato crop (ASTER)

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Tomato is a key crop worldwide, particularly in the Mediterranean Basin. Its market value in the EU represents about 7 billion euros. Tomato can be grown in open fields or protected conditions, and short or long production periods can be possible due to the large availability of commercial varieties. Regardless of the type of cultivation or the duration of the cycle, increasing quantities of external inputs (pesticides, fertilizers, herbicides) are required to cope with: i) nutrition issues linked to the deployment of the soil; ii) resident and invasive species made more aggressive by the climatic change and the development of resistance to pesticide; iii) water limitation which is more critical in arid and semi-arid climates of the Mediterranean area due to climatic change. The PRIMA project "ASTER" aims to build up a management model for small tomato producers of the Mediterranean Basin based on the application of main agroecology principles such as (i) the conservation and the enhancement of functional biodiversity both above and belowground, to increase and exploit the ecosystem services (protection, nutrition, pollination) in alternative to the use of external synthetic inputs; (ii) the sustainable control of main pests and pathogens to reduce the environmental impact of plant protection practices; (iii) the circularity of the production chain to approach the "zero waste" objective. ASTER will improve the tomato resilience in the economy of all Mediterranean Basin countries where it can be grown in open fields or protected systems, during the entire annual season, particularly in small farms.

Keywords: aboveground biodiversity, belowground biodiversity, natural derived compounds, biological control, pollination

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### P272. Phenology and distribution of billbugs (*Sphenophorus* spp.) in sod farms

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The billbugs, *Sphenophorus* spp. (Coleoptera: Curculionidae) are important pests attacking turfgrass. Evaluation of linear pitfall trap captures revealed *Sphenophorus venatus vestitus* as the major billbug species (> 98%) in the sod farms of central Georgia, USA. Also, seasonal billbug captures were influenced by turfgrass phenology (e.g., early and late growth stage and fully grown turfgrass). The numbers of *Sphenophorus* spp. collected were significantly greater in the fully grown turfgrass than in the early- and late-growth stages. Adult billbugs were sampled from harvested and nonharvested areas of sod farms by using linear pitfall traps. A significantly greater number of billbug adults were captured from the nonharvested than from the harvested sod. *Sphenophorus* spp. adults actively emerged from the harvested and nonharvested sod areas. The spatial distribution analyses revealed a significant aggregation pattern for adults and larvae. The average ranges of spatial dependence for larval and adult samples were 3.9 m and 5.4 m, respectively. Implications of this new information for managing billbugs in sod farms will be discussed.

**Keywords:** turfgrass, Curculionidae, linear pitfall trap

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## P273. Parasitoids of *Nezara viridula* (Linnaeus, 1758) in Hungary

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The southern green stink bug *Nezara viridula* (Linnaeus, 1758)- believed to have originated in eastern Africa and Mediterranean- has spread all over the world through commercial activities. The southern green stink bug (SGSB) is highly polyphagous, feeding on more than 100 plant species of more than 30 families but preferring legumes and brassicas. In Hungary, SGSB was first reported in early 2000's, since then its parasitoids has not been reported. In this study, we investigated on wild egg masses of *N. viridula* for their potential parasitoids in different areas of Hungary during 2022. The collected parasitoid samples were identified with molecular technology. The parasitoids collected from the wild egg masses of *N. viridula* were mainly *Trissolcus basalis. T. basalis* is globally distributed and a potential parasitoid of *N. viridula* however, the distribution of *T. basalis* within Europe has been rather sparsely documented and their presence were reported in France, Italy, Spain, Slovenia, Bulgaria, Germany, Portugal, Montenegro, Cyprus, and Belgium. In Hungary, *T. basalis* has not yet been reported previously from eggs of *N. viridula,* therefore this study is the first report in Hungary.

**Keywords:** *Nezara viridula, Trissolcus basalis*, parasitoid, natural enemy

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## P274. Optimizing decision-making potential, cost and environmental impact of traps used for monitoring of olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)

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This work aimed at optimizing olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) monitoring and integrated management, to ensure optimal and less-costly decision-making and timely intervention. For that, two years field trials in Andalusia (Spain) were carried out to optimize trap model, color, and size, together with the trap density, to accurately determine pest spatial distribution and damage curve as a function of the olive cultivar. Among the six trap models selected, Mcphail and yellow sticky traps outperformed the other four models for fly monitoring, whereas Mcphail traps caught the higher number of natural enemies. Therefore, the sticky trap model was selected to unravel the effect of the color on both pest and natural enemy catches, with the yellow one outperforming the white, green, and blue in pest monitoring with lower impact on natural enemies. Regarding yellow sticky trap catching surface, even if 20x25 and 10x25 cm single-size traps were equally efficient for olive fly monitoring, the large double-sided yellow sticky trap catches significantly more natural enemies than the small double-sided trap, a key result for developing a less-costly and environmentally friendly monitoring system. On the other hand, for the six selected olive varieties, it was demonstrated the cultivar-based shape of the damage curve. Finally, it was determined that 15 traps per hectare optimized the estimation of the pest spatial distribution. On the overall, the present research provides key information for new trap design and olive fly forecasting and IPM development.

Keywords: sustainability, monitoring systems, tephritids, olive fruit fly, control, economic threshold

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## P275. Neonicotinoid insecticides and its alternatives against soil pests from sunflower crops

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Due to the restrictions imposed by the European Commission in Regulation (EU) No 485/2013, that forbids the use of clothianidin, imidacloprid and thiamethoxam, Romania is facing an alarming increase in the population density of some soil pests, the most dangerous being *Tanymecus dilaticollis* and *Agriotes* spp., no insecticides being available for sunflower seed treatment. Our study aims to identify alternative methods and means to control pest populations of *Tanymecus dilaticollis, Opatrum sabulosum* and *Agriotes* spp. in sunflower crop. Studies were performed in three experimental locations in Romania, where sunflower is one of the main crops and the mentioned pests exceed the economic threshold. As chemical treatments, it was applied seed treatment with Langis + Microfert insecticide, supplemented with two treatments in vegetation with Mospilan 20SP and Faster Delta. As biological treatments, it was performed soil treatment with *Beauveria bassiana* in two doses and seed treatment with Biosem, a biological product based on neem oil. Sunflower crop has been affected by *Agriotes* spp. larvae and adults of *Tanymecus dilaticollis* and *Opatrum sabulosum*, the most intense attack of *Agriotes* spp. was registered to the seed in emergence. To keep pests below economic damage threshold, 2 treatments were also applied in vegetation, one with Faster Delta and one with Mospilan 20 SP. Best results were registered in the variants of seed treatments with Langis + Microfert, followed by vegetation treatments with Faster Delta. Biological control with *Beauveria bassiana* and Biosem did not ensure protection of sunflower from the attack of targeted pests.

Keywords: sunflower, neonicotinoids, alternative means, *Tanymecus dilaticollis*, *Agriotes* spp

## P276. Peptides and metabolites against *Aculops lycopersici* and *Bemisia tabaci* in tomato

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The tomato russet mite *Aculops lycopersici* and the whitefly *Bemisia tabaci* are key pests in tomato crops causing serious yield losses if left uncontrolled. Methods other than chemicals are required to control the populations of both pests, whereas biological control with the release of predators is not successful against *A. lycopersici*. In this study, we assessed the effects of a series of compounds of plant origin, such as the peptide systemin and metabolites when applied as soil drench, with the aim to enhance tomato resistance against *A. lycopersici* and *B. tabaci* via plant defense elicitation. Plants were infested with a standard number of mites or whitefly females after being treated with the peptide, the metabolites, or not. After two weeks or five days, we assessed the number of live *A. lycopersici* individuals or *B. tabaci* females, respectively. In the latter case, we also counted the number of eggs on each plant. In the case of systemin, different application methods such as spraying or seed treatment, and different application doses, timing and combinations with beneficial microbes were assessed. Our results highlight the potential of peptides and metabolites application in pest control, as well as the need for further research in this field.

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**Keywords:** metabolites, plant defense, tomato, tomato russet mite, systemin, whiteflies

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## P277. Plant-mediated defences induced by an omnivorous mirid bug do not prevent transmission of a begomovirus by *Bemisia tabaci*

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Mirid bugs (Miridae) are omnivorous insects feeding both on herbivorous prey and plants. As such they not only control herbivores by direct consumption but also by the induction of anti-herbivore defences mediated through plant feeding. These direct and indirect effects result in lower population densities of herbivores like whiteflies and thrips. These herbivores, in turn, play a major role in the transmission of plant viruses. However, whether plant-mediated defences induced by mirid feeding can influence transmission and spread of plant viruses by herbivores is not fully understood. Here, we studied the induction of plant defences by a mirid bug (*Dicyphus hesperus* Knight) and evaluated the impact of this plant-omnivore interaction on transmission of tomato yellow leaf curl virus (TYLCV) a begomovirus transmitted by the whitefly *Bemisia tabaci* Gennadius. We observed a reduced preference of whiteflies to colonize plants exposed to mirids. However, the plant defences induced by *D. hesperus* did not prevent virus transmission under controlled greenhouse conditions. On the contrary, plants exposed to mirid bugs accumulated a similar amount of TYLCV as plants not previously exposed to mirids. Overall, in our system of study, induction of plant defences by an omnivorous mirid did not influence TYLCV transmission by whiteflies on tomato.

**Keywords:** plant defences, plant-virus-vector interactions, whitefly, tylcv, mirid, plant virus

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## P278. The long and challenging road to capitalize on knowledge of plant-based extracts

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Europe has planned to halve pesticide use by 2030 as part of the Farm to Fork strategy to achieve the European Green Deal. Global warming combined with all year-round availability of host plant for a majority of pests is likely to favor the installation of invasive alien species (IAS) in Europe. The reduction of the range of authorized chemical molecules, initially intended for the control of native species, challenges research to propose sustainable solutions to manage IAS.
Plant-based products are an alternative to synthetic products, already adopted in organic agriculture, such as *Tanacetum cinerariifolium* (natural pyrethrum), formulated as aqueous, organic extracts, or essential oil. The knowledge-based system Knomana has identified, in the scientific literature, 397 plant species used or experimented to protect 64 crop species in Africa against pests and diseases. A knowledge mining software is being developed, based on a method derived from artificial intelligence, i.e. formal concept analysis, to propose to the final user (e.g. producer, technician) potentially usable local plants. As some IAS are coming from the African continent, using Knomana can allow European researchers to identify interesting plant species present in Europe, or plant extracts to be imported from Africa, to control IAS once their official approval is obtained. Examples are given with the Noctuidae species *Spodoptera littoralis* and *Helicoverpa armigera,* already present in Europe, or the fall armyworm, *Spodoptera frugiperda*, that has been observed on maize in many African countries since 2016.

**Keywords:** biopesticide, botanicals, insect pests, Knowledge base

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## P279. Preferences of snap bean cultivars by the sweetpotato whitefly, *Bemisia tabaci* for egg laying and development

The sweetpotato whitefly, *Bemisia tabaci* Gennadius Middle East-Asia Minor 1 (MEAM1), is a global pest on vegetables that inflicts losses in crop production. Losses result from bot whitefly damage and disease transmission. Chemical insecticides are routinely used by vegetable growers to control MEAM1 but this pest has developed considerable resistance to most insecticide labels. Therefore, alternative plant protection strategies are required, such as using host plant resistance. In this study, 24 local and commercially available cultivars of snap bean (*Phaseolus vulgaris* L.) in the southern United States were determined for their susceptibility to MEAM1 during three seasons (2020 fall, 2021 spring, and 2021

fall). In each season, the number of MEAM1 adults, eggs, and nymphs was assessed weekly for 6 weeks, and snap bean yield was evaluated at the end. During the 2020 fall, the number of adults per leaf was highest on Week 1 (22 adults/leaf) but was not significantly different among the cultivars. The least number of eggs/leaf disk was observed on the 'Jade' cultivar. Cultivars 'Gold Mine', 'Golden Rod', 'Long Tendergreen', and 'Royal Burgundy' supported a significantly lower number of nymphs/leaf disk. Overall, the number of eggs and nymphs was highest on Week 2 and Week 4, respectively. Snap bean yield was significantly higher on cultivars 'Affirmed', 'Momentum', 'PV-857', 'Sybaris', and 'Tema'. In the spring and fall of 2021, temperature and relative humidity were positively correlated with whitefly infestations. These results provided valuable information for whitefly management on snap beans based on host plant resistance and yield.

## P280. Plant defensive activation through exposure to volatiles in citrus: effect on HLB and its main vectors

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Huanglongbing (HLB), or citrus greening, is a bacterial disease severely limiting citrus production in most citrus-growing regions worldwide. Fortunately, the disease and its two main insect vectors, the psyllids *Trioza erytreae* and *Diaphorina citri*, are absent in the Mediterranean citrus industry. Recent work has shown that activating plant defenses can limit HLB, mainly triggered by the salicylic acid (SA) signaling pathway. Our research group has developed a novel technique based on plant communication to activate the immune system of citrus. This activation is achieved by exposing the plants to specific organic volatiles they recognize as an alert signal to an imminent attack and preparing for it by inducing their defenses. Six volatiles were individually exposed to eight citrus species using a polymer diffuser. Almost all combinations of volatile and citrus species could overexpress the SA pathway. The volatile Z(3)-hexenyl propanoate [Z(3)-HP] had a good performance and was therefore studied for its effect on the biology of both HLB vectors in defensively activated plants. The oviposition of both psyllids was reduced by more than 50% in defensively activated plants. In addition, the behavior of their parasitoids, *Tamarixia dryi* and *T. radiata*, was studied in response to this defensive activation. Both parasitoids were attracted to plants activated by (Z)-3-HP exposure. Finally, this work also presents preliminary results on the effect of defensive activation through exposure to (Z)-3-HP on the acquisition and multiplication of HLB.

Keywords: *Diaphorina citri*, *Tryoza erytreae*, *Tamarixia dryi*, *Tamarixia radiata*, (Z)-3-hexenyl propanoate

## P281. Phenotyping methods for aphid resistance of wheat

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*Sitobion avenae* and *Rhopalosiphum padi* are two of the most economically important cereal aphids in Europe. They damage plants by feeding injury during phloem feeding, excreting honeydew and vectoring Barley Yellow Dwarf Virus. Recently, aphid infestations have become more severe due to warming climate and the rise of insecticide resistance. This has driven the need to find aphid resistance genes in wheat for use in integrated pest management. The study aimed to develop high throughput methods to screen wheat genotypes for aphid resistance by assessing aphid life history traits and behaviour. Aphid behaviour, mortality, reproduction, and relative growth rate were assessed on 29 commercial wheat varieties. Olfactometer bioassay using headspace samples and a rapid antixenosis resistance phenotyping identified wheat genotypes that were significantly less attractive to winged *S. avenae* and *R. padi.* Genotypes exhibiting high antibiosis resistance causing nymph mortality were also found. Electrical penetration graph (EPG) bioassay was performed to observe aphid feeding activity on the identified resistant varieties and susceptible varieties. Development of mapping population with contrasting resistance and susceptibility from the assessed panel of genotypes will allow for the identification of Quantitative Trait Locus (QTLs) for aphid resistance.

**Keywords**: aphid, *Sitobion avenae*, *Rhopalosiphum padi*, wheat, insect resistance

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## P282. Acquisition and inoculation of a begomovirus is influenced by host resistance to the virus and naturally occurring virus variants

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The Sweetpotato whitefly, (*Bemisia tabaci* Gennadius), transmits tomato yellow leaf curl virus (TYLCV), which is in the genus *Begomovirus*. TYLCV is a serious constraint in tomato production worldwide. TYLCV resistance in tomato is governed by semi-dominant genes (*Ty*$_{1\text{-}6}$), and resistant cultivars with one or more genes are widely planted to mitigate losses. TYLCV resistant cultivars upon infection do not exhibit hypersensitive resistance but instead exhibit systemic infection. TYLCV-induced phenotype often tends to be less severe in resistant than in susceptible cultivars. Research in our laboratory has focused on how resistant cultivars influence TYLCV acquisition and inoculation by whiteflies, and how these processes could influence TYLCV epidemics in the farmscape/ landscape. Results indicated that planting resistant cultivars might not reduce initial virus transmission by whiteflies but suggested that resistant cultivars could function as poor inoculum sources than susceptible cultivars and suppress TYLCV epidemics over time. Sequencing of TYLCV genomes from TYLCV and resistant cultivars showed substantial and consistent variations, thereby reiterating the ‘quasispecies’ nature of TYLCV. These variants were later established in the greenhouse through single-whitefly inoculations. Two variants

with single nucleotide polymorphisms and amino acid truncations were selected, and PCR-based assays were established to distinguish and quantitate them. In addition, whole genome cloning was used to establish genome identities and validate detection and/or quantitation methods. Ensuing virus acquisition and inoculation experiments revealed that variants differentially interacted with host cultivars and whiteflies. Excitingly, variants' interactions with whitefly acquisition were not density dependent and/or congruent. All results will be sequentially discussed.

**Keywords:** host resistance, vector fitness, virus transmission, vector fitness

### P283. Approaches to control *Popillia japonica* adults: Does the entomopathogenic fungal species *Beauveria pseudobassiana* have potential as control agent?

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The scarab beetle *Popillia japonica* is an invasive, polyphagous beetle that has infested regions in northern Italy and southern Switzerland, where it causes major crop damage. Recently, we discovered that *Beauveria pseudobassiana* is a dominant pathogen of *Melolontha melolontha*, a well-known pest in orchards, grasslands and certain crops in central Europe. The aim of this study was to test the control potential of *B. pseudobassiana* on the related *Popillia japonica.* We tested the infection and survival rate of *P. japonica* adults and larvae following dip-infection in individual conidia solutions ($10^7$ spores/ml) of four different *B. pseudobassiana* strains and a *M. brunneum* strain (BIPESCO5) registered for control of different scarabs. Death and fungal outgrowth were monitored in 75 individuals per treatment during four weeks. All the fungal strains resulted in significantly reduced *P. japonica* medium survival, i.e., 5-6 days compared to 22 days for the control. Mycosis was observed in 69-92% of the infected individuals. Of these 50-73% represented the applied strains, as shown by microsatellite marker genotyping. The remaining strains and the strains obtained from control individuals represented natural infections among the field-collected beetles. Tests with *P. japonica* larvae revealed 2-8% mortality after 70 days with no significant difference among treatments. Our results indicated that *B. pseudobassiana* isolates are as virulent as the registered *M. brunneum* strain, which reveals a high potential of this fungus for control of *P. japonica* adults. Next, we will test whether adult *P. japonica* may become infected by feeding on *B. pseudobassiana* treated leaves.

**Keywords:** Japanese beetle, *Beauveria pseudobassiana*, biocontrol agent, entomopathogenic fungus, bio-assay

## P284. Pilot application of bait sprays for the control of *Bactrocera oleae* with Unmanned Aerial Vehicle

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The olive fruit fly *Bactrocera oleae* is the most devastating pest of olives in the Mediterranean region. Pest control is primarily based on bait or canopy sprays. However, applying area-wide bait sprays can be extremely problematic due to restricted access to orchards or high-ground inclination. Using Unmanned Aerial Vehicles (UAV) for bait applications could overcome such issues. Therefore, a three-year field trial was conducted in olive groves of diverse morphology in the region of Crete, comparing the efficacy of standard ground bait applications vs UAV-mediated bait sprays. In each region, plots of olive groves were sprayed using one of the two approaches. Three replications and an untreated control plot were predicted per spray method and region. The insecticide spinosad formulated as concentrated bait (Success 0.24CB) was applied in all cases. Sprays were performed according to population density of the trap network. The efficacy was based on adult catches in McPhail traps (3 traps per plot) and olive fruit infestation level at weekly and biweekly intervals, respectively, in each plot. In 2020 & 2021 overall *B. oleae* population density was generally very low to extract valid conclusions. However, in 2022 population density was substantial (max 4000 caches /week) resulting in olive infestation levels up to 40%. Nonetheless both parameters exhibited similar trends in the respective plots suggesting similar pest control efficacy levels by both spray methods. Parameters in terms of water usage and duration of application were also investigated and were substantially reduced in UAV applications.

Keywords: *Bactrocera oleae,* Unmanned Aerial Vehicle, bait sprays, spinosad, olives

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## P285. *Cydia pomonella* remote monitoring in apple orchards using a new a smart trap prototype

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Smart traps for remote monitoring can facilitate insect pests' detection, increasing spatial and temporal resolution while reducing the need for field visits. Traps equipped with image acquisition systems rely on detection algorithms for the recognition and counting of target species. It is necessary, therefore, to ensure high-quality pictures by modifying the standard trap in order to comply with minimum focusing distance and field of view, while providing a trapping efficiency comparable to the standard systems on which phenological forecasting models and action thresholds are based. We developed and validated a smart trap prototype selecting the apple crop pest *Cydia pomonella* as a case study. We

considered design factors to improve trapping efficiency and compared automatic and manual counting for each collected image to implement the recognition algorithm. Since 2022, several field comparison trials were conducted to test different prototypes (4 colors and 2 designs), comparing their trapping capacity with the orange delta trap, which is considered the current standard monitoring tool for *C. pomonella*. Additionally, a detection algorithm (software) was developed by using open-source programs to automatically identify and count *C. pomonella*. The following parameters were calculated for each picture collected: false positive, false negative, accuracy, sensitivity and precision. Preliminary results suggest that delta traps performed better than the prototype (p-value<0.05) and color do not affect the trapping efficacy toward *C. pomonella* (p-value>0.05), while it affects the number of non-targets (p-value>0.05). The results of the 2023 trials, still in progress, will be presented in the poster.

**Keywords:** codling moth, pest monitoring, integrated pest management, automatic trap

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### P286. High-throughput isolation of fluorescent *Anopheles stephensi* larvae using the Biosorter large particle cytometer

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Union Biometrica's flow cytometers enable high-throughput analysis and sorting of large objects like insect larvae, nematodes, cell cluster and seeds. Objects are funneled one at a time through a flow cell, where optical density and fluorescence measurements are acquired via one or more laser beams. These parameters can be combined to select specific subpopulations of objects and dispense them in the receptacle of choice. Importantly, our patented air sorting devices operate at low pressures to gently deliver the specimen and maximize the survival of living organisms for further downstream applications. Our instruments proved effective in separating male and female *Anopheles sp* mosquitoes and identifying *Drosophila Melanogaster* larvae based on their fluorescent reporter expression. Here, we used a Biosorter large particles flow cytometer to isolate of *Anopheles stephensi* mosquito larvae that carry gene drive constructs inducing simultaneous DsRed, AmCyan and ZsYellow expression. Selected larvae expressing each reporter were dispensed into a 96 well plate and the sorting accuracy was assessed by fluorescent microscopy. These results confirm the versatility of the Biosorter as a high-throughput alternative to the visual selection of fluorescent larvae under a stereoscope. Because gene drive systems are gaining ground in the field of pest control, this approach can be translated to other environmentally-relevant insect species.

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### P287. Integrated management of the *Bactrocera oleae* using modern electronics, automations, and IoT technologies: The case of Stavies in Crete

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The olive fruit fly (*Bactrocera oleae*) is the major enemy of olive cultivation, with economic losses that exceed 30% in Greece. Due to the weaknesses of the National Pesticide Program (NPP), farmers additionally apply cover sprays with harmful consequences to the olive agro-ecosystem, such as (a) killing of other entomofauna, especially the beneficial insects, (b) development of insect resistance to pesticides, (c) olive oil contamination with pesticide residues, and (d) biodiversity balance disruption, etc. In the proposed paper, the use of modern electronics, automations, and IoT technologies are demonstrated and are going to be applied – for the first time on a large scale (pilot olive grove of “Stavies”) – for the modernization, optimization, and precision of application of *improved bait sprays*. Bait sprays will be thus implemented in a rational, optimal, and consistent way, minimizing the impact of human intervention on their effectiveness. The amount of solution sprayed per tree will be fully controlled and regulated by automatic spraying devices. A specialized and dedicated information system will also be developed to represent the detailed spraying data and all the necessary information related to the complete pest management procedure for direct supervision and timely/valid decision-making by the scientific staff. The platform will also provide the capability to be accessed by farmers for transparency, consistency, and unbiasedness. The proposed approach promotes the ideas of viable and sustainable agriculture by significantly reducing the use of harmful pesticides. It enhances the production of olive oil with special quality characteristics, optimal taste, and increased commercial value. The project is implemented by the Operational Group “DacoTech” (M16ΣΥΝ2-00358) funded under M16 of the RDP 2014-20 in Crete, Greece.

Keywords: *Bactrocera Oleae*, improved bait sprays, IoT, automations

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## P289. Biologically produced pheromones control *Helicoverpa armigera* and *Plutella xylostella*

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The development of a novel technology for the biological production ofpheromones from yeast fermentation, reduces significantly production cost, allowing farmers to protect field crops against pests in a sustainable way. Biological production of low-cost pheromones will enable the rapid adoption of pheromone-based pest management products that can be used as effective, affordable and non-toxic alternatives to insecticides. To validate and compare the performance of the biologically produced pheromones with those chemically synthesised, electrophysiological, behavioural and field tests were performed on cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and on diamondback moth *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae). Laboratory behavioural bioassays and field tests of the biopheromones in cotton and cabbage fields in Greece, confirm their activity. Specifically, in monitoring and sex communication disruption tests biopheromones were equally effective as the

commercially available chemically synthesised ones. For the sex communication disruption of *H. armigera* we developed a flowable polymer which adheres to plants, protects the labile pheromone molecules and controls their release in the environment. The resulting polymer blobs act as non-retrievable dispensers and can be easily distributed either manually or by means of UAVs. Respectively, for the sex communication disruption of *P. xylostella* we developed and used a novel hydro-degradable pheromone dispenser that disintegrates gradually emitting pheromone continuously throughout a growing season, eliminating the cost of manually replenishing or removing dispensers. By harvest the dispensers are gone dissolved by irrigation and rain. Pheromone production from yeasts is an innovative, ground-breaking technology expected to strongly influence the plant protection products industry.

**Keywords:** biologically produced pheromones, non-retrievable dispensers, hydro-degradable dispensers, UAV pheromone application

## P290. Transgenic potato plants expressing aegerolysin complexes confer resistance against Colorado potato beetle

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Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera: Chrysomelidae; CPB) is one of the most devastating insect pests of potato causing important yield losses worldwide. To combat this pest, various crop protection strategies have been employed, including chemical insecticides, entomopathogens, and biotechnological products, such as the proteinaceous endotoxins from *Bacillus thuringiensis* (Bt) and double stranded RNAs. However, CPB has already demonstrated rapid resistance development against chemical pesticides and biopesticides, including Bt toxins, highlighting the importance of integrating multiple modes of action for its effective management in the future. Recently, lipid-binding aegerolysin proteins derived from edible oyster mushrooms (Pleurotus sp.) have gained interest as new biopesticides and a solution with potential to resist CPB resistance development. These aegerolysin proteins, in combination with their protein partner pleurotolysin B, interact specifically with sphingolipid-enriched insect membranes, resulting in pore formation in the CPB midgut. This unique mechanism of action exhibits potent and selective toxicity towards CPB, making it a potential breakthrough in pest control strategies. We will present the strategy used to obtain potato plants expressing a bioinsecticidal aegerolysin-based protein complex and show the results of feeding trials with the transgenic plants that demonstrate its effective protection against CPB infestation. In addition, we will showcase the genome-wide expression response of CPB larvae exposed to the protein complex, providing insights into its physiological impact on the pest.

**Keywords:** novel biopesticides, plant-incorporated-protectant (PIP), *Leptinotarsa decemlineata*, *Solanum tuberosum*, larval toxicity, transgenic crop

## P291. Using the principles of ECOstacking to develop ecologically-based IPM approaches in apple agroecosystems in New England

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ECOstacking is the assembling of ecosystem services to achieve functional biodiversity. One ecological approach to pest management involves manipulating the habitat by means of planting non-crop plants. Non-crop plants can be trap crop plants that can pull the pests away from the cash crop, and insectary plants that can attract and boost natural enemy populations. In 2018, we initiated a long-term project aimed at developing a permanent, low-cost, trap cropping system for multiple apple pests by grafting selected perimeter-row trees with six apple cultivars that are attractive to key pests including plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), and apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae). This long-term study aims at manipulating insect pest behavior through host plant preference by increasing plant diversity at the local scale. Here, we report the results of 2021 and 2022 research concerning plum curculio. Significantly more oviposition injury was observed in fruit sampled from grafted trees relative to non-grafted trees. Because branches were grafted in 2018 and grafted branches continue to grow, then further research is needed over multiple years to confirm whether multicultivar-grafted trees can be used as “trap crop” to bring multiple pests to those trees. This type of IPM approaches can be readily implemented to support ecologically-based pest management via ECOstacking in support of more sustainable crop production.

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## P292. Proximal remote sensing in detecting damage by cereal leaf beetle (*Oulema* sp.) in winter wheat

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Cereal leaf beetles (CLB; Oulema sp.) are major pests of wheat. The typical symptoms of both adults and larvae are thin lines where the larvae peel off the epidermis of the leaf, while the adults chew completely through the leaf, producing longitudinal slits. Damage from infestation is evident in the decline in chlorophyll content and the extent of leaf tissue loss, which reduces photosynthetic efficiency. This results in yield losses as high as 40%, especially during drought. Recently, remote and proximal sensing systems have been used to detect various stress factors in crops. These methods rely on hyperspectral sensors to detect changes in plant reflectance due to variations in leaf structure and pigments. They are suitable for rapid assessment of physiological responses of plants to pest infestation and are therefore an important component of crop protection strategies and site-specific pest management. Therefore, our objective was to determine the reflectance patterns of wheat infested by CLB using proximal hyperspectral measurements. As CLB triggers loss of chlorophyll content, this leads to a decrease in absorption and an increase in reflectance of radiation throughout the visible region of the spectrum (400-700 nm) with a pronounced green peak at 550 nm. Based on the spectral measurements of healthy and infested leaves, a cluster analysis was performed to distinguish healthy from insect-infested plants. Our results suggest that hyperspectral measurements of leaves are a reliable method for detecting stress from insect infestations, with great potential for larger-scale application using multispectral drone or satellite imagery.

Keywords: cereal leaf beetles, winter wheat, remote sensing, proximal sensing, hyperspectral data

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## P293. Image-based detection and identification for smart pest management using deep learning: The case of *Bactrocera spp*. and *Ceratitis spp*. fruit flies in Ghanaian mango orchards

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AI-based tools in automated visual identification of insects are rapidly gaining popularity in entomological data collection. In agriculture, these methods can be utilized in smart pest management for detecting and monitoring pest populations, potentially in real-time, enabling targeted use of insecticides, for example. We trained a state-of-the-art object detection model, yolov7, using images of pests of mango orchards. The target insects were captured on yellow sticky traps associated with specific sexual pheromones in Tamale, Ghana. The traps were set in the field for one week before being photographed in situ using smartphone cameras. The dataset consists of images with annotated examples in two different genera of fruit flies: *Bactrocera* and *Ceratitis*. Some of the classes had more representation in the data than others. This class imbalance is common when collecting insects, but also a classic problem in machine learning. Learning tends to be biased towards the majority class, which can lead to poor performance in the rarer classes. We compare different methods of data pre-processing and loss functions with the base model to select the best model using evaluation metrics more sensitive to class imbalance. Our results show that low-effort data collection is sufficient for training an automated identification system capable of reliably identifying and quantifying pest insects.

Keywords: precision agriculture, smart pest management, automated identification, object detection

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## P294. Experimental development and implementation of a research program against the Moroccan locust *Dociostaurus maroccanus* Thunberg (Orthoptera: Acrididae) in Aghios Efstratios island

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Locusts (Orthoptera: Acridoidea) are of great ecological significance since they are part of the ecosystems both in the role of consumers and prey. Excessive growth of the locust population can lead to outbreaks that create significant problems to the crops. Locust outbreaks are caused by various factors such as precipitation, overgrazing and deforestation. Managing locust outbreaks is challenging due to their extensive distribution and high reproductive capacity. Moreover, climate change has made it difficult to predict future outbreaks. The small island of Agios Efstratios in Greece has suffered from outbreaks by the Moroccan locust *Dociostaurus maroccanus* (Orthoptera: Acrididae) since 2016. Their voraciousness has led to habitat destruction, which affected the livelihoods of inhabitants, most of whom are employed in livestock farming. Considering the fact that the island belongs to the NATURA2000 network, a specialized program was applied in order to control locusts' population and restore the biodiversity of the island. Data from a surveillance drone and scouting teams were combined to create a map of the locations of the locust bands. Three active ingredients were used (lambda-cyhalothrin, diflubenzuron and spinosad), through backpack ultra-low-volume sprayers, vehicle-mounted sprayer. Moreover, a spraying UAV was used in steep slopes which were inaccessible for the other teams. The success of the method was evaluated by recording the population of the Moroccan locust and calculating the Simpson biodiversity index. Population of *D. maroccanus* was reduced and the ecosystem of the island has come back into balance, as shown by the increase in biodiversity.

**Keywords:** *Dociostaurus maroccanus* outbreak, control program, UAV, biodiversity

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## P295. Integrated management of the *Bactrocera oleae* using modern electronics, automations and IoT technologies: The case of Roumata in Crete

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The olive fruit fly, *Bactrocera oleae*, is considered to be the major pest of olive tree cultivations, causing a particularly increased quantitative and qualitative degradation of the produced olive oil. Due to climate change to subtropical and human intervention in the agroecosystem of the olive grove, high pest populations appear early in spring, threatening the forthcoming olive production. A representative example is the monumental olive grove of "Palaia Roumata" in Crete, Greece. In this olive grove, there is a high risk of producing olive oil with degraded quality characteristics due to increased pest infestation or insecticide residues from the intensive use of chemicals (applied by cover and/or bait sprays). At the same time, resistance to insecticides has also been reported. In this paper, the optimal combination of mass trapping (which is considered a biological control method) with precision and *improved bait sprays* (low-volume sprays using electronic equipment and automation systems) is proposed and presented, thus suggesting an environmentally friendly, comprehensive, efficient, and effective management of the insect [Integrated Pest Management (IPM)]. This approach minimizes the use of agrochemicals by 50% and contributes to the protection of the biodiversity of the olive-agroecosystem, thus fully compatible and consistent with the European Green Deal. The project is implemented by the Operational Group

“PROTOTYPE OLIVE ORCHARD OF ROUMATA” (M16ΣΥΝ2-00380), funded under M16 of the RDP 2014-20 in Crete, Greece.

**Keywords:** *Bactrocera oleae,* mass trapping, improved bait sprays, IoT, automations

# Session 16:
## Stored Product Protection

## P349. Utilizing nitrogen as a phosphine resistance breaker in stored product protection

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Modified atmospheres are regarded as a highly promising method for the effective control of insect infestations during the post-harvest stages of durable agricultural commodities. In the current study, we evaluated the effectiveness of modified atmospheres in commercial nitrogen chambers for phosphine-susceptible and -resistant stored product insect populations. After each nitrogen application, mortality data were collected, and all insects were kept under controlled conditions (26°C, 65% relative humidity) for 65 days to observe their progeny production capacity. In all cases, complete parental mortality was observed, while there was no progeny production for most of the trials, with some exceptions. To evaluate the effectiveness of the method in areas with no available sensors, we developed a computational model to predict the nitrogen concentration. Simulation results revealed that nitrogen can easily penetrate the treated commodities, and that its concentration is uniform. Additionally, the simulation model showed that temperature levels do not affect nitrogen concentration profiles. Overall, our results indicate that nitrogen generators creating low oxygen atmospheres can be used to control stored product insects with varying susceptibility to phosphine and can be adopted as an alternative method to control stored product insects.
This research is part of the project «Management of entomological infestations in the stored products by using innovative technologies» (Project code: KMP6-0081034) that is co-funded by Greece and European Union by the Action «Investment Plans of Innovation» in Central Macedonia under the framework of the Operational Program «Central Macedonia 2021-2027».

**Keywords:** modified atmospheres, nitrogen, low oxygen, stored product insects, phosphine resistance

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## P351. Sublethal effects of *Acmella oleracea* hexane extract on various behavioral traits of *Prostephanus truncatus*

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Botanical insecticides can represent sustainable alternatives to synthetic pesticides for pest control. *Acmella oleracea* (L.) exhibits insecticidal properties that deserve special attention. Herein, we investigated the sublethal effects of *A. oleracea* hexane extract (HE) on the motility, mating, and

laterality behavior of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), which is one of the most destructive pests of stored maize and cassava. In toxicity experiments, the lethal concentrations $LC_{10}$, $LC_{30}$, and $LC_{50}$ of *A. oleracea* HE were calculated. Furthermore, locomotor behavioral assays were carried out to assess the impact of being exposed to $LC_{10}$ and $LC_{30}$ of *A. oleracea* HE on adult mobility. Results showed that insect locomotor ability was significantly affected in terms of velocity, duration of walking, duration of stops, number of short stops, duration and number of upside down, if compared to control beetles. The mating success of *P. truncatus* males was negatively affected by their exposure to $LC_{10}$ and $LC_{30}$ of *A. oleracea* HE in comparison with the control males. Regarding lateralization, males of *P. truncatus* were found right biased at population level during their sexual interactions. Overall, this study reveals that low concentrations ($LC_{10}$ and $LC_{30}$) of *A. oleracea* HE had negative impact on the mobility and mating behavior of *P. truncatus* adults, issues that merit consideration in the implementation of sustainable management protocols against this stored product species.
This study was supported by the COST Action 20132- Urban Tree Guard- Safeguarding European urban trees and forests through improved biosecurity, through a Short-Term Scientific Mission (STSM Request Reference No. E-COST-GRANT-CA20132-a64f6e35) of Maria Boukouvala at the Department of Agriculture, Food and Environment, University of Pisa.

**Keywords:** stored-maize pest, plant-derived pesticide, motility, laterality, copulation success

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## P352. Usage of insect-based chitosan for the preservation of fresh cherry tomatoes

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The main commercial source of chitin, the second most abundant biopolymer on Earth, are the crustacean exoskeletons, that are no longer sustainable. Insect-derived chitin has received increasing attention, as an alternative and more sustainable source. Among insects, the Diptera *Hermetia illucens* is a very promising source as larvae can feed on organic waste and bioconvert it into larval biomass, while breeding waste (pupal exuviae and dead adults) can be used to extract chitin. Chitosan, its main deacetylated derivative, due to its antimicrobial, antioxidant and film-forming capacity, it's one of the most promising biopolymers to use as edible coatings, that reduce dehydration and microbial/fungal spoilage by acting as barriers, able to delay ripening and senescence. Due to its content of healthy compounds, tomato (*Solanum lycopersicum*) is one of the most consumed and cultivated fruits worldwide. Chitosan produced from *H. illucens* pupal exuviae was used. was applied by spraying and by dipping on the tomatoes at two concentrations (0.5% and 1 %); then, they were stored at two temperature conditions: room temperature and 4°C. The weight loss, physico-chemical parameters, total phenolics, total flavonoids and variation in antioxidant activity of the tomatoes were studied for a 30-day storage period. The spraying method, compared to the dipping technique, was more effective in reducing weight loss and pH variation. Chitosan coatings were effective in reducing the pH variation of the tomatoes. No significant differences in the protective action between chitosan derived from *H. illucens* and commercial chitosan were found.

**Keywords:** chitosan, biofilm, fruit shelf life

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## P353. Food impact on immediate and delayed mortalities of three insecticides applied on concrete against *Alphitobius diaperinus* small and large larvae

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In the current study we examined the immediate and delayed mortalities of piperonyl butoxide+acetamiprid+d-tetramethrin, deltamethrin, and etofenprox applied on Petri dishes covered with concrete against *Alphitobius diaperinus* small and large larvae, in the presence or absence of food. Immediate mortality was noted 1, 3, 5, and 7 days post-exposure, while delayed mortality was noted 7 days post-transport of the larvae alive to concrete dishes without pesticide. Piperonyl butoxide+acetamiprid+dtetramethrin killed 84.4% (with food) and 100.0% (without food) of small larvae after a 7-day exposure. It caused low to moderate mortalities to large larvae, not exceeding 67.8% at the same exposure interval. Regarding deltamethrin, 100% of small larvae were dead after the exposure for 3 and 5 days at dishes without and with food respectively. Deltamethrin killed 100% and 98.9% of large larvae 5 days post-exposure on dishes with and without food respectively. Concerning etofenprox, 97.8% and 80.0% of small and large larvae died in dishes without food, 7 days post-exposure respectively. Delayed mortality varied among the three tested insecticides. Piperonyl butoxide+acetamiprid+d-tetramethrin killed up to 85.0% of small larvae in the presence of food, but in the case of large larvae with food present it did not exceed 33.5%. Deltamethrin caused 0% mortality to large larvae in dishes with food. Etofenprox caused 100% mortality of small larvae (without food) and 24.0% of large larvae (with food). Overall, deltamethrin killed almost all larvae, regardless of their size and presence of food, ≤5 days post-exposure.

**Keywords:** etofenprox, deltamethrin, piperonyl butoxide+acetamiprid+d-tetramethrin, surface application

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## P354. Identification of dominant insect species in storage facilities of specific dried fruits in Greece, Turkey, and Israel

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Early detection of the presence of stored product insects is the basis of sampling in storage facilities. Trapping is the most useful technique in stored product pest management and can be used as a viable technique to optimize the time for insecticidal applications. In the present study, the dominant species were monitored in different storage facilities, located in Turkey, Israel, and Greece using different traps and attractants. The results of the present study illustrate different species and capture numbers for each facility and country. More specific, for Greece the dominant species on currants were:

*Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), *Carpophilus freemani* Dobson (Coleoptera: Nitidulidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae). For Turkey on figs, were: *Carpophilus hemipterus* L., *O. surinamensis*, *Ephestia cautella* (Walker) and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and for Israel on dates were: *C. hemipterus* and *Stegobium paniceum (L.) (Coleoptera: Anobiidae).* The results of this survey demonstrate trapping is an important tool for detecting and monitoring stored product insects as part of an integrated pest management program.

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**Keywords:** trapping, identification, dried fruits, storage facilities

## P355. Efficacy of *Carlina acaulis* essential oil nanoemulsion as wheat protectant against adults and larvae of three Tenebrionidae pests

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*Carlina acaulis* root essential oil (EO) was encapsulated in a nanoemulsion (NE) (6% EO (w/w)) and tested against *Tribolium castaneum, Tribolium confusum* and *Tenebrio molitor* adults and larvae when applied on wheat kernels. The NE was tested at two concentrations (500 and 1000 ppm). Mortality was determined after 4, 8, and 16 h and daily from 1-7 days. The 1000 ppm killed 93.9% and 98.9% of *T. castaneum* and *T. confusum* larvae, 7 days post-exposure respectively. *Tenebrio molitor* larvae were tolerant since only 18.9% of the exposed individuals were killed at the same dose and exposure. The concentration 1000 ppm killed 85.2% of *T. molitor* adults after 7 days of exposure. Adult mortalities of *T. confusum* and *T. castaneum* were low, not exceeding 21.4% and 23.3% respectively, at the end of the experiment, at 1000 ppm. *Carlina acaulis* EO-based NE is an effective green insecticide for both adults and larvae, depending on the target insect species and its life stage, leading towards advanced pest management strategies.

**Keywords**: *Tribolium castaneum*, *Tribolium confusum*, *Tenebrio molitor*, stored grain, developmental stage, stored-product

## P356. Insecticidal effect of three insecticides applied on different surfaces for the control of three stored-product beetle species

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We evaluated the efficacy of alpha-cypermethrin, deltamethrin and spinosad on concrete, ceramic, plastic and metallic surfaces against found major stored-product beetle species, i.e. the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). For alpha-cypermethrin, deltamethrin and spinosad the dose rates applied to surfaces were 0.01, 0.05, and 0.1 mg of active ingredient (Al)/cm$^2$, respectively. All three insecticides were applied at the label dose, while the bioassays were carried out against the adult stage of the three insect species. Mortality was assessed after 3, 7 and 14 days of exposure. The results of the present study revealed that the AI with the highest efficacy for all species was spinosad, while the most tolerant species for all AIs evaluated was *T. confusum.* Surface type was significant for all species, except for *T. confusum*. Concrete reduced the efficacy of deltamethrin, while ceramic reduced the efficacy of spinosad. Regarding the overall data, spinosad and deltamethrin can effectively control the species tested here, but there are certain factors that affect their efficacy, such as the exposure interval and the type of the surface.

This research is part of the project "*Management of entomological infestations in the stored products by using innovative technologies*" (Project code: KMP6-0081034, acronym: InsectStop) that is co-funded by Greece and European Union by the Action "Investments Plans of Innovation" in Central Macedonia under the framework of the Operational Program "Central Macedonia 2014–2020".

Keywords: surface treatment, insecticides**,** stored-product insects, mortality, efficacy

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## P357. Influence of grain type on the efficacy of graphene for the control of *Tribolium castaneum*, *Sitophilus zeamais* and *Sitophilus oryzae*

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The efficacy of three graphene formulations against adults of three major stored grain beetle species, the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae), the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in three grain commodities was evaluated under laboratory conditions. The tested grains were soft wheat, paddy rice and maize. Graphene was applied at three dose rates: 0 (control), 500 and 1000 ppm and bioassays were carried out at 25 °C and 60% r.h. Mortality of the exposed adults was assessed after 7, 14 and 21 days of exposure while progeny emergence was recorded 65 days after the last exposure interval. The grain type affected the insecticidal activity of graphene. All three graphene formulations were effective against the tested insect species and the increase of dose rate notably increased insect mortality in all grain commodities. The results of the present study show that graphene is effective in a variety of grain commodities, and its utilization as a grain protectant can be further investigated.

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**Keywords**: stored product insects, graphene, grain type, commodity, insect mortality

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### P358. Insecticidal potential of lavender essential oil against some stored grain cereals pests

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Annually, grain losses during storage can reach 50% of total cereal production due to the action of specific pests and the proper treatment of the grain and harvest is essential to reduce these damage. Synthetic insecticides used for the removal of these pests, had not only developed resistance but also caused harmful effects for humans and the environment mainly due to their slow degradation in the environment and toxic residues in the products. Among natural products, essential oils from plants belonging to several species have been widely tested against diverse insect pests to evaluate their repellent properties and fumigant toxicity being a valuable natural resource since they, unlike conventional pesticides, present no risk to humans and the environment. The lavender essential oil used in the experiments was tested on the larvae and adults of the two harmful species *Sithophilus granarius* and *Tribolium castaneum* existing in the stored grain (wheat and corn seeds) using fumigation and repellency test carried out at five concentration (doses) and different exposure times. Lavender oil, suppressed egg hatching and subsequent development of eggs to larvae and adults of *S.granarius.* Adults of *S. granarius* and *T. castaneum* showed similar susceptibilities to the contact toxicity of lavender oil at the LD50 level, but *S. granarius* was more susceptible than *T. castaneum* at the LD95 level. Lavender oil may be a potential protectant for stored grain by controlling different life stages of *T. castaneum* and *S. granarius* through contact actions and as a fumigant.

**Keywords:** natural insecticide, wheat, maize, contact toxicity, stored pests

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### P359. Usage of insect-based chitosan for the preservation of fresh fruits

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Due to its properties, chitosan is suitable for use as a natural biopolymer coating material to preserve the quality and extend the shelf-life of fresh foods. Chitosan-based active films against microbial contamination and spoilage are successfully used in fruit and vegetable packaging. Currently, the main commercial chitin and chitosan source is the waste stream from the crustacean industry. However, crustacean low availability and sustainability make insects, particularly bioconverters, a promising alternative source of chitin and chitosan. This work is focused on the exploitation of one of the side streams generated from *Hermetia illucens* farming, pupal exuviae. Chitosan from pupal exuviae of H. illucens were used for the formulation of coating solutions to be applied to fresh fruit. *Prunus armeniaca*, *Prunus persica* and *Persica vulgaris* were coated with 0.5% and 1% bleached and unbleached chitosan and stored at both room temperature and 4°C. The effect of the chitosan coating was evaluated by determining changes during storage occurred in weight loss, total soluble solids content, pH and mould development. The obtained results revealed that insect chitosan is as effective as or better than the commercially chitosan in maintaining more stable some post-harvest physicochemical parameters. The effects are more evident on fruits stored at controlled temperature. No relevant differences were found between the tested concentrations, nor between decolorized and not decolorized chitosan. These preliminary results provide an encouraging starting point for validating pupal exuviae from *H. illucens*, for the chitosan production and use in the agri-food industry.

**Keywords:** chitosan, biofilm, fruit shelf life

## P360. Insecticidal effect of contact insecticides applied on fortified rice for the control of stored product insects

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We examined the efficacy of pirimiphos-methyl, deltamethrin and cypermethrin on rice fortified with spearmint against major stored product beetle species, i.e. the red flour beetle, *Tribolium castaneum* (Herbst) (Coleptrera: Tenebrionidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptrera: Silvanidae) and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichychidae). All three insecticides were applied at the label dose, while the bioassays were carried out against the adult stage of the four target species, with the exception of *T. castaneum* and *O. surinamensis*, for which larvae were also tested. The vials were kept in controlled conditions at 26ºC and 55% relative humidity. Mortality was assessed after 3, 7, 14 and 21 days for each species. After the termination of this interval, all individuals were removed from the treated fortified rice and remained at the same conditions for 65 days. Based on our results, the least susceptible species was *T. castaneum*, while the most susceptible species was *S. oryzae*. Regarding the overall data, deltamethrin and cypermethrin can effectively control the species tested here, but there are certain factors that affect their efficacy, such as the exposure interval.

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH—REATE-INNOVATE (project code: T2EΔK-03726).

**Keywords:** fortified rice, stored product insects, mortality, progeny production, insecticides, spearmint

## P361. Insecticidal effect of different diatomaceous earth formulations for the control of stored product beetles

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Diatomaceous earths are promising alternatives over the use of traditional insecticides. For the assessment of ten diatomaceous earth formulations (namely as AGR, DD, ELC, SLEC, D3, D5, D6, TRH, D7, D8), laboratory bioassays were carried out for the control of a wide range of stored product insect species. The species tested were the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the granary weevil, *Sitophilus granarius* (L.) (Coleoptrea: Curculionidae), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptrera: Tenebrionidae), the confused flour beetle *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptrera: Silvanidae), the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptrea: Tenebrionidae). Different dose rates, i.e. 0 (control), 300, 500, and 1000 ppm, were used for each of the aforementioned dust formulations. Mortality of the exposed individuals was measured after 3, 7, 14, and 21 days of the exposure. Moreover, progeny production was counted 65 days later. Based on our results, three out of the ten dust formulations used (DD, AGR, TRH) were very effective for the control of all stored product beetle species. The present study provide data that encourage the utilization of DE in stored product protection, as an integrated pest management tool.

This research is part of the project «Integrated management of insect infestations in stored animal feed: Feed without pesticides» (Project code: KMP6-0088130) that is co-funded by Greece and European Union by the Action «Investment Plans of Innovation» in Central Macedonia under the framework of the Operational Program «Central Macedonia 2021-2027».

**Keywords:** diatomaceous earth, dust formulation, mortality, stored product beetles, inert materials

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## P362. Efficacy of Apiaceae essential oil nanoemulsions against five arthropod pests

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Apiaceae produce essential oils (EOs) that are effective against a wide range of noxious insects. Due to issues dealing with EOs stability and persistence, in the present study they were encapsulated using nanocarriers. We evaluated new EO-based nanoemulsions (NEs) derived from *Pimpinella anisum* and *Trachyspermun ammi*, against *Tribolium castaneum*, *Tribolium confusum*, *Tenebrio molitor*, *Trogoderma granarium* adults or larvae, and *Acarus siro* adults or nymphs. The two EO-based NE was tested on wheat (500 and 1000 ppm). Mortality of arthropod was recorded after 4 h, 8 h and 16 h and daily from 1 to 7 days. *Tribolium castaneum* larvae showed 100% mortality on wheat sprayed with 1000 ppm 4% (w/w) NE based on *P. anisum* EO after 6 days of exposure. Complete mortality occurred in *T. confusum* larvae after 7 days at 1000 ppm when exposed to 8% (w/w) NE based on *T. ammi*. Furthermore, 89.4% of *A. siro* adults exposed to 1000 ppm of 8% (w/w) *T. ammi* EO-based NE, were killed after 7 days. Binary toxicity tests of EO-based NE combination were also carried out. A percentage of 97.8% *T. molitor* adults died after 7 days in 1000 ppm 3% (w/w) *T. ammi* + 3% (w/w) *P. anisum* EO-based NE and 98.6% of adults of *T. granarium* died at 3% (w/w) *T. ammi* + 3% (w/w) NE based on *P. anisum* after 7 days of 1000 ppm. The recorded mortalities indicate that EO-based NEs are effective management tools as wheat protectants against a wide spectrum of stored-product insects.

**Keywords:** Apiaceae, stored product insects, nanoemulsions, essential oils

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## P363. Changes in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) fitness when different developmental stages are exposed to chlorfenapyr

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This study deals with the exposure effect of different population developmental stages of *T. castaneum* to chlorfenapyr using life table statistics and a survival analysis method. Eggs, larvae and parental females of *T. castaneum* were exposed to chlorfenapyr, and their births and deaths were recorded daily. The exposure of eggs and larvae was harmful as neither of them completed their development. In contrast, progeny survival, biological traits as well as life table parameters were not significantly different compared to the control treatment when parental females were exposed to chlorfenapyr. The developmental period of eggs, larvae, and pupae for the control treatment were 4.66, 25.85, and 5.00 days, respectively, while those exposed to chlorfenapyr were 4.76, 25.71, and 5.26 days. The longevities of adult female progenies were 87.33 days and 104.22 days for the control and chlorfenapyr treatments, respectively. The longevities of adult male progenies were 76.87 days and 91.87 days for the control and chlorfenapyr treatments, respectively. The mean net reproductive rate was 14.3 and 9.3 females/female for the control and chlorfenapyr treatments, respectively. The intrinsic rate of increase was 0.038 and 0.028 females/female/day, the intrinsic rate of increase was 1.039 and 1.029, the mean generation time was 70.0 and 76.9 days and the doubling time was 18.5 and 24.9 days, respectively. The findings of the current research effort will help to the rational use of insecticidal applications on the basis of the effects of insecticides to all developmental stages of the target insects.

**Keywords:** *Tribolium castaneum,* chlorfenapyr, population, longevity, mortality

## P364. The pyrrole derivative chlorfenapyr causes immediate and delayed mortality to adults and larvae of *Alphitobius diaperinus* on concrete

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This study evaluates mortalities of adults and larvae of *A. diaperinus* on concrete treated with chlorfenapyr at half label or label doses. Regarding immediate mortality, 97.8% of adults, exposed on concrete treated with label dose when food was absent, were killed after 7 days. At the same exposure interval, 32.2% of adults died on concrete treated with half label dose of chlorfenapyr with the presence of food. All small larvae died on concrete treated with label dose when food was absent after 1 day of exposure. All other dose/ food combinations provided complete mortality after 3 days of exposure. All large *A. diaperinus* larvae were killed at all tested dose/food combinations after 3 days of exposure. The delayed mortalities of adults exposed on concrete treated with label dose were 36.7 (presence of food) and 100.0% (absence of food).

**Keywords:** lesser mealworm, developmental stages, surface treatments

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## P365. Quality control of *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) as pupal parasitoids for biological control of *Piophila casei* (Diptera: Piophilidae) in ham productions.

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Ham products play a fundamental role in the Italian economy, and attention to the problems of this sector is essential. The products of this sector can be attacked by parasites, including arthropods, which can cause direct and indirect damage. *Piophila casei* (L.) (Diptera: Piophilidae) a parasite of cheese and meat, is currently responsible for critical hygiene problems in ham factories. The trophic activity of the larvae of this pest on the products causes serious direct damage; moreover, adults are potential vectors of various bacteria, including *Clostridium botulinum*. Another source of damage is human ingestion of the larvae, which resist to the action of gastric juices and digestive enzymes, potentially causing myiasis in the intestine. It is not allowed to use any type of insecticide in aging rooms, so biological control can represent a potential alternative. In this study, we investigate quality parameters as successful rate of parasitism (SP), degree of parasitism (DP), sex-ratio (SR), life-span (LS) and sex-ratio (SR) of two pupal parasitoids of Diptera: *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae), currently the only known pupal parasitoid of *P. casei*, and *Muscidifurax raptor* (Girault and Sanders) (Hymenoptera: Pteromalidae).

Our research confirmed *P. vindemiae* efficacy to parasitize *P. casei* and reported, for the first time, *M. raptor* as a pupal parasitoid of this Piophilidae. The parameters investigated can be considered useful for pre-release evaluation and crucial for mass-rearing programs.

**Keywords:** hams, parasitoids, biological control, quality control, stored product protection

## P366. Using long-lasting insecticide-incorporated netting protects bulk storage and processing facilities while minimizing fumigations

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Long-lasting insecticide-treated netting has successfully been used to impair mobility and prevent infestation by stored product beetles after harvest. Understanding how to integrate insecticide netting with existing integrated pest management (IPM) tactics at food facilities can improve commodity protection. In our study, we used 3 MT grain bins or a rice processing facility, and in former, 60 perforated buckets were filled with 500 g of uninfested wheat as surrogate miniature silos while the latter had netting on an intake manifold for the facility. Miniature silos were protected by, or small removable metal squares from the manifold contained insecticide-treated netting (with 0.3% α-cypermethrin, BASF), control netting (no insecticide), or no netting (negative control). Half of each treatment was randomly assigned to receive a fumigation if above Federal Grain Inspection Service-mandated tolerances, while the remainder were not fumigated in the silos. Monthly samples of grain from silos were taken, while netting was tested for ability to induce mortality of stored product insects from the rice facility. Insects and grain quality were recorded in silos. We recorded and compared the length of protection by each fumigation and the number of fumigations required by treatments with and without netting in bulk storage. Overall, we found silos with insecticide-treated netting had fewer insects, damage, and required fewer fumigations than controls. Netting remained effective regardless of cleaning at the rice processing facility. Our results demonstrate that insecticide-treated netting can be used for effective IPM programs to enhance the efficacy of existing tactics at food facilities.

**Keywords:** insecticide netting, *Tribolium castaneum*, red flour beetle, postharvest, stored products, IPM, behavior

## P367. Density dependent development of the yellow mealworm *Tenebrio molitor*

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Insects can provide a promising alternative protein source for animal feed rich in fibers, minerals and lipids. Insect rearing is economically viable, since they can be fed with organic waste and agricultural by-products. The aim of this study was to evaluate the effect of a diet enriched with functional ingredients of aromatic plants of the Greek flora on the survival and development of larvae of the yellow mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae), in the context of larval density induced stress. Particularly, wheat bran enriched with 5, 10 or 20% of a prepared mixture containing post-distillation residues of aromatic plants of the Greek flora, e.g. oregano, thyme, sage and rosemary, linseed oil, rock-samphire, industrial cannabis and olive paste by-product was evaluated as insect feeding substrate, whereas wheat bran alone served as control. Groups of 25, 50, 75 and 100 first instar larvae were separately collected, weighed, and put in plastic cylindrical vials to test if larval density had any effect on the survival and development of the larvae. Our results show that larval survival is not density dependent. Larvae fed with 5% enriched substrate showed the highest survival rate, followed closely by those fed with the control and 10% substrates. Our study highlights the effect of larval density on the individual larval weight. The lower the density, the highest larval weight was observed. Highest weight was that of larvae reared on 5% mixture. Slightly lower was the weight of those reared on control substrate, followed by the 10 and 20% mixtures.

This research has been co-financed by the European and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (T2EΔK-02356, InsectFeedAroma).

**Keywords:** edible insects, circular economy, functional ingredients, survival, larval weight

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## P368. Inclusion of *Tenebrio molitor* (Coleoptera: Tenebrionidae) reared on substrates enriched with post-distillation residues of aromatic-medicinal plants in fishfeed: cellular responses in the liver and the muscle of *Sparus aurata*

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Insects are currently emerging as a potential alternative animal protein source in fishfeed due to numerous benefits such as the high nutritional content and the sustainable mass production. Despite several studies on fishes concerning fishmeal substitution with insect meal, nutritious enhancement of the latter through supplementation of aromatic-medicinal plants (AMP) in insects' rearing substrate has yet to be fully investigated. Nevertheless, dietary changes may compromise nutrients' sufficiency and bioavailability, thus leading to nutrient stress. The present study aims to evaluate the expression of heat shock proteins (HSPs) and mitogen-activated protein kinases (MAPKs) and the apoptotic machinery in the liver and the white muscle of *Sparus aurata* following dietary inclusion of insect meal from *Tenebrio molitor* (Coleoptera: Tenebrionidae) reared on substrates enriched with AMP residues. Three isoproteic and isoenergetic diets were formulated: a standard fishmeal diet (CONTROL) and two diets with 10% insect meal from insects reared either on conventional substrate (IM) or on substrate enriched with a mixture of post distillation residues of AMP (IFA). According to the immunoblot analysis, both insect meal diets significantly induced HSPs expression in the examined tissues, with the exception of HSP90 in muscle where AMP inclusion resulted in non-significant changes compared to control. Significant p38 MAPK activation was apparent in the liver of IFA group compared to control, while apoptosis was modulated in a tissue specific manner.

This research has been co-financed by the European and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (T2EΔK-02356, InsectFeedAroma).

**Keywords:** fishfeed, nutrient stress, apoptosis, aromatic-medicinal plants, yellow mealworm, aquaculture

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## P369. Dietary inclusion of *Tenebrio molitor* (Coleoptera: Tenebrionidae) reared on substrates enriched with post-distillation residues of aromatic-medicinal plants as modulator of the internal metabolism in pigs

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Utilization of insect meal in animal feed encompasses various advantages including a high nutritional profile rich in protein. The dietary substrate may exert ameliorative effects on the nutritional value of insects. In this context, the metabolic capacity in the liver and the intestine of piglets was evaluated by determining the activities of the enzymes l-lactate dehydrogenase (L-LDH), citrate synthase (CS), and 3-hydroxyacyl-CoA dehydrogenase (HOAD), following dietary inclusion of insect meal from *Tenebrio molitor* (Coleoptera: Tenebrionidae) reared on substrates enriched with aromatic-medicinal plants (AMP) residues. Three isoproteic and isoenergetic diets were formulated: a standard control diet and two diets with 10% insect meal from insects reared either on conventional substrate (IM) or on substrate enriched with a mixture of post distillation residues of AMP (IFA). The diets were fed to weaned piglets (12 piglets per group) for a period of 42 days. The L-LDH activity was elevated in the

intestine and the liver of dietary group IFA compared to the other two groups. Regarding HOAD, increased activity was observed in the intestine of group IFA, while in the liver a reduction was evident in both IM and IFA groups, compared to the control. Furthermore, compared to the control CS activity in the intestine was increased in group IFA and decreased in group IM, while non-significant changes were observed in the liver.

This research has been co-financed by the European and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (T2EΔK-02356, InsectFeedAroma).

**Keywords:** animal feed, metabolic enzymes, aromatic-medicinal plants, yellow mealworm, pigs

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## P370. Agri-food residues as rearing substrates for *Tenebrio molitor* (Coleoptera: Tenebrionidae): effects on growth, cellular responses and antioxidant capacity

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Edible insects have recently gained momentum as an alternative source of protein, energy, fat and fiber for both human and domestic animals. Due to the poikilothermic nature, insects are able to grow on organic waste streams and efficiently convert them into high-quality biomass. Additionally, rearing substrates may shape the nutritional profile of insects. Thereby, the aim of the present study was to investigate the growth performance, and cellular and antioxidant status of *Tenebrio molitor* (Coleoptera: Tenebrionidae) reared on wheat bran (WB), rice bran (RB) and corncob (CC) by-products supplemented with 0, 10% or 20% essential oils distillation residues (EODR). For this purpose, levels of heat shock proteins (HSPs) and mitogen-activated protein kinases (MAPKs), and apoptosis were determined by immunoblot analysis, while antioxidant capacity by the activity of superoxide dismutase (SOD), glutathione reductase (GR) and catalase. Agri-food substrates had no adverse effects on oviposition, while 20% EODR supplementation reduced larvae mortality in CC. Compared to WB, MAPKs activation decreased in RB and CC, while HSPs induction was more apparent in the RB substrate. The antioxidant potential of *T. molitor* displayed non-significant changes in CC and RB compared to WB, while EODR supplementation exerted various effects on enzymes.

This research has been partially funded by EPAnEK (European Regional Development Fund of European Union and by national funds) and is implemented through the General Secretariat for Research and Innovation (GSRI) in the framework of the project T6YBΠ-00174 “Insect meal as an alternative source of protein in the aquafeed for fish farming” (Entomo4fish).

**Keywords:** agri-food residues, antioxidant potential, apoptosis, essential oils, yellow mealworm

## P371. Evaluation of antimicrobial activity of lipids extracted from *Tenebrio molitor*

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Antimicrobial products deriving from natural sources, especially those made from new sources and environmentally friendly ingredients, are gaining more and more attention since the problem of antimicrobial resistance has become worse in recent decades. Due to the nature of their fatty acids, insect lipids can be categorized as naturally occurring antimicrobial substances. The purpose of this study was to investigate the antibacterial characteristics of crude insect fat of the coleopteran *Tenebrio molitor*. Lipids were isolated by Soxhlet extractor from *T. molitor* larvae, fed with standard diet (wheat bran and fresh vegetables for hydration), and adults and tested against *Escherichia coli* and *Micrococcus flavus*. Lipids from larvae showed antibacterial activity against *M. flavus*, whereas no samples had any activity on *E. coli*. In order to understand if the specific fatty acid composition can influence the different antimicrobial activity, samples were analyzed by gas chromatography. The antibacterial activity shown during the trials, could be attributed to the most detected fatty acids (oleic, linoleic and stearic acids), that are reported to have antibacterial effects also combined with each other. Lipids can be used as new feed/food additives, that can also substitute or support current antibiotics.

**Keywords:** lipid, antimicrobial effect, yellow mealworm

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## P372. Evaluation of food substrates on larval development and nutrimental parameters of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

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A plethora of studies have investigated the effects of different diets on the development and the nutritional profile of the *Tenebrio molitor* larvae, one of the most important alternative food and feed sources. However, after its approval by the European Union as a novel food further efforts should be devoted to evaluate diets which will promote safety and increase or differentiate the nutritional value of the reared *T. molitor* larvae. In this study the effects of different food substrates on *T. molitor* larvae development and on their nutritional profile were evaluated. Wheat bran (standard diet) was used as rearing substrate alone (control) or mixed with either flax seed or carob flour at 75:25 or 87.5:12.5 ratios (w/w). According to our results, among the treatments the larval weight was significantly reduced in comparison to the control only in the case that the flax seed was used at a high ratio. However, when the larvae fed on a diet with high ratio of carob flour their weight was highest among the treatments although did not differ significantly to them. In conclusion, the carob flour affects positively the weight gain of *T. molitor* larvae, while all the used diets affect their nutritional value in different ways. This research show that, the parameter of diet on *Tenebrio molitor* larvae rearing must be taken into account especially when they are intended for human consumption, because affects their growth and their nutritional value.

**Keywords:** flax seed, carob flour, weight, nutritional value, protein, fat and fatty acid profile

### P373. Evaluation of antimicrobial activity of lipids extracted from *Hermetia illucens* reared on different feeding substrates

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As the antimicrobial resistance issue is constantly getting worse, there is a new interest in antimicrobial solutions deriving from natural sources, especially those made from cutting-edge and environmentally friendly materials. Insect lipids can be categorized as naturally occurring antimicrobial compounds. Indeed, due to their fatty acid composition, they may represent new antimicrobial molecules in response to the need to find and use new substances to fight antibiotic resistance. In order to assess the antibacterial efficacy of *Hermetia illucens* lipids, we extracted lipids from larvae fed on different substrates, in order to determine if and how the feeding substrate affects the antimicrobial activity. Following the extraction by Soxhlet method, antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria. As differences in antimicrobial activity were detected among larvae fed on different substrates, the specific fatty acid composition was analyzed by gas chromatography, in order to understand if this component varies according to the substrate. Statistically significant differences were detected in five fatty acids between lipids showing antibacterial activity and lipids not showing any activity. Literature data reports undeniable antibacterial activity of these fatty acids, confirming the hypothesis that they can contribute to the antimicrobial activity of *H. illucens* lipids. Lipids can be used as new feed/food additives, that can also substitute or support current antibiotics.

**Keywords:** lipid, antimicrobial activity, fatty acids, black soldier fly

## P374. Larval development of the superworm *Zophobas morio* (F.) (Coleoptera: Tenebrionidae) on substrates enriched with functional ingredients of aromatic and pharmaceutical plants of the Greek flora

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The interest in utilizing insects as a source of food and feed has been rapidly increasing. The inclusion of bioactive compounds and active ingredients in insect diets has been identified as a potential key factor for enhancing their growth and performance. The current study aims to investigate the growth and development of *Zophobas morio* (F.) (Coleoptera: Tenebrionidae) larvae, when fed on substrates enriched with functional ingredients derived from aromatic and medicinal plants of the Greek flora. The study evaluated the potential of wheat bran supplemented with two mixtures (A and B) at varying concentrations (10%, 20%, and 30%) as a substrate for insect feeding. The control group was provided with unenriched wheat bran. Mixture A comprised post-distillation residues obtained from various aromatic plants such as oregano, thyme, sage, and rosemary, as well as industrial cannabis, linseed oil, rock samphire, and olive paste by-product. Mixture B consisted of Mixture A augmented with essential oils derived from the aforementioned aromatic plants. Enrichment of the substrate with 20% of mixture A positively affected the development of *Z. morio* larvae and led to higher final larval weight compare to the control. In the case of mixture B, all enrichment rates resulted in higher final weight. The objective of our findings is to facilitate the utilization of insects as a source of food and feed by augmenting their feed with functional ingredients possessing favorable characteristics.

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (Project Code: T2EΔK-02356, Acronym: InsectFeedAroma).

**Keywords**: circular economy, edible insects, insects as food and feed, insect protein

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## P375. Asymptotic Estimated Digestibility, a new indicator to overcome challenges related to faeces collection and ingesta quantification in *Hermetia illucens* larvae

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Black soldier fly larvae (BSFL; *Hermetia illucens*) can quickly convert organic substrates into body proteins and lipids. Due to its high conversion efficiency, this specie has gained significant attention in the field of insects as feed and food. However, there is a lack of understanding of the digestive processes underlying this potential. The main challenges in assessing digestibility in BSFL are accurate feed intake measurement and proper faeces collection, as larvae feed and excrete in the same moist substrate. In this study, a new indicator called Estimated Digestibility (ED) was introduced, which measures the difference between distributed feed and frass macronutrient weight, divided by macronutrient weight in distributed feed. ED was evaluated at increasing larval densities to ensure complete feed ingestion and frass free from refused feed. Initial diet and frass samples were analyzed for dry matter (DM), starch, nitrogen, ether extract (EE), neutral detergent fibre, acid detergent fibre, acid detergent lignin, ash and energy. The results showed a sigmoidal pattern for ED of all fractions except fibres, with asymptotic ED of 80.3±1.3% for DM, 99.0±2.3% for starch, 78.6±1.1% for nitrogen, 95.3±1.5% for EE, 58.4±1.0% for ash and 80.6±1.2% for energy. This new indicator is a way of measuring digestibility in insects living in their feeding substrate. It offers insight into the digestive efficiency of BSFL.

**Keywords:** insect nutrition, alternative protein, insects as feed, macronutrient, density, Diptera

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## P376. Black soldier fly as a tool for the valorization of tomato waste

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The tomato is one of the most developed horticultural products in Italy and the tomato industry, particularly cannery industry, with 2.5 M tons/y produces enormous quantities of waste for which safe and cost-effective disposal must be developed. In particular, the re-use of washing and sorting residues, consisting of green leaves and stems and defective raw tomatoes (unripe, green or damaged), which represent 2-3% of the weight of the starting material, has been little studied.
In this work, tomato production residues were used as a substrate for the growth of larvae of *Hermetia illucens* (Diptera: Stratiomyidae), also known as the black soldier fly (BSF), that can grow on numerous organic substrates, converting them into valuable larval biomass rich in proteins and lipids. For this aim, different types of tomato wastes, such as stem, red or green tomatoes, leaves, seeds and peeling waste, were fed to groups of 500 BSF neonate larvae, with three replicates per each experimental trial. Growth parameters, bioconversion and substrate reduction indexes were then evaluated, and the larvae were analyzed to assess their final chemical composition. Results highlighted the ability of BSF to grow on the tomato waste, particularly those consisting of peeling waste (peel and seeds), or where tomato fruits were present in a higher percentage. In order to re-use waste biomass to obtain high added-value products, also in line with the EU's plan on the circular economy, tomato waste deserves further investigation for its exploitation for the production of BSF larvae.

**Keywords:** edible insects, *Hermetia illucens*, circular economy, vegetable waste

## P377. Subchronic oral dose toxicity evaluation and allergen of freeze-dried powder of *Locusta migratoria* as a novel food source

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The migratory locust, *Locusta migratoria* (Orthoptera: Acrididae), is a well-known edible insect which may serve as new source of human food and animal feed. However, potential toxicity and food safety of *L. migratoria* had not been investigated extensively until now. Therefore, in this study, we aimed to investigate toxicity of freeze-dried powder of *L. migratoria* (fdLM) in accordance with the OECD guidelines and GLP conditions and identify allergic components in ELISA and PCR techniques. Male and female SD rats were divided into four groups, and fdLM suspended in distilled water was administered once daily by oral gavage to these groups at the doses of 0 (vehicle control), 750, 1500, and 3000 mg/kg/day for 13 weeks. No toxicological changes in mortality, clinical signs, body and organ weights, food consumption, ophthalmology, urinalysis, hematology, clinical biochemistry, gross findings, and histopathological examination was observed in this subchronic study. In addition, fdLM did not induced increases of serum immunoglobulin E and 21 homologous proteins were not detected under our present conditions. In conclusion, the NOAEL (no-observed-adverse-effect level) for fdLM was 3000 mg/kg/day and no target organ was identified in both sexes of rats. Collectively, these results suggest that fdLM is safe with no adverse effects and offers the potential of its use for safe consumption in human food and feed.

**Keywords:** *Locusta migratoria*, edible insect, food safety, adverse effects, allergen

## P379. Study on the application of *Protaetia brevitarsis* larva excrement as organic fertilizer

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This study was performed to assess *Protaetia brevitarsis* larva excrement as an organic fertilizer for corn cultivation. Furthermore, the study investigated insect communities in each treatment. In 2009, the corn growth rate was worst in soil treated with *P. brevitarsis* larva excrement and in 2010, the corn growth rate was worst in untreated soil. From the outcome of the study, *P. brevitarsis* larva excrement could be utilized as organic fertilizer for corn cultivation.

**Keywords:** corn growth rate, insect, *Protaetia brevitarsis*

## P380. Feed particle size effect on larval growth of *Alphitobius diaperinus* and *Tenebrio molitor*

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The lesser mealworm, *Alphitobius diaperinus* (Panzer), and the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), are two insect species that have received a lot of scientific and commercial interest recently, due to their potential to be used as highly promising nutrient sources in aquaculture, poultry, and swine diets. They are also among the first insect species that have received EU-wide authorization for human consumption. In order to optimize their production and rearing processes, the chemical and nutritional features of mealworm feed are thoroughly investigated, but the physical properties of the feed are poorly understood. The size of the feed's particles is a physical property that may considerably affect the feed intake, as it has been already shown for several insect species. As a result, the primary goal of this research was to evaluate the effect of feed with four different particle sizes (100 – 250 µm, 500-600 µm, 850-1000 µm and <2000 µm) on larval the growth and development of *T. molitor* and *A. diaperinus*, in terms of their development time, feed consumption and weight gained, as well as the overall feed conversion effectiveness. The results of this research provide conclusive evidence that feed of smaller particle size substantially affects the development of both species and that this factor may significantly increase production. The research work was partially supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship Number: 11297).

**Keywords:** lesser mealworm, yellow mealworm, larval growth performance, mass rearing, feed conversion efficiency

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## P381. Waste orange peels as a feed additive for the enhancement of the nutritional value of *Tenebrio molitor*

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With the growing interest in sustainable food sources, edible insects are gaining attention as an economical and ecologically-friendly source of protein. One of the first insects identified as edible was the *Tenebrio molitor* (Coleoptera:Tenebrionidae), which has the potential to be used as a substitute for conventional protein sources in a variety of foods. The aim of this study was to enhance the nutritional value of *T. molitor* larvae using a food industry by-product, and to promote, at the same time, the circular economy via insect rearing. Specifically, albedo- a part of the orange peel that is often discarded – was mixed with wheat bran (10%, 17.5% and 25% w/w albedo) and tested, along with a control group fed only with bran. The results showed that increasing the percentage of albedo in the *T. molitor* diet led to a subsequent increase in the larvae's carotenoid and vitamin A content (up to 198%), vitamin C content (up to 46%), and protein and ash content (up to 32% and 26.5%, respectively). Moving forward, the findings of this study can inform the development of sustainable insect farming practices that utilize food industry by-products as a feed source, ultimately contributing to more sustainable and resource-efficient food systems. Furthermore, this study can serve as a foundation for future research examining the use of other food industry by-products in insect rearing and their effects on the nutritional quality of edible insects.

Keywords: *Tenebrio molitor*, orange albedo, proximate composition, vitamin A, vitamin C, proteins

## P382. Integration of alternative proteins in the Mediterranean food and feed value chains: the CIPROMED perspective

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Current European agricultural production systems are heavily dependent on protein imports, to cover mainly the nutritional needs for livestock animals and farmed fish, as well as for human consumption. Due to this dependency, European farming systems are fragile and vulnerable to rapid disruptions of supply chains. To tackle this issue, EU and particularly Mediterranean countries are in search of competent, sustainable, locally available alternative protein sources. In this framework, the PRIMA project CIPROMED [«Circular and Inclusive utilisation of alternative PROteins in the MEDiterranean value chains»] aims to propose alternative protein sources for the Mediterranean food and feed value chain. Particularly, the ground-breaking objective of this PRIMA project is the integration of insect farming and microalgae cultivation in the Mediterranean supply chains, together with the management and valorisation of agri-industrial side-streams, as well as their transformation and fermentative bioconversion into valuable resources. The recovery of ingredients that are based on these natural

resource-based substances will be performed through applying cascades of processes enabling the sequential extraction and fermentation of alternative ingredients. The suitability of the new protein ingredients in prototypes of food and feed, will be demonstrated in order to provide validated food and feed products, assured by a systemic LCA-based sustainability assessment. To achieve these goals, CIPROMED aims to adjust novel protein production to the unique Mediterranean conditions, creating a new, socio-economically feasible and environmentally sustainable alternative protein production system located in Mediterranean countries.
This research is supported by the EU-PRIMA program project CIPROMED (Prima 2022 – Section 1).

Keywords: alternative nutrient sources, circular economy, insects as food and feed, legumes, microalgae, side-streams

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## P383. Insects for upcycling nutrients from food waste and agri-food byproducts into animal feed, fertilizers, and soil amendments – Towards pilot implementation in Greece

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Insects and their gut microbiota constitute natural nutrient bridges from organic residues to higher animals. Harnessing this ecosystem service and integrating it into our global food system can contribute to securing adequate nutrition for our growing population and alleviating related environmental and geopolitical crises. A rapidly growing sector is doing just that: Selected insect species, reared on food waste and agri-food byproducts are processed into food and feed ingredients, to replace environmentally impactful protein meals such as fish- or soy meal, while the residual material is used for fertilization and soil amendment. Greece provides the ideal context for implementing this technology, with abundant food waste, rich variety of nutritious byproducts, a permissive climate for insect rearing and a strong demand for animal feed raw materials, fertilizers, and soil amendments. Accordingly, we propose to:

1) Adapt the technology to the Mediterranean climate and the locally available food waste and agri-food byproducts, focusing on two insect species, the black soldier fly, and the yellow mealworm, approved within the EU for this purpose.
2) Implement the technology at pilot scale to characterize economic feasibility as well as safety and quality of the resulting insect-based products.
3) Showcase the pilot facility and products to a variety of stakeholders to facilitate acceptance and uptake of the technology in the country.

## P384 Utilization of local agricultural by-products from Greece as sustainable feed ingredients of *Tenebrio molitor* diets

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One of the main barriers to the further development of the insect sector is the high production cost, i.e., the labor, infrastructure, and feed cost. The exploitation of cheap, largely available organic by-products as insect feed ingredients could provide a means to partly alleviate the feed cost. Agro-industrial production systems produce a considerable amount of waste that constitutes a big pool of mostly underrated resources. In this study, compound diets based on agro-industrial by-products locally produced in Greece, i.e., by-products of lupin, triticale, oat, barley, and pea, were formulated and subsequently evaluated for the rearing of *Tenebrio molitor* larvae in two series of trials. The first trial was conducted at a laboratory scale, i.e., using vials with 50 newly-hatched larvae that were reared on the different diets. The second trial was conducted at a pilot-scale using crates with 10,000 larvae. The results of both the lab and the pilot-scale trial showed that several of the Greek by-products tested are suitable as feeding substrates for the larvae of *T. molitor*. Remarkably, the diets that contained lupin, oat, triticale, or barley by-product efficiently supported the growth and development of *T. molitor* larvae. However, diets that contained the pea by-product did not efficiently support larval development. The integration of circular economy and zero waste principles with insect farming through the valorization of locally produced agricultural by-products could be adopted by small farms, generating additional income for farmers in the Mediterranean countries.

This research is supported by the EU-PRIMA program project ADVAGROMED (Prima 2021 – Section 2).

Keywords: circular economy, edible insects, yellow mealworm, feed conversion efficiency, larval development

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# Bioprotection Symposium

## Combining chemical ecology and conservation biological control: flower-associated microbes impact nectar traits with consequences for insect parasitoids

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Conservation biological control aims to enhance populations of natural enemies of insect pests in crop habitats, typically by intentional provision of flowering plants which can provide naturally-occurring non-host food resources, such as nectar and/or pollen. In order to feed on such plants, foraging parasitoids need to find suitable flowers and it is known that floral scent is used for their orientation. Chemical ecology investigations on the attractiveness of flowers is therefore an important aspect of parasitoid efficacy that should be taken into account in the selection of flowering plants to be used in engineering agricultural habitats. However, studies have demonstrated that nectar-dwelling microbes, which are regarded as omnipresent in flowers in a landscape, influence the variations in flower nectar traits, including those that are used by parasitoids to discriminate the presence or absence of suitable flower nectars. We thus provide an overview of floral compounds as semiochemicals from a multitrophic perspective, and we focus on the remaining questions that need to be addressed to move the field forward.

**Keywords**: chemical ecology, parasitoid food sources, agroecosystems

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## Enhancing ecosystem service provision for EcoStacking: an oilseed rape exemplar

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Insect pest management in oilseed rape (OSR) is challenging for farmers due to the lack of available, effective insecticides. Failure to control insect pests has led to a dramatic decline in OSR cropping in Europe and the need for imports of less sustainable alternatives such as palm oil. Sustainable bioprotection practices that fit the EU's 'Green Deal' principles are urgently required. A combination of in-crop and off-crop management practices that support ecosystem providers has the potential to enhance pest regulation, as well as pollination and other ecosystem services, helping to reduce or negate insecticide use. We will describe innovative work using molecular and image-based methods to identify the most important natural enemies of crop pests in OSR, and the plants which support them best for bespoke flower-margin design. We will also present results on pest and beneficial community

network analysis derived from DNA-based methods and how different in-crop agronomic practices such as reduced tillage and companion planting affect pest regulation and pest pressure. We propose that 'ecostacking' these practices could help to re-establish the viability of the crop and maintain productivity.

**Keywords:** pest regulation, pollination, flower strips, semi-natural habitat, companion planting

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## Novel bioprotection through bio-inspired pest control technologies

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To meet the challenges of feeding a predicted population of 9.8 billion people by 2050, a paradigm shift is required in how we grow our crops. These practices will need to be both resilient and environmentally sustainable, posing negligible risks to non-target organisms, and in particular ecosystem service providers. Whilst biological control is a valuable component of any integrated pest management strategy, its efficacy is often very variable. Success is dependent upon both the ecological context and prevailing environmental conditions. It is therefore highly desirable to complement biological control agents (BCAs) with sustainable control tools of natural origin, which themselves have low impact on the environment and non-target organisms. To achieve this objective, attention has turned to the exploitation of molecules and genes present within BCAs, themselves refined by long co-evolutionary processes, which are used to kill the host or to disrupt its physiology and reproduction. Current research being carried out seeks to develop novel, highly specific biopesticides based on knowledge obtained from the understanding of the molecular mechanisms underlying associations between plants, insects and pathogens, and by mimicking the natural antagonistic strategies of the BCA. Examples of this approach based on exploiting molecules from insect antagonists that target either the central nervous system or immune system of the pest insect, or the use of RNAi to silence essential genes in target insects, will be presented. Such molecules represent good candidates for biopesticide development.

**Keywords** biopesticides, RNAi, immune system, central nervous system, pest insects, biological control agents

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## Fitting parasitoids into ecological networks to improve pest control in crops

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Abstract: Studying parasitoids can provide insights into global diversity estimates, climate change impacts, and agroecosystem service provision. However, this potential remains largely untapped due to a lack of data on how parasitoids interact with other organisms. Ecological networks are a useful tool for studying and exploiting the impacts of parasitoids, but their construction is hindered by the magnitude of undescribed parasitoid species, a sparse knowledge of host ranges, and an under-representation of parasitoids within DNA-barcode databases (estimates suggest <5% have a barcode). Here, I show how DNA metabarcoding can be used to construct the host–parasitoid component of multilayer networks. While the incorporation of parasitoids into network-based analyses has far ranging applications, I focus on its potential for assessing ecosystem service provision within agroecosystems.

Keywords: DNA barcoding, host-parasitoid network, pest control

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## Setting the scene for a scientifically sound risk assessment of low-risk pesticides

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Synthetic pesticides have been the cornerstone of modern agriculture for decades. However, their use has been associated with serious concerns regarding human health and the decline of the quality of our natural water resources. This has shifted attention in recent years to biopesticides or so-called low risk pesticides of biological origin. Low risk pesticides (LRP) include groups that they have already representatives in the market like (i) plant extracts (including plant-derived pure compounds) (ii) semiochemicals, like pheromones and allelochemicals, (iii) microbial pesticides (bacteria, fungi and viruses). In addition, new microbial solutions (phages, protists, SynComs) and ds-RNA pesticides are emerging, low-risk solutions expected to reach the market in coming years. Despite on-going regulatory efforts by the European Commission and OECD and the recent implementation of Guidance for microbial pesticides (November 2022), we are still lacking a concrete risk assessment (RA) scheme relevant to LRPs, a point which blocks LRPs reaching the EU market. To address this major regulatory gap the European Commission has funded the project RATION whose main goal is to develop a novel RA scheme, supported by the necessary guidance on methods and tools, tailored to the specific characteristics of established and emerging LRP solutions. RATION has already identified the key regulatory issues per LRP group and is going to provide decision-making procedures, evaluate the potential implementation of approaches used by other regulatory frameworks (e.g. Qualified Presumption of Safety (QPS) in Food or Feed Additives), and develop novel tools for the efficient and scientifically sound assessment of the risk associated with the use of LRPs. Such examples constitute the development of tools to define pathogenicity, infectivity, antimicrobial resistance gene carriage (and transferability) and secondary metabolites biosynthetic pathways in microbial pesticides based on whole genome sequence or novel bioinformatic tools for design and *in silico* testing of the off-target toxicity of ds-RNA molecules for use in crop protection.

**Keywords:** low-risk pesticides, biopesticides, risk assessment, microbial pesticides, botanicals, ds-RNA

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## Agroecology and landscape management to reinforce ecosystem services

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Sustainable and resilient agricultural systems are needed to feed and fuel a growing human population. However, the current model of agricultural intensification which produces high yields has also resulted in a loss of biodiversity, ecological function, and critical ecosystem services. Along with intensification, landscape simplification exacerbates biodiversity losses which leads to reductions in ecosystem services on which agriculture depends. In recent decades, considerable research has focused on mitigating these negative impacts, primarily via management of habitats to promote biodiversity and enhance services at the local scale. While it is well known that local and landscape factors interact, modifying overall landscape structure is seldom considered due to logistical constraints. I propose that the loss of ecosystem services due to landscape simplification can only be addressed by a concerted effort to fundamentally redesign agricultural landscapes. Designing agricultural landscapes will require that scientists work with stakeholders to determine the mix of desired ecosystem services, evaluate current landscape structure, and implement targeted modifications to achieve desired goals.

**Keywords:** sustainable agriculture, insects ecosystem services, habitat management

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## Volatile interaction between undamaged plants suppress aphid in cultivar mixtures

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Combining different cultivars in mixtures to increase within field diversity has been suggested as a promising strategy to reduce aphid pressure. The effects of cultivar mixtures on pest suppression are mixed, as in some studies the aphid population size was reduced, while in other studies there were no effects. Understanding these inconsistent effects of cultivar mixtures on aphids is important from both an ecological and practical perspective. Volatile organic compounds are important mediators of mutualistic interactions between plants as they can prime defense responses in neighboring plants. We investigated the effects of cultivar mixtures on aphid suppression and possibility of volatile plant interactions as underlying mechanism that may contribute to observed aphid responses. Our studies have provided new evidences that volatile interaction between specific cultivars can have implications on neighboring plant growth and can prime defense responses. These induced responses significantly reduced aphid feeding, performance settling and population development. These bottom up trophical effects were observed only in certain cultivar mixtures, showing that plant-plant interactions depend on genotypes involved. The results from our studies on volatile interactions between cultivars in mixtures will contribute to development of the sustainable pest management strategies and implementation of crop production systems characterized by higher levels of integration.

**Keywords:** aphid-plant interactions, volatile induced defense, host plant finding, priming, aphid feeding behavior

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## Insect multitrophic interactions and bioprotection

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Reduction of pesticide use in agriculture requires the sustainable exploitation of ecosystem services provided by functional biodiversity, and an increasing availability of low-impact tools and strategies of pest control.

Current research being carried out seeks to develop novel, highly specific biopesticides based on knowledge obtained from the understanding of the molecular mechanisms underlying multitrophic associations among plants, insects, and their natural antagonists.

This approach allows to use biocontrol agents beyond the organism level, to develop control strategies based on their virulence factors or on molecular technologies that reproduce their negative impact on pests. Host regulation factors and strategies adopted by insect natural antagonists are a nearly untapped source of bioinsecticide molecules currently being explored to develop bioinspired pest control tools for sustainable plant protection.

Moreover, an in-depth understanding of the mechanisms underlying insect multitrophic interactions allows the definition of protection strategies for beneficial insects and the ecosystem services they provide.

**Keywords:** natural antagonists, biological control, RNAi, biopesticides, beneficial microorganisms, pest control

## Tomato prosystemin as novel source of bioactive peptides

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The in-depth studies over the years on the defence barriers by tomato plants have shown that the systemin peptide controls the response to a wealth of environmental stress agents. This multifaceted stress reaction seems to be partially related to the intrinsic disorder of its precursor protein, prosystemin (ProSys). Since latest findings show that ProSys has biological functions besides systemin sequence, we investigated if this precursor includes additional peptide motifs able to trigger stress-related pathways. Candidate peptides were identified by bioinformatic analysis and synthesized to test their capacity to trigger defence responses in tomato plants (*Solanum lycopersicum*) against different biotic stressors. The results show the presence of peptide repeats along ProSys amino acidic sequence which are involved in plant immune reactions against pathogens and insect pests. Notably, mass spectrometry analyses of peptides extracted from transgenic plants overexpressing ProSys identified 3 repeated motifs from *in vivo* leaves. Our data shed light on unrecognized functions of ProSys, mediated by multiple biologically active sequences which could provide new valuable tools for sustainable plant protection.

**Keywords:** biotechnology, plant defence, transgenic plants

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## Honeydew: the hidden and sweet driver of interactions in biological control

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Honeydew is the sugar-excretion product of plant-feeding hemipterans, such as aphids, coccids, whiteflies, and psyllids. This sugar source is exploited by many insects, including biological control agents such as parasitic wasps and predators. Compared to other sugar sources present in agricultural lands, honeydew is highly accessible and abundant in nearly all crops and seasons. However, honeydew is not only consumed by biological control agents, but also by pests, hyperparasitoids and ants. Besides arthropods, many fungi and bacteria grow on honeydew in agroecosystems, changing its composition. Therefore, honeydew mediates the interaction between many insect species. These interactions may affect directly or indirectly the honeydew producer and the honeydew feeder in both a positive and negative way. Herein, we first review the main ecological interactions driven by honeydew as a food source and as a semiochemical. Then, we propose several strategies to exploit this knowledge and improve biological control of pests in agroecosystems.

**Keywords:** hemipterans, parasitoids, predators, ants, mutualism

## The new approach to biocontrol

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The objective of ensuring food security and access to healthy food for the growing population promotes the reduction of the use of pesticides in agriculture.

Biocontrol aims at not causing any negative impact on the environment, non-target species and human health. In contrast to plant protection based only on chemical products, biocontrol considers the holistic approach of understanding the ecosystem of the farm, the stages of cultivation, the cycle of pest insects or diseases and their behavior, as well as the influence of cultural practices on plant health. In this context, Corteva is dedicated, firstly, to the development of innovative biocontrol tools of different types and, secondly, to the integration of these new tools with existing and also in development conventional plant protection products from its pipeline, understanding the best practices in building IPM strategies to ensure efficient pest control, sustainable productions and at the same time, reduce the risks of resistance appearance to the to the conventional fewer tools that will be available on the market in the coming years.

**Keywords** biological control, biopesticides, resistances, pest control

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## Ecological Innovations provide bioprotection alternatives to pesticides

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In nature, pest and disease outbreaks are extremely rare. This is due to the complex interaction webs in natural ecosystems that tend to stabilize populations. In contrast, the simplified agricultural cropping systems encourage pest and disease outbreaks. By studying and understanding natural systems, we can identify novel solutions and strategies to control agricultural pests and diseases. Three examples will be presented that show how such nature inspired bioprotection solutions can provide effective alternatives for crop protection.

i) The use of specifically composed landscape elements targeted to organisms providing bioprotection. Large scale projects have shown that informed use of Functional AgroBiodiversity (FAB) on farms increases pest natural enemies, can help suppress weeds, reduces pesticide use by 90 %, and even increase increase yields by 10-30%. ii) Ants can be a severe problem in fruit production when they visit and guard aphids, mealybugs and other honeydew producing pests. By using sugar feeders, we show that it is possible to distract ants from their pest guarding and turn them into pest predators instead. This simple pest solution has been proven to be effective in citrus (mealybugs and aphids), vineyards (mealybugs) and apple (aphids). iii) A new species of predatory mites (*Pronematus ubiquitous*) has been shown to be highly effective in protecting crops against powdery mildew as well as small pests. This is the first example of arthropods providing effective disease control and promises to revolutionize biological disease control.

These three examples show that basic ecological research can inspire successful bioprotection innovations that provide commercially viable alternatives to chemical pesticides

**Keywords:** functional agrobiodiversity, pest predators, trophic network

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