European PhD Network "**Insect Science**" XV Annual Meeting

Firenze, 13-15 November 2024

PROGRAMME & BOOK OF ABSTRACTS

CREA Centro di Ricerca per la Difesa e la Certificazione
Via Lanciola 12/a - Cascine del Riccio (Firenze)

PROGRAMME

SESSIONS ON:

- INSECT PEST CONTROL (13 & 14 November)
- INSECT BIOLOGY (13 & 14 November)
- INSECT ECOLOGY AND CONSERVATION (14 & 15 November)

Wednesday 13 November 2024

13:30 **Registration**

14:00 **Welcome address**

14:10 **Senior scientist lecture:** Emmanuelle Jaquin Joly - INRAE, Sorbonne University
Reverse chemical ecology targeting insect odorant receptors: new avenues for pest control

14:40 - 16:30 **SESSION ON INSECT PEST CONTROL**
Oral Presentations (regular & short talks)
Chair: TBD onsite

14:40 - 14:55 R Sara Amoriello - CREA, University of Siena
Entomopathogenic nematodes for biological control of *Popillia Japonica* larvae: current status and future prospects

14:55 - 15:10 R Aurora Bozzini - University of Padova
Multispectral drone images for the early detection of *Ips typographus* infestations: assessment over large forest areas in the South-Eastern Alps

15:10 - 15:15 S Matilde Case - University of Padova
Development of new technologies for the study and management of the European spruce bark beetle *Ips typographus*

15:15 - 15:30 R Maria Rosaria Chianese - University of Napoli Federico II
Evaluation of frass of *Hermetia illucens* as elicitor of plant defenses against aphids

15:30 - 15:45 R Corentin Clavé - University of Napoli Federico II, CNRS-University of Tours
Endophytic colonisation of *Beauveria bassiana* in tomato plants: mechanisms and consequences for the control of the lepidopteran pest *Spodoptera littoralis*

15:45 - 16:00 R Maria Giovanna De Luca - University of Napoli Federico II
Three different endophytic microorganisms are effective control agents of *Spodoptera littoralis* noctuid moth larvae

16:00 - 16:15 R Nicolò Di Sora - Tuscia University
Advancements on the management of *Toumeyella parvicornis* (Cockerell, 1897): biology, distribution and control strategies

R Regular Presentation (15 min, discussion included) S Short Presentation (5 min, discussion included)

16:15 - 16:30 R Giovanni Jesu - University of Napoli Federico II
Sublethal effects of prosystemin biologically active repeat motifs on *Spodoptera littoralis* larvae

16:30 **Coffee break**

16:50 - 18:30 **SESSION ON INSECT BIOLOGY**
Oral Presentations (regular & short talks)
Chair: TBD onsite

16:50 - 17:05 R Ilaria Armenia - University of Insubria
Impact of protein-to-carbohydrate ratio on the immune response of black soldier fly larvae

17:05 - 17:20 R Laura Rita Besana - University of Padova
Color vision in longhorn beetles: from electroretinogram recordings to practical applications

17:20 - 17:35 R Sara Caramella - University of Insubria
Effects of lead intake on *Hermetia illucens* immune system and development

17:35 - 17:50 R Marilù Cardinale - University of Napoli Federico II
Cryptocephalinae beetles (Coleoptera, Chrysomelidae) host a symbiont from the Morganellaceae family: prevalence, localization and potential roles for the host

17:50 - 17:55 S Marta Chignola - Free University of Bolzano
***Myzus mumecola*: insights into the biology of the newly invasive apricot aphid**

17:55 - 18:00 S Emanuele Crepet - University of Milano
Across the Spiderverse: comparative analysis of metals in spiders' mouthparts (Arachnida, Araneae)

18:00 - 18:15 R Giovanna De Leva - University of Napoli Federico II
An immunosuppressive virus causes gut dysbiosis in honey bees

18:15 - 18:30 R Aya Mohamed Attia Elsayed - University of Torino
Insect gut microbiome as a resource to improve insect mass rearing

Thursday 14 November 2024

09:00 **Senior scientist lecture:** Massimo Nepi - University of Siena
The power of plants: insect manipulation in nectar-mediated interactions

09:30 - 11:10 **SESSION ON INSECT ECOLOGY AND CONSERVATION**
Oral Presentations (regular & short talks)
Chair: TBD onsite

09:30 - 09:35 S Sara Basiglio - University of Milano
Assessing the impact of different chemical pollutants on the gut microbiome of different pollinator species

09:35 - 09:50 R Sara Boschi - University of Siena

Population genetics and the role of dispersal barriers in the Antarctic springtail *Kaylathalia klovstadi* (Collembola, Isotomidae)

09:50 - 10:05 R Carla Libia Corrado - Sapienza University of Roma
Potential effects of an organic biostimulant on the tri-trophic interaction zucchini-ZYMV-aphid vector

10:05 - 10:20 R Paola Costagliola - University of Napoli Federico II
Arthropod biodiversity in Italian agroecosystems: monitoring approaches and factors of influence

10:20 - 10:35 R Claire Duménil - Free University of Bolzano
The impact of endophytic communities on the volatile organic compound profile of sunflower *Helianthus annuus* and its detection by honey bees

10:35 - 10:50 R Jay Darryl Ermio - University of Palermo
Neglected microbes in floral nectar: influence of filamentous fungi on nectar traits and parasitoids olfaction and performance

10:50 - 10:55 S Marco Falasco - University of Padova
Environmental and habitat influences on the gut microbiota of two European pollinators

10:55 - 11:10 R Alberto Fassio - University of Torino
New insights into the biology and parasitism rate of *Aphelinus mali*, parasitoid of the wolly apple aphid *Eriosoma lanigerum*

11:10 **Coffee Break**

11:30 - 13:05 **SESSION ON INSECT PEST CONTROL**
Oral Presentations (regular & short talks)
Chair: TBD onsite

11:30 - 11:45 R Stefano Galvagni - University of Trento, Fondazione Edmund Mach
Impacts of *Scaphoideus titanus* disturbance vibrational signal on grapevine defence mechanisms

11:45 - 12:00 R Vito Antonio Giannuzzi - University of Perugia
Assessing attraction of lures and dispensers on the efficiency of *Halyomorpha halys* trapping

12:00 - 12:15 R Valeria Grande – CNR, University of Torino
Sustainable *Varroa destructor* management influences honeybee viral dynamics

12:15 - 12:20 S Badr-Eddine Jabri – University of Torino
Effects of insecticide application on the gut microbiome of *Scaphoideus titanus*: shifts in endosymbiont composition and diversity

12:20 - 12:35 R Samah El Moussaoui - University of Trento
Biotremology for pest management, new solutions for stink bugs and true bugs

12:35 - 12:50 R Roberto Masturzi – Tuscia University
Impact of pest control management of phytophagous and predatory mites in hazelnut orchards in Central Italy

12:50 - 13:05 R Sara Paola Nastasi - University of Milano

Humic substances-based nanoparticles as a novel nano-delivery system for an RNAi-mediated control strategy against *Spodoptera littoralis*

13:05 **Lunch**

14:15 **Senior scientist lecture:** Dana Ment - Agricultural Research Organization ARO
Lifestyle switches in entomopathogenic fungi – from pest control to plant benefit and beyond

14:15 - 15:30 **SESSION ON INSECT PEST CONTROL**
Oral Presentations (regular & short talks)
Chair: TBD onsite

14:15 - 14:30 R Adriana Poccia - University of Perugia
Behavioural response of *Philaenus spumarius* to different compounds from essential oils

14:30 - 14:35 S Luca Rasi - University of Bolzano
Developing innovative digital solutions for the automatic detection and discrimination of *Drosophila suzukii* and *Drosophila melanogaster*

14:35 - 14:40 S Chiara Sciandra – CREA, University of Siena
Testing chitosan-based Fosetyl-Al nanocrystals in *Meloidogyne javanica* control

14:40 - 14:55 R Maria Carmen Valoroso - University of Milano
Characterization of the responses mounted by *Aedes albopictus* larvae exposed to the entomopathogen *Bacillus thuringiensis*

14:55 - 15:10 R Giorgio Vicari - University of Pisa
A regional plan for the management of the alien hornet *Vespa velutina* (Hymenoptera: Vespidae) in Tuscany

15:10 - 15:25 R Francesco Volpe - University of Torino
The egg parasitoid *Ooctonus vulgatus* (Hymenoptera: Mymaridae) in Northwestern Italy

15:25 - 15:30 S Marzia Zagallo - Free University of Bolzano
***Wolbachia*: endosymbiotic bacteria of insects and their potential use for sustainable pest control**

15:30 **Coffee Break**

15:50 - 17:15 **SESSION ON INSECT BIOLOGY**
Oral Presentations (regular & short talks)
Chair: TBD onsite

15:50 - 16:05 R Frédéric Manas – IRBI, University of Tours
Seminal fluid proteome and female post-mating responses in the black soldier fly *Hermetia illucens*

16:05 - 16:20 R Martina Marzari - University of Insubria
Evaluation of the growth performance of BSF larvae on a polyethylene terephthalate (PET)-contaminated substrate

16:20 - 16:25 S Marianna Olivadese - University of Bologna
A humanistic approach to entomology: tracing the imprint of the past on modern

challenges

16:25 - 16:40 R Veronika Pavlasova - Newcastle University
Identification and characterization of membrane transport proteins in *Buchnera aphidicola*: insights into nutrient exchange in aphid symbiosis

16:40 - 16:55 R Davis Roma - University of Milano
Exploring *Hermetia illucens* larval gut microbiota for innovative plastics biodegradation strategies

16:55 - 17:10 R Elia Scabbio - University of Padova
Using X-ray tomography to study ambrosia beetles

17:10 - 17:15 S Marta Villa - University of Milano
Tiger mosquito, *Aedes albopictus*, larval salivary glands characterization

Friday 15 November 2024

09:00 **Senior scientist lecture:** Andrea Becchimanzi – University of Napoli Federico II
Parasites, pathogens and microbiota: a stressful job for bee's immunity

09:30 - 12:10 **SESSION ON INSECT ECOLOGY AND CONSERVATION**
Oral Presentations (regular & short talks)
Chair: TBD onsite

09:30 - 09:45 R Andrea Ferrari - University of Milano
First insights into the role of urbanisation in shaping the aculeate wasp community in an Italian metropolis

09:45 - 10:00 R Eleonora Vittoria Fontana - University of Torino
How do fungal mutualists affect social behaviour of a facultatively eusocial ambrosia beetle?

10:00 - 10:15 R Camille Heisserer - University of Tours, CNRS
A transposable element potentially involved in ecological speciation

10:15 - 10:30 R Aziza Hussein - University of Bari Aldo Moro
α-Taxonomy identification and morphs of relevant Aphrophoridae from Palestine

10:30 - 10:45 R Alice Lenzi – CREA, University of Siena
Research on saproxylic beetles, from community to single species studies: a case study in a Spanish National Park

10:45 - 11:00 R Paolo Masini - University of Perugia
Investigation of olfactory cues for host-location in European ecto-parasitoids *Sclerodermus cereicollis* and *Sclerodermus domesticus* (Hymenoptera: Bethylidae)

11:00 - 11:15 R Mariangela Milordo - University of Catania
Effects of plant-mediated soil microorganisms on the olfactory response of insect natural enemies in tomato

11:15 - 11:20 S Giovanni Naro - University of Milano
First report of *Leptoconops noei* in Tuscany inland and new morphological findings

R Regular Presentation (15 min, discussion included) S Short Presentation (5 min, discussion included)

11:20 - 11:35 R Francesco Patriarca - University of Milano
Sublethal effects of agricultural chemicals on pollinators: insights from the POLYPOLL project

11:35 - 11:50 R Rosa Ranalli - University of Milano, NBFC
Pollination networks in urban areas: a pathway to sustainable and biodiverse cities

11:50 - 11:55 S Chiara Francesca Trisoglio - University of Milano
Histological alterations in honeybee gut suggest caution in using sewage sludge as soil improver in agriculture

11:55 - 12:10 R Laura Zavatta - University of Bologna
Monitoring of pollinators supported by GIS: territorial classification and ecological restoration based on bee and hoverfly diversity

12:10 - 12:20 **Discussion on PhD education and future careers and meeting closure**

12:20 - 13:10 **Brunch**

We kindly request that all speakers respect the designated time limits assigned in the program to ensure smooth proceedings. Thank you for your cooperation.

More information is also available at https://www.accademiaentomologia.it/scuole/

The organizers

Gianfranco Anfora, Morena Casartelli, Eric Conti, Francesca Romana Dani, Giuseppino Sabbatini Peverieri, Sauro Simoni

REGULAR & SHORT PRESENTATIONS

◆

ABSTRACTS

(in alphabetical order by last name of the 1st author)

Entomopathogenic Nematodes for Biological Control of *Popillia Japonica* Larvae: Current Status and Future Prospects

R

S. Amoriello[1,2], G. P. Barzanti[1], F. Paoli[1], L. Marianelli[1], P. F. Roversi[1], G. Torrini[1]

[1]CREA - Research Centre for Plant Protection and Certification, Italy; [2]University of Siena - Department of Life Sciences, Italy

The Japanese beetle (*Popillia japonica* Newman), first detected in Northern Italy in 2014, is considered one of the most dangerous quarantine pests for the entire European territory due to its potential environmental, economic, and social impact. As its larvae spend most of their life cycle in the soil, entomopathogenic nematodes (EPNs) offer a promising biological control option. *Heterorhabditis bacteriophora* (strain POP 16) was detected in the soil of outbreak area and selected as the most virulent native strain based on preliminary laboratory virulence assays. Field trials were conducted in the Piedmont region from 2021 to 2024 to assess POP 16's effectiveness in controlling the *P. japonica* larval population and its persistence in the soil.

P. japonica larval populations were significantly reduced in plots treated with EPNs, with over 90% fewer larvae observed compared to untreated areas and *H. bacteriophora* persisted in the soil for up to two years after treatment. The effects of EPNs on non-target soil biota were also evaluated and no significant negative impacts were observed in EPN-treated soil compared to the control. However, some non-target edaphic arthropod taxa showed increased abundances, suggesting potential long-term changes in the soil ecosystem.

Regarding the future prospects: identify the most promising symbiotic bacterial strains associated with EPNs for controlling *P. japonica* and study their genomes could reveal the loci responsible for entomopathogenesis. These further in-depth investigations are essential for developing novel, environmentally friendly control measures for the control of *P. japonica*, such as bioinsecticides based on bacterial strains isolated from EPNs.

Impact of Protein-to-Carbohydrate Ratio on the Immune Response of Black Soldier Fly Larvae

R

I. Armenia[1], S. Caramella[1], E. Mengotti[1], D. Bruno[1], G. Tettamanti[1,2]

[1]*University of Insubria, Department of Biotechnology and Life Sciences, Varese, Italy;*
[2]*Interuniversity Center for Studies on Bioinspired Agro-environmental Technology (BAT Center), Portici, Italy*

Black Soldier Fly (BSF) larvae, *Hermetia illucens,* are increasingly recognized as a valuable source of protein and fat for animal feed production. However, BSF rearing on decaying organic material exposes the larvae to various microorganisms, potentially compromising the quality of insect-derived products. Enhancing the immune system of BSF larvae is therefore critical for ensuring the safety and quality of BSF-based bioproducts. This study investigates how varying the dietary protein-to-carbohydrate (P:C) ratio influences the immune response of BSF larvae when exposed to Gram-negative and Gram-positive bacteria.

To this aim, larvae were reared on diets with different P:C ratio and challenged with a mix of *Escherichia coli* and *Micrococcus luteus*. Their immune response was assessed by measuring both cellular (haemocyte count and phagocytosis) and humoral markers (lysozyme activity and antimicrobial peptide expression). Our results showed significant differences between diets, with larvae exhibiting stronger immune responses when reared on high P:C ratio diet. Specifically, cellular markers and antimicrobial peptide expression were significantly elevated in larvae fed on protein-rich diet. These findings highlight the important role of substrate nutritional composition in enhancing the immune response of BSF larvae and provide insights into optimal dietary parameters for improving both larval growth and resistance to bacterial infections.

Assessing the impact of different chemical pollutants on the gut microbiome of different pollinator species

S

S. Basiglio[1], F. Patriarca[1], R. Ali[1], E. Holzer[1], S. Casini[2], F. Sgolastra[3], D. Lupi[1], F. Mapelli[1], E. Crotti[1]

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[2]University of Siena - Department of Physical, Earth and Environmental Sciences, Italy;
[3]University of Bologna - Department of Agricultural and Food Science, Italy

Over the last decades, there has been a reduction in the diversity of bees and other pollinators, marked by declines or extinction of certain species. The exposure to chemical pollutants has been identified as a primary factor contributing to the reduction of pollinators. Exposure to anthropogenic chemicals may indirectly compromise animal health by disrupting the gut microbiota, which is essential for insect health. In fact, the gut microbiome of insects plays specific roles in various evolutionary, biological, and physiological processes, including nutrition and development. Most research examining the impact of agrochemicals on pollinators has concentrated on honeybees and bumblebees and on few selected molecules. Hence, little is known so far about the impact of various chemical pollutants on other insect pollinators, for which the structure and function of the microbiota are also poorly understood. Thus, this study, which is part of the POLYPOLL project (https://site.unibo.it/polypoll/it), aims to assess the effects of different categories of chemical pollutants on the gut microbiome of different pollinator species, i.e. *Apis mellifera*, *Osmia bicornis,* and *Eristalis tenax*. The study focuses on the taxonomic composition of the gut microbiome and the abundance of bacterial and fungal communities present in the gut. Initial data on the gut bacterial community of *O. bicornis* indicate that bacterial abundance is significantly reduced when specimens are exposed in laboratory trials to chemical pollutants, i.e. copper chloride and Boscalid, administered singularly and in combination. Further data must be collected to enhance understanding of the effects of various pollutant categories on the gut microbiome of pollinators.

Parasites, pathogens and microbiota: a stressful job for bee's immunity

SENIOR LECTURE

A. Becchimanzi

University of Napoli Federico II - Department of Agricultural Sciences, Italy

Honey bees (*Apis mellifera*) are essential pollinators for many ecosystems and agricultural crops. However, the health of bee populations is increasingly threatened by various stressors, both biotic and abiotic, which negatively affect bee's immunity, leading to increased mortality and colony collapse. The honey bee immune system plays a central role in a complex network of interactions, not only defending against pathogens and parasites but also maintaining gut homeostasis by distinguishing between beneficial gut symbionts and harmful species. Here we explore the interactions between the bee immune system and biotic stressors, focusing on the ectoparasite *Varroa destructor* and the immune-suppressing viral pathogen Deformed Wing Virus (DWV). We employed a range of molecular tools, including transcriptomics and RNA interference for studying *V. destructor* salivary blend, and proteomics and metagenomics, for studying DWV mechanisms of replication and pathogenesis. We show that biotic stressors adopt diverse strategies to evade and suppress the immune response of honey bees, including the alteration of metabolic and gut homeostasis. Understanding these interactions is crucial for developing sustainable strategies to support bee health and mitigate the risks posed by modern agricultural practices.

Color vision in longhorn beetles: from electroretinogram recordings to practical applications

R

L. Besana[1], G. Cavaletto[1], G. Santoiemma[1], D. Lazar[1], G. Belušič[2], D. Rassati[1]

[1]University of Padova - Department of Agronomy Food Natural Resources Animals and Environment, Italy;
[2]University of Ljubljana - Department of Biology

Longhorn beetles (Coleoptera, Cerambycidae) constitute a family of more than 25,000 species worldwide and are among the most significant groups of invasive forest insects. The wood-boring habit of larvae allows them to often escape routine inspections of wood and wood-packaging materials at entry points and to become major pests in the invaded environment. Biosecurity surveys commonly employ traps baited with pheromones and host-plant volatiles; however, recent investigations on color stimuli showed that color can also be a crucial factor for improving catches of some species. For this reason, we set up a trapping experiment involving eight different partners in Italy, France, Poland, Canada, and the US. We tested four different trap colors (black, red, yellow, white) and a trap that combines all the four colors on the same panels to investigate whether it is possible to save money and resources by using multiple visual stimuli on the same trap. Preliminary results demonstrated a species-specific response of longhorn beetles to the different visual stimuli. In addition, we performed electroretinogram recordings on a few species of longhorn beetles to investigate their retinal sensitivity to color. Some species showed an ancestral condition of dichromacy, while others clearly demonstrated complex mechanisms for color vision, including a set of three different photoreceptors and neuronal mechanisms that fine-tune sensitivity to different colors.

Population genetics and the role of dispersal barriers in the Antarctic springtail *Kaylathalia klovstadi* (Collembola, Isotomidae)

R

S. Boschi[1], C. Cucini[1], E. Cardaioli[1], F. Frati[1], F. Nardi[1], A. Carapelli[1]

[1]*University of Siena - Department of Life Science, Italy*

Isolation and extreme environmental conditions have had a significant impact on terrestrial invertebrate biodiversity in Antarctica, with glacial cycles further reducing animal diversity to a limited number of species, among which springtails are well represented, albeit with low taxonomic diversity. Glaciers may act as major barriers to dispersal, inducing high levels of genetic divergence between isolated populations. In the Victoria Land (Continental Antarctica), the Tucker Glacier has been proposed as an example of such a barrier, although different species/taxa may be influenced in different ways by the same geographical barriers. We tested if geographic distance between populations, major glacier tongues and seawater channels are responsible for shaping the distribution of *Kaylathalia klovstadi* (Collembola; Isotomidae) in Northern Victoria Land. We performed mitochondrial DNA haplotype analyses for fragments of cytochrome c oxidase subunit 1 (*cox1*) and ATP synthase 6 (*atp6*) in 62 individuals from six populations of *K. klovstadi*. Comparison of genetic data provides new insights into the genetic diversity and distribution patterns of *K. klovstadi* populations by increasing the number of sampling sites and analyzed specimens, and the introduction of an additional molecular marker in the population genetics study. We challenge previous assumptions on the role of the Tucker Glacier as an important physical barrier to dispersal of springtails in Northern Victoria Land. Our results reconsider the role of physical barriers in shaping genetic diversity of some springtail taxa, among which the species under study.

Multispectral drone images for the early detection of *Ips typographus* infestations: assessment over large forest areas in the South-Eastern Alps

R

A. Bozzini[1], S. Brugnaro[2], L. Huo[3], G. Morgante[1], H.J. Persson[3], V. Finozzi[4], A. Battisti[1], M. Faccoli[1]

[1]University of Padova - Department of Agronomy Food Natural Resources Animals and Environment, Italy; [2]Geologist Flight Instructor and Remote Sensing, San Giorgio delle Pertiche, Padua, Italy; [3]Department of Forest Resource Management, SLU University, Umeå, Sweden; [4]Regione del Veneto, U.O. Fitosanitario, Treviso, Italy

European forests face increasing threats due to climate change-induced stressors, which create the perfect conditions for bark beetle outbreaks. The most important spruce forest pest in Europe is the European Spruce Bark Beetle (*Ips typographus* L.). Effective forest management of these beetles' outbreaks necessitates timely detection of recently attacked spruce trees (*i.e.*, when tree crowns don't show visible symptoms yet), which is challenging given the difficulty in spotting early symptoms on infested trees, especially on large areas. This study assesses the detectability of recently infested trees over large spruce dominated areas (20-60 ha, > 1,600 trees) using high-resolution drone multispectral imagery.

A drone-borne multispectral sensor captured images weekly during June 2023. The analyses of a set of vegetational indices allowed to observe the recently infested trees' reflectance features at each site, comparing them with those of unattacked trees.

Results showed that it was possible to separate the spectral signal of infested and unattacked trees during the final developmental stage of the beetles first generation and before tree crown discoloration, despite the limitations due to imagery processing over large areas and lack of extensive information from the field. Key performing vegetation indices included NDRE (Normalized Difference Red Edge index) and GNDVI (Green Normalized Difference Vegetation Index). This remote early-detection approach could make a great contribution to the development of tools for the automatic diagnosis of bark beetles' infestations and provide useful guidance for the management of areas suffering pest outbreaks, especially if integrated with satellite imagery.

Effects of Lead Intake on *Hermetia illucens* Immune System and Development

R

S. Caramella[1], D. Spanu[2], O. Santoro[1], D. Bruno[1], I. Armenia[1], G. Tettamanti[1,3]

[1]*University of Insubria, Department of Biotechnology and Life Sciences, Varese, Italy*
[2]*University of Insubria, Department of Science and High Technology, Como, Italy*
[3]*Interuniversity Center for Studies on Bioinspired Agro-environmental Technology (BAT Center), Portici, Italy*

Hermetia illucens (Diptera: Stratiomyidae), known as black soldier fly (BSF), is a saprophagous insect with significant potential as a source of proteins for animal feed. BSF larvae can grow on decaying organic waste and are thus potentially exposed to heavy metals that may impact insect's health and bioaccumulate in the derived products.
The aim of this project is to investigate the impact of lead chloride ($PbCl_2$), a heavy metal present in organic waste, on the development and health of BSF larvae. To this aim, larvae will be reared on a substrate mimicking the Organic Fraction of Municipal Solid Waste (OFMSW), spiked with varying concentrations of $PbCl_2$.
Following analysis of the insect growth performance, markers of both cellular and humoral branches of the immune system were evaluated. In addition, the potential effects of lead on the antimicrobial activity of the hemolymph was assessed against Gram-positive and Gram-negative bacteria. Moreover, the concentration of lead ions (Pb^{2+}) in the rearing residue and in insects was measured with Inductively Coupled Plasma Optical Emission Spectrometry.
Our results indicate that the bioaccumulation rate of lead is correlated to the dose administered to the substrate and that high concentrations of $PbCl_2$ negatively affects larval growth and survival. Lead contamination significantly impacts on some immunological markers, such as lysozyme activity, the number of circulating haemocytes, encapsulation rate, and antibacterial activity.
This study provides new insights into the adverse effects of heavy metals on BSF health and contributes to delineate a species-specific risk profile for BSF in feedstuff.

Cryptocephalinae beetles (Coleoptera, Chrysomelidae) host a symbiont from the Morganellaceae family: prevalence, localization and potential roles for the host

R

M. Cardinale, M. Brunetti, P. Costagliola, M. Montagna, G. Magoga

University of Napoli Federico II - Department of Agricultural Sciences, Italy

Bacteria play a crucial role in the adaptation of phytophagous insects to their host plants. In leaf beetles (Coleoptera, Chrysomelidae), both primary symbionts and gut bacteria aid in detoxifying plant metabolites, breaking down recalcitrant plant compounds, and supplementing diet with vitamins. Recently, we identified a bacterium from the Morganellaceae family (Enterobacterales) associated with multiple species of the Cryptocephalinae subfamily, suggesting it may act as a mutualistic symbiont for these insects. This ongoing study aims to: i. determine the prevalence of this bacterium in Cryptocephalinae; ii. identify its location within the insect body; and iii. assess its potential functional role for the host. DNA was extracted from 32 Cryptocephalinae species, the 16S rRNA gene amplified using specific primers and the obtained amplicons Sanger sequenced. Dissections of Cryptocephalinae adults and larvae were conducted to search for specialized symbiont-hosting structures and fluorescence in situ hybridization will be performed to localize the symbiont within the insect body. Genome sequencing of this bacterium will be also conducted to identify specific genes that might provide benefits to the host. Eight species were found positive to the bacterium, including *Cryptocephalus flavipes*, where it was detected in eggs laid by positive adults and larvae (first to third instars), suggesting vertical transmission across generations. Dissections revealed the presence of gastric caeca in the adult midgut, potential sites for bacterial housing, observed in both bacterium-positive and bacterium-negative species.

Development of new technologies for the study and management of the European spruce bark beetle *Ips typographus*

S

M. Case

University of Padova - Department of Agronomy Food Natural Resources, Animals and Environment, Italy

This presentation is about the planned research project on the bark beetle outbreak in the southeastern Alps and its management. The aim of this study is to understand the factors behind the expansion of the bark beetle *Ips typographus* following the "Vaia" windstorm in October 2018 and to test a strategy to combat outbreaks. Two approaches will be used: first, reconstructing the timeline of attacks to identify hotspots and their evolution, considering both satellite images and various abiotic factors (soil pH, temperature, slope, water content, carbon and nitrogen concentrations), especially focusing on how temperature influences attacks and the spread to higher altitudes. This will create a database for predicting future attacks. The second approach targets the insect and associated fungi (e.g., *Endoconidiophora polonica, Grossmania penicillata*) by testing their sensitivity to natural substances in lab experiments. The goal is to identify effective, low-impact molecules which could undermine this symbiosis and weaken the attacks. This project aims to provide forest management recommendations and to support the recovery of affected areas in the southern Alps, particularly in the Veneto region. However, the expected results will also benefit other Alpine areas, and they will be useful both for the economic component of Norway spruce forests, and for forests that are part of protected areas, in which the greatest interest is the biodiversity component.

Evaluation of frass of *Hermetia illucens* as elicitor of plant defenses against aphids

R

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The sustainable management of agri-food waste is a challenging but critical environmental goal. Larvae of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) are well known for their role in processing such residues by producing flour for feed and frass, which can be usefully used as biofertilizer in agriculture. The high chitin content of frass makes it also suitable as elicitor of plant defenses against pathogens and insects, hence making frass also a potential sustainable crop protection tool. However, very few studies have tested this application of frass against insect pests. This study aims to assess the effects exerted by *H. illucens* frass on an aphid-plant system as a starting point for the development of new strategies for sustainable plant protection through the rational manipulation of trophic interactions. The used frass was also characterized and evaluated to partially replace commercial peat in the production of potted plants to verify its biostimulating effect. Tomato plants (Dwarf San Marzano), grown on a commercial substrate enriched with different concentrations of frass (0, 1, 2, 4 and 8 g/kg soil) produced by *H. illucens* larvae fed on a vegan diet, showed a reduced total biomass and alteration of gas exchanges parameters (stomatal conductance, transpiration, water utilization efficiency and CO_2 assimilation). The frass-exposed plants induced in a dose-dependent manner a reduction of development, survival rate, fecundity and longevity in the aphid *Macrosiphum euphorbiae* (Hemiptera: Aphididae). Contrary to expectations, the results show that adding frass to the soil leads to a reduction in plant growth, accompanied by increased resistance to sap-sucking insect attacks. Further studies are needed to identify the concentrations of frass that can induce the desired defense-eliciting effect that does not negatively affect plant growth.

***Myzus mumecola*: insights into the biology of the newly invasive apricot aphid**

S

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Italy, 2016: an aphid species is found in apricot orchards that does not belong to any of the previously identified pests. As for many invasive insects, concern arose over its threat towards local ecosystems and economy. Identified as *Myzus mumecola* (Hemiptera: Aphididae), an aphid native to Eastern Asia, it rapidly invaded neighbouring countries like France, Germany, Serbia, Hungary and Czech Republic, causing damages to leaves and shoots of the plant. With Italy being the highest producer of apricots in Europe and South Tyrol providing its well-known "Vinschger" variety, there is an urgent need to explore the behaviour and impact of this poorly studied pest. Initial phylogenetic characterizations of *M. mumecola* based on mitochondrial DNA were carried out to confirm the correct identification. Moreover, we performed laboratory trials to study the life cycle of this aphid species. Feeding behavioural tests and a molecular plant DNA analysis will be carried out to determine the summer hosts and potential alternative hosts of the pest. Finally, the endosymbiont community will be compared among different generations to understand their potential role for the life cycle of *M. mumecola*. Here we present our first results about the ecology of this emerging pest species and its potential secondary host plant, aimed at contributing to the development of targeted pest control practices.

Endophytic colonisation of *Beauveria bassiana* in tomato plants: mechanisms and consequences for the control of lepidopteran pest *Spodoptera littoralis*

R

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Beneficial microorganisms can promote plant growth and defence barriers, offering a valuable alternative to synthetic agrochemicals. *Beauveria bassiana* is one of the most important entomopathogenic fungi and can colonize a wide variety of plant species as an endophyte, limiting the growth and survival of plant pests and pathogens. Here we further contribute to this research topic by studying the effect of tomato plants colonization by *B. bassiana* on *Spodoptera littoralis* (Lepidoptera, Noctuidae) survival, growth and immune response. Endophytic colonization of plants did not affect the survival of larvae feeding on them, but induced larval weight increase, higher pupal mortality and lower adult fecundity. Interestingly, encapsulation and nodulation responses of larvae fed with *B. bassiana* colonized plants were reduced, while, in contrast, phagocytosis slightly increased. This reduced immune competence was associated with an increased sensitivity toward entomopathogens. To better understand the mechanisms underlying such reduced immune competence, plant tissues, gut content metabolites and gut microbiota have been analyzed. 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.

Potential effects of an organic biostimulant on the tri-trophic interaction zucchini-ZYMV-aphid vector

R

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Biostimulants are substances that promote plant growth and efficiency of nutrient uptake by improving plant and/or rhizosphere characteristics. The effect of a biostimulant containing alfalfa, brown algae, and molasses extracts on the interaction among zucchini plants (*Cucurbita pepo* L.), zucchini yellow mosaic virus (ZYMV) and aphid vectors was evaluated. Several crop traits were compared between healthy and ZYMV-infected plants, which were treated or untreated with biostimulant. Treated plants had increased growth with higher values of reproductive parameters (number of flowers and fruits) even in infected conditions. After treatment, a decrease of ZYMV titre and a progressive slowdown of symptom severity were observed in plant, together with the up-regulation of some defence genes. The effect of biostimulant on the settling preference and life traits of the aphid vector *Myzus persicae* was also investigated. Aphid choice test experiments using detached zucchini leaves showed that fewer specimens settled on biostimulant-treated plants, with and without ZYMV. When the aphids were caged on both infected and healthy potted plants, the biostimulant treatment led to reduce aphid survival and decrease offspring production, regardless of the infection conditions. Volatile organic compounds (VOCs) were collected and analysed by GC-MS, and then used in olfactometer experiments comparing different combinations of treatment and infection conditions. When allowed to choose freely between treated and untreated plants, aphids preferred untreated plants independently of their healthy status, confirming a potential role of biostimulants in repelling the vector. Taken together, these findings suggest that biostimulant treatment can reduce disease risk in *C. pepo* crops by potentially contributing to the concurrent control of ZYMV and its vector.

Arthropod biodiversity in Italian agroecosystems: monitoring approaches and factors of influence

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Preserving arthropod biodiversity in agroecosystems is essential for maintaining ecosystem services and environmental health. However, factors like agronomical practices and landscape structure can affect arthropod biodiversity. This project aims to identify the most effective approaches for monitoring arthropod biodiversity in agroecosystems and evaluate the impact of agronomic practices and landscape structure on epigean and hypogean arthropod taxa. Vineyards and olive groves were selected as model agroecosystems, with two 30 km^2 study areas selected. In each area, 15 experimental fields were surveyed using Malaise traps, pitfall traps, and soil cubes (processed via Berlese funnels) to collect arthropods. Soil cores were also collected to estimates biodiversity using environmental DNA (eDNA). Sampling occurred in both spring and autumn. Arthropods identification is carried out through DNA metabarcoding, targeting COI and 18S rRNA genes, both for bulk samples (traps and Berlese) and for the eDNA (soil cores). Sample processing is ongoing, and once results will be available, landscape characteristics and agronomical practices will be correlated with arthropod diversity estimates. Furthermore, the efficiency of soil eDNA for monitoring arthropod biodiversity will be evaluated by comparing the diversity estimates from metabarcoding with those derived from morphotaxa-based approaches, using the arthropods collected with Berlese funnels and pitfall traps. Additionally, in a more restricted area, the efficiency of airborne DNA for arthropod monitoring is tested by comparing the diversity estimated derived from air samples with those obtained by collecting arthropods with Malaise traps.

Across the Spiderverse: comparative analysis of metals in spiders' mouthparts (Arachnida, Araneae)

S

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Spiders are a group of widespread generalist predators, principally insectivorous. This study aims to compare for the first time the presence of metals on the feeding structures (fangs, cheliceral teeth and serrula) of Araneomorph spiders and exploring how this feature varies in relation with morphological and ecological traits of the species (body length and functional guild). A total of 127 spiders (39 species distributed among 20 families) were analysed. The presence of zinc, manganese, calcium and magnesium was searched through Energy Dispersive X-ray Spectroscopy. Calcium was recorded in all the mouthparts, manganese only in teeth, whereas magnesium was generally absent only in fangs. Zinc was found in all the mouthparts, including the serrula, where its presence is documented here for the first time. The highest zinc and manganese levels were found in fangs and teeth respectively, in accordance with previously published works. However, through Kruskal-Wallis test followed by Bonferroni-adjusted Dunn's paired comparisons, it was found that only zinc and manganese levels were significantly higher in feeding structures than in not-feeding control body parts. Furthermore, the greatest zinc levels in fangs occur in the family Salticidae. Through General Linear Models, it was found that teeth's manganese % was positively associated with body length, whereas calcium % was higher in in the serrula and the fangs of free-hunters than in web-makers, because probably the former require a stronger prey holding. In addition, zinc levels in both fangs and serrula did not significantly vary with the ecological traits, likely due to phylogenetic inertia.

An immunosuppressive virus causes gut dysbiosis in honey bees

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The decline in honey bee health represents a globally significant problem due to the remarkable impact of these pollinators on the environment and human economy. Reduced bee survival results from a multifactorial syndrome triggered by several stress factors that may interact synergistically. A common factor in collapsing colonies is the high load of parasites and pathogens, such as Deformed Wing Virus (DWV), an endemic immunosuppressive virus that causes covert infections, kept in check by the bee's immune system unless other stressors weaken antiviral defences. Given the vital role of the gut microbiota in supporting immunity and maintaining host homeostasis, this study investigates the effects of DWV infection on honey bee gut microbiota. Using bacterial 16S rRNA amplicon sequencing, we profiled gut bacterial communities in bees under natural and experimental infection conditions. Results revealed a DWV-induced dysbiosis, characterized by reduced *Lactobacillus spp*. and increased *Rhizobiaceae* (Proteobacteria). *Lactobacillus spp.*, known for their beneficial symbiosis and potential immunomodulatory functions, were significantly depleted in highly infected bees, suggesting that DWV-induced dysbiosis may exacerbate the immunocompromission syndrome. The comprehension of DWV effect on microbiota will deepen our knowledge about immunosuppressive strategies used by viruses for host exploitation and will allow us to define blends of probiotic microorganisms which may help to rescue the decay of honey bee immune competence.

Keywords: Deformed Wing Virus, immune response, bee microbiota, dysbiosis, *Lactobacillus spp*.

Three different endophytic microorganisms are effective control agents of *Spodoptera littoralis* noctuid moth larvae

R

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The intimate mutualism established between plants and endophytic microorganisms is often capable of promoting plant growth and defense, providing protection against different biotic stress agents, including pest populations. The strategic use of these microorganisms in agriculture offers a viable alternative to synthetic agrochemicals, enabling their gradual reduction, with positive impacts on food safety and the environment. Here, we selected the well-known beneficial fungus *Trichoderma afroharzianum* strain T22, the entomopathogenic fungus *Beauveria bassiana*, and the entomopathogenic bacterium *Bacillus thuringiensis* to obtain colonized tomato plants (respectively named T22-, Bb- and Bt-plants) and assess how these microorganisms can influence the biology of the lepidopteran pest *Spodoptera littoralis* either directly or indirectly through plant-mediated effects. Larvae fed on Bt-plant leaves showed a significant reduction in survival rate up to the third larval instar, reduced pupal survival, and decreased adult longevity and fertility. Instead, the survival rate and weight of larvae fed on T22-plant leaves progressively decreased from the fourth instar onward, compared to control larvae. This resulted in a developmental delay with negative effects on subsequent stages. Otherwise, Bb-plants did not affect larval survival, but larvae displayed increased weight, which was associated with higher pupal mortality. In addition, all three plant treatments impaired the cellular immune response of the larvae, generating immunosuppressed phenotypes more susceptible to sublethal doses of a commercial bioinsecticide containing Bt.
These results contribute to the understanding of the functional mechanisms underlying multitrophic interactions among beneficial endophytic microorganisms, plants, and insects, which will allow the development of new "bio-inspired" strategies for crop protection.

Advancements on the management of *Toumeyella parvicornis* (Cockerell, 1897): biology, distribution and control strategies

R

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Toumeyella parvicornis, the pine tortoise scale, is a significant pest that affect pine trees. During the last decade, has expanded its distribution even in the European continent as an alien species affecting mainly *Pinus pinea*. In some Italian regions, hundreds of stone pine trees have already been lost due to it. In the light of this concerning scenario, it has been investigated the biology, the distribution, and some feasible containment measures for this harmful pest. The species has been *i)* morphologically and molecularly characterized providing useful DNA barcoding tools, which made simpler its identification; *ii)* its potential distribution has been assessed through Species Distribution Modelling, allowing the detection of areas potentially at risk of occupation by the species in the future; *iii)* containment strategies have been evaluated: low-impact control strategy such as endotherapy, testing its efficacy on the pest, the duration of the beneficial effect and the best techniques to be used or *iv)* biological control strategy through the use of predators with a focus on local ladybugs species, considering the possibility to select candidate for an open-field release.
The outcomes of these studies highlight the crucial role of the investigations on effective management strategies to face invasive pests such as *T. parvicornis*. Their implementation in the available management guidelines for the pest may apport important improvements, providing valuable insights for mitigating the impact of that invasive species and safeguarding stone pine stands.

The impact of endophytic communities on the volatile organic compound profile of sunflower *Helianthus annuus* and its detection by honey bees

R

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Plant Growth-Promoting Bacteria (PGPB) and Rhizobacteria (PGPR) are widely present in the rhizosphere and contribute to the health and performance of associated plants. Their effect can be extended to the volatile organic compounds (VOCs) released by plants and used in insect-plant communication. In recent decades, several anthropic factors have degraded the agroecosystem soil, thereby reducing the contribution of the microbial population to plant and ecosystem health and biodiversity. Enhancing the positive plant-microorganism interactions is thus a primary goal of crop management. However, evidence is lacking on the role that PGPB and PGPR exert on the chemical ecology of plants, notably in attracting pollinators. The hypothesis, at the basis of our project, is that PGPB and PGPR improve soil fertility and quality, and directly impact plant-pollinator interactions by modifying plant VOCs, nectar and pollen composition, thereby playing a key role in pollinator health and ecosystem functionality. One of our objectives is to measure the effects of these endophytic communities on the VOC profile of different sunflower cultivars and how these are detected by honey bees, *Apis mellifera*. Ancient and hybrid cultivars of sunflower were grown with different endophytic communities. VOCs were collected from the blooming flowers using closed-loop stripping analysis. Extracted VOCs were presented to the honey bee antenna in coupled gas chromatography-electroantennography experiment. Then, the antennally active compounds were identified using coupled gas chromatography-mass spectrometry. This work is supported by the project "IMPLiCIT - Improving soil-plant-insect interactions to promote pollinators" funded by the MIUR Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN) Bando 2022- grant 2022NMAPEL.

Biotremology for pest management, new solutions for stink bugs and true bugs

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Urgent action is needed to develop resilient and sustainable strategies that reduce crop yield losses from pests while minimizing the negative impacts of pest management on human health and the environment. Our aim focuses on exploring alternative methods such as behavioural manipulation and mating disruption to create biorational pest management strategies, in this case for the southern green stink bug (SGSB), *Nezara viridula*. Recordings of male and female pairs, as well as individuals, from Italian and Spanish populations confirmed the presence of two types of vibrations: female calling songs (FCS) and female courtship songs (FCrS). Based on these findings, we initiated playback experiments using an E-shape artificial arena. The results showed that males responded more frequently to FCS, exhibiting searching behaviour and calling songs. We then refined our focus to two distinct FCS signals—one at 64 Hz and another at 128 Hz. The playback experiments revealed a significantly higher rate of correct responses with the 128 Hz FCS, which we selected for further semi-field and field trials. A specific company provided us with Shindo traps equipped with the chosen audio. We measured the vibrations from the traps to ensure the audio signal was accurate when deployed in the semi-field and field experiments. And now the setup for semi-field experiment is ready and trials began.

Insect gut microbiome as a resource to improve insect mass rearing

R

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The gut microbiome plays vital role in health and fitness. Olive fruit fly, *Bactrocera oleae* is a major pest in olive cultivation, and is reared for Sterile Insect Techniques programs. Olive fly has a gut microbiome dominated by the symbiont '*Candidatus* Erwinia dacicola', but after prolonged rearing the symbiont tends to be lost, leading to impaired fitness. This study investigates the importance of *E. dacicola* and possible bacterial substitutes in the gut microbiome of *B. oleae* through transcriptomic sequencing and qPCR analysis, aiming to identify microbial consortia crucial to be redelivered for insect health and rearing success. We conducted transcriptomic sequencing of the gut microbiome in parental (from the field) and F1 (lab-reared) generations to identify differentially expressed genes (DEGs) in response to *E. dacicola* reduction and performed taxonomy assignment to determine microbial composition. Additionally, we used antimicrobial treatment to simulate prolonged lab rearing, and qPCR was employed to compare the expression of *E. dacicola* with and without antimicrobial treatment. Our results revealed that *E. dacicola* is a core member of the olive fly gut microbiome, with DEGs linked to nutrient metabolism, stress response, and immune function identified between generations. Antimicrobial treatment significantly reduced the abundance of *E. dacicola*. Moreover, when *E. dacicola* starts to be lost among gut microbial composition, other taxa increase in F1 generation and complement part of metabolic processes, suggesting a compensatory role. These findings underscore the dynamic nature of the olive fly gut microbiome and the critical role of *E. dacicola* in maintaining its balance.

Neglected microbes in floral nectar: Influence of filamentous fungi on nectar traits and parasitoids olfaction and performance

R

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Floral nectar is ubiquitously inhabited by a multitude of microorganisms. Fermentation by nectar-inhabiting microbes can change several nectar traits via the production of microbial Volatile Organic Compounds (mVOCs) and other primary metabolites. Although there is growing evidence on how yeasts and bacteria influence the foraging behavior and performance of pollinators and parasitoids, the potential role of other microbial taxa that can potentially colonize floral nectar has been largely neglected. Here, we investigated how filamentous fungi isolated from the floral nectar of buckwheat, *Fagopyrum esculentum*, affect nectar scent and quality and, subsequently, the olfactory responses and performances of two co-occurring egg parasitoids, *Trissolcus basalis* and *Ooencyrtus telenomicida*. In olfactometer assays, 2 out of 6 fungal strains, namely *Cladosporium* sp. SAAF 22.2.11 and *Cladosporium* sp. SAAF 22.3.29 induced attraction in the parasitoids. Specifically, *O. telenomicida* displayed olfactory responses to both *Cladosporium* spp.-fermented nectars, while *T. basalis* only responded to nectar fermented by *Cladosporium* sp. SAAF 22.2.11. In the performance assay, parasitoid longevities and survival rates were not significantly affected after feeding on individual fermented nectar, except for the survival of *T. basalis* that fed on nectar fermented by *Cladosporium* spp. SAAF 22.2.12 and SAAF 22.3.29 were significantly reduced. Chemical analyses highlighted both qualitative and quantitative differences in the VOCs emitted by different fungus-fermented nectars and the presence of primary metabolites caused by microbial fermentation. By demonstrating that parasitoids were affected by nectar fermented by filamentous fungi, we contribute to uncovering interactions between flower-visiting insects and flower-associated microbes.

Environmental and habitat influences on the gut microbiota of two European pollinators

S

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Pollinators are recognized as key contributors to crop pollination worldwide, and their recent decline has made pollinator conservation a critical challenge. In social insects, the gut microbial community is closely linked to essential functions such as longevity, fecundity, and overall health. This suggests that disruptions in microbial associations may significantly impair pollinator fitness. Flowers serve as a primary microbial source for pollinators, and the surrounding environment, particularly floral diversity, can strongly influence the composition of the microbiota in eusocial bees. It has been widely documented that bee biodiversity declines with increasing land-use intensity, leading to the loss of floral diversity and nesting sites. Therefore, the landscape can influence both the composition and diversity of pollinator microbiota.

This study aims to investigate the role of several environmental factors, such as landscape, geolocation, and climate, in shaping the bacterial communities associated with two model European pollinators, *Halictus scabiosae* and *Bombus pascuorum*. The microbiota of specimens collected from different landscapes (agricultural and semi-natural sites) across five European countries (Italy, Switzerland, the Netherlands, Germany, and the UK) will be investigated using a meta-barcoding approach. The results will provide a comprehensive understanding of the ecological factors influencing pollinators' microbiota composition and their subsequent impact on insect fitness.

The insights gained will support the development of strategies aimed at enhancing microbial diversity in pollinators, indirectly improving their fitness, and contributing to biodiversity conservation with positive economic implications.

New insights into the biology and parasitism rate of *Aphelinus mali*, parasitoid of the wolly apple aphid *Eriosoma lanigerum*

R

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The woolly apple aphid (WAA), *Eriosoma lanigerum* (Hemiptera: Aphididae), has recently become an increasing threat to apple orchards also due to the withdrawal of several active substances, such as neonicotinoids and organophosphates. Native to North America, WAA completes its life cycle on apple trees in Europe by colonizing roots, trunks, and branches. The coevolved and specialized endoparasitoid *Aphelinus mali* (Hymenoptera: Aphelinidae), which was introduced in Italy about a century ago in a successful programme of classic biological control, is primarily responsible for biological control of this pest. From 2021 to 2024, surveys were conducted in apple orchards (both organic and IPM) in Northwest Italy to assess: *i)* presence and parasitism rate of *A. mali* against WAA by visual inspection of plants and use of yellow sticky traps, and *ii)* overwintering survival of parasitoid by winter collecting and rearing under natural and controlled conditions of WAA colonies. The presence of *A. mali* was consistently higher in organic apple orchards; the first adults were captured from mid-March in all years. Despite these early captures, the activity of the parasitoid was evident from about mid-June, reaching maximum values, close to 100%, in July and August. From overwintering WAA colonies, *A. mali* adults emerged at a rate varying from 8% to 44% under natural conditions and from 13% to 54% under controlled conditions, with a female-biased sex ratio. Based on results, further research is needed to better understand *A. mali*'s biology and behaviour and to enhance WAA control throughout the season.

First insights into the role of urbanisation in shaping the aculeate wasp community in an Italian metropolis

R

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Urbanisation is causing changes in land use and climate, with documented impacts on insects. Among urban insects, aculeate wasps provide important ecosystem services such as regulating arthropod populations. However, the impact of urbanisation on wasp communities has been largely neglected, especially on a large scale and with intensive sampling efforts. Here, wasps were hand-netted at 10 sites in the metropolitan area of Milan (Italy), with each site covered by 5 sampling rounds (May to September 2023). Urbanisation was described in terms of mean site temperature, grassland patch density, and tree cover connectivity. Accordingly, sampling sites were categorised as “less urbanised”, “urbanised” and “highly urbanised”. The preliminary results aim to (i) describe the aculeate wasp community, and (ii) investigate whether this community is changing in response to urbanisation. A total of 739 individuals spanning 22 genera and 42 (morpho)species were sampled. The most abundant genera were *Vespula*, *Polistes* and *Cerceris*. Wasp genus-landscape networks suggest that highly urbanised areas may be dominated by solitary, ground-nesting genera. In addition, community composition is significantly influenced by changes in mean temperature. More wasps (N), higher estimated species richness (q0), and a higher Shannon index were found at sites with higher grassland patch density (less urbanised). These preliminary analyses suggest that urbanisation affects aculeate wasp communities, and that more extensive and coherent urban grasslands are likely to be beneficial for wasps. This study will be further developed in other cities to identify generalisable trends to inform better conservation practices for wasps in urbanised landscapes.

How do fungal mutualists affect social behaviour of a facultatively eusocial ambrosia beetle?

R

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The fruit-tree pinhole borer, *Xyleborinus saxesenii* Ratzeburg (Coleoptera: Curculionidae, Scolytinae), is among the most widespread ambrosia beetle in temperate environments. It is well studied because it is a rare example of an insect exhibiting fungiculture and social behaviour; comparable to attine ants and macrotermitine termites. Two nutritional fungal mutualists, *Dryadomyces sulphureus* Procter & de Beer and *Raffaelea canadensis* Batra, develop inside *X. saxesenii*'s nests within wood and there is evidence that these fungi have different ecological niches. Having two fungal mutualists may facilitate wood degradation, prolong the longevity of nests and thus beetles generational overlap, which may have profound effects on social behaviour. However, so far, very limited studies have been conducted on this subject. To fill this gap, we created monosymbiotic beetle lines and tested the effects of individual fungi on the social investment of beetle offspring. Here we shall discuss our results in the light of social evolution and mutualism theory.

Impacts of *Scaphoideus titanus* disturbance vibrational signal on grapevine defence mechanisms

R

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A new environmental-friendly approach, called vibrational mating disruption, is under development against the leafhopper *Scaphoideus titanus* on grapevine. This method involves the transmission to plants of a disturbance vibrational signal to interfere with the mating communication of the insects. Plants react to mechanical stimuli and can induce defense mechanisms. For example, substrate-borne vibrations emitted during chewing by caterpillars of *Pieris rapae* can induce defense responses in *Arabidopsis thaliana*. This study aims to analyze the possible side-effects of the disturbance vibrational signal on defense mechanisms and physiological processes of grapevine plants. The disturbance vibrational signal was transmitted to potted grapevine plants (variety: Pinot Noir) for 53 days without interruption under controlled conditions and plants of the same cultivar were left untreated as control. To simulate the field setting of disturbance noise transmission, treated plants were in contact with a scaled trellis system, where metallic wires were vibrated using a shaker. During the treatment, stomatal conductance, chlorophyll content, photosynthetic activity, and growth parameters were monitored. Leaf samples were collected at four time points (1 day, 16 days, 28 days, and 50 days) and RNA was extracted from leaves of treated and control plants. Following reverse transcription, the expression analysis of defense-related genes was carried out by quantitative real-time PCR. The results will provide better insights on plant responses to substrate-borne vibrations and side-effects of the disturbance vibrational signal on grapevine plants.

Assessing attraction of lures and dispensers on the efficiency of *Halyomorpha halys* trapping

R

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The brown marmorated stink bug, *Halyomorpha halys* (Stål), is a worldwide pest native to East Asia, with a wide host range that includes many cultivated plant species, leading to significant economic losses on a global scale. In order to tentatively contain its spread, different approaches have been used, including IPM (Integrated Pest Management) strategies. Monitoring pest populations is the starting point for developing an appropriate control strategy. The most commonly used cue to attract *H. halys* is its two-component aggregation pheromone (10,11-epoxy-1-bisabolen-3-ol, also called Murgantiol) in combination with the synergist methyl (2E,4E,6Z)-2,4,6-decatrienoate (MDT). The aim of this research is to evaluate the most effective combination of Murgantiol and MDT with different types of adjuvants on three types of dispensers (blister pack, wax tablets and ecoflex polymer). Field trials were conducted during summer-autumn 2023 and 2024. The effectiveness of the different combinations was evaluated on the basis of the number of *H. halys* individuals captured by the sticky traps and those actively collected in the surrounding vegetation. The most efficient combination will be considered to improve mass trapping of *H. halys* and to develop a future push-pull strategy.

Sustainable *Varroa destructor* management influences honeybee viral dynamics
R

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Viral infections and *Varroa destructor* infestations are major health threats to honeybee (*Apis mellifera* L.). Key viruses include the acute bee paralysis virus complex (ABPV-IAPV-KBV), deformed wing virus (DWV-A/B/C), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), and sacbrood virus (SBV). Severity of virus infections is influenced by viral load, strain virulence, and coexisting stressors, particularly *V. destructor*, which acts as a vector for viral transmission. Applying oxalic acid during the broodless phase is a sustainable method for controlling *Varroa* and limiting secondary viral infections. Treatments are applied in summer following artificial brood interruption and in winter, when brood is naturally absent. However, climate change has led to the persistence of brood, and thus *Varroa* and viruses, in northern Italy and southern France. This study aims to compare the efficacies of artificial brood interruption in autumn followed by oxalic acid treatment and oxalic acid treatment alone as long-term sustainable strategies for *Varroa* and viral infection control in honeybee colonies during winter. While managing the colonies according to the two strategies viral loads in 140 colonies across both countries were quantified by qRT-PCR over three seasons. Additionally, selected viral samples underwent sequencing to characterize strains. Preliminary results suggest brood interruption reduces colonies coinfected by two or more viruses and the absence of KBV and DWV-C in infected colonies. This research could deepen our understanding of virus ecology in honeybee colonies, providing insights for developing seasonally adaptive strategies to manage *Varroa* and reduce viral infections through sustainable means.

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A transposable element potentially involved in ecological speciation

R

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Endoparasitoid wasps lay their eggs and develop at larval stages inside a specific host. Recent studies have shown that endoparasitoid wasp species often constitute a complex of sister species, each one resulting from the adaptation to a particular host. This project aims to determine whether a transposable element (TE), mobile DNA sequence, is involved in the ongoing speciation of two populations of the parasitoid wasp, *C.congregata* (CcC and MsT), that specialize on different hosts and often occur in sympatry. The CcC population parasitizes the caterpillar *Ceratomia catalpae* which lives on the catalpa tree, and MsT population parasitizes the caterpillar *Manduca sexta* which lives on tobacco. Recent analysis indicates these two host races are beginning a speciation process. Interestingly, a reproductive defect is observed which could contribute to reinforcing barriers between the two populations. The cross between CcC females and MsT males gives a fertile offspring while the reciprocal cross (MsT females X CcC males) gives a nearly sterile offspring showing ovarian atrophy. We hypothesize that this phenotype corresponds to hybrid dysgenesis previously described in *Drosophila*, which would be induced by a TE present and active in CcC and not in MsT wasps. Hence, the TE could contribute to speciation by limiting genetic exchanges between the two populations.
Here, we present results identifying TE candidate potentially involved in dysgenesis. We study TE repression in the head compared to repression in the gonads. Finally, we characterize and compare the piRNA production regions of the two wasp populations.

α-Taxonomy identification and morphs of relevant Aphrophoridae from Palestine

R

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Species in the worldwide distributed Family Aphrophoridae (Amyot & Audinet-Serville, 1843), spittlebugs, play a vector role of *Xylella fastidiosa,* and the interplay among the plant pathogen with the diseasing plants poses a significant threat to agriculture worldwide.
Spittlebugs may display genetically inherited color patterns, leading to specimen misidentification, name confusion, and unnecessary entity description. Artifacts due to greasy individuals in collections are also pretty confounding, requiring oil removal and cleaning to evaluate the collected. We scrutinized male genitalia to identify genera or species to propose the α-taxonomy identification and morph recognition for relevant Aphrophoridae from Palestine (West Bank territory), where *Xylella fastidiosa* is not found.
The material was conventionally swept and card-glued. A detergent-soap mixture cleared the genitalia, and PVA mounted the parts in thick slides. Our findings add some knowledge to candidate Xf vectors in the area.

Effects of Insecticide Application on the Gut Microbiome of *Scaphoideus titanus*: Shifts in Endosymbiont Composition and Diversity

S

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Flavescence Dorée (FD) is a severe grapevine disease transmitted by *Scaphoideus titanus*, whose management relies heavily on insecticide use. However, insecticides can alter the gut microbiome of the vector, potentially influencing resistance mechanisms. This study analyzes the microbiome composition of *S. titanus* juveniles collected from two vineyards located in Piedmont (northwestern Italy) in the summer of 2023, before and after insecticide exposure with flupyradifurone or acetamiprid, respectively. High-throughput sequencing of the V3-V4 regions of the 16S rRNA gene revealed changes in the microbiome composition, with increased relative frequencies of two endosymbionts, namely *Candidatus* Sulcia*,* and *Candidatus* Cardinium, in samples after insecticide application. Alpha diversity analysis showed a significant shift in the samples from the vineyard treated with acetamiprid. Quantitative PCR (qPCR) confirmed a substantial rise in these endosymbionts post-treatment in these insects, while no significant changes were observed in samples coming from the vineyard treated with flupyradifurone. Our findings highlight an interaction between insecticide exposure and gut microbiome composition in *S. titanus*, suggesting a potential role of endosymbionts in supporting insect capability to overcome xenobiotic-related stress.

Keywords: Flavescence Dorée, *Scaphoideus titanus*, Insecticide, Gut microbiome, Endosymbionts, *Candidatus Sulcia*, *Candidatus Cardinium*, metabarcoding, qPCR analysis

Reverse chemical ecology targeting insect odorant receptors: new avenues for pest control

SENIOR LECTURE

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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, leading to specific behaviors. Interfering with OR functioning thus appears as a promising solution to disturb pest insect behavior. 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. These approaches have the potential to accelerate the discovery of new OR agonists/antagonists. As a proof-of-concept, we targeted ORs from a crop pest moth, the cotton leafworm. Both ligand-based and sequence-based virtual screenings 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 the insect odor space, and iii) the development of novel behavioural disruptors for pest insect control.

Sublethal effects of Prosystemin biologically active repeat motifs on *Spodoptera littoralis* larvae

R

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Prosystemin is a 200-amino acid precursor expressed in Solanaceae plants, that releases, in response to wounding and herbivore attack, a peptide hormone called Systemin. Recent studies have shown that Prosystemin is an intrinsically disordered protein, containing disordered regions within its sequence that have biological functions beyond those of Systemin. Prosystemin harbors multiple biologically active repeat motifs (RMs), which trigger immune responses in plants against pathogens and pest insects. In this study, we investigated the sublethal effects of treating tomato plants with the "G1" peptide, the most active Prosystemin RM, on 4th instar larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae). Although no differences were observed in the survival rates of larvae fed on G1-treated plant leaves compared to those fed on control plant leaves, there was a significant lethal effect during pupation, with the emergence of deformed pupae unable to develop into adults. Among the few adults that successfully emerged, a notable alteration in sex ratio was observed. We demonstrated that these developmental alterations were the result of disrupted normal functioning of the prothoracic glands and altered expression of genes encoding Insulin-Like Precursor Polypeptides in brain tissues, both of which are involved in pupation. Our findings highlight a pronounced effect of the G1 peptide on the development of *S. littoralis* larvae, suggesting its potential use in pest control.

Research on saproxylic beetles, from community to single species studies: a case study in a Spanish National Park

R

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The current climatic crisis is causing disordered and unpredictable variations of the natural environment, with implications even in protected areas. Nevertheless, some of these effects are slow to develop, modify the ecosystem gradually, and thus result to be hardly detectable at first glance, even if leading to significant effects. Saproxylic beetles is a highly diversified group, both in terms of species and played ecological roles; due to the dependence on deadwood and ancient trees, many species are considered worthy of protection or at extinction risk. Therefore, a deeper knowledge of such taxa, as well as population dynamics across space and times is needed, by adopting both a "community" and "single-species" approach.

This given, we present the results from a study aimed at assessing potential shift in abundance, morphology and phenology over time in a saproxylic beetles inhabiting the Cabañeros National Park in Spain, by comparing data collected in two one-year sampling campaigns (2009-2010 and 2021-2022). Results showed changes in species abundance and body size for ca. 20%. Moreover, also the phenological pattern of some species differs between the two sampling years, as it can be observed also at community level. These results suggest a shift in several biological traits within the saproxylic beetle community and have produced important insight for estimating the impact of ongoing climatic changes to the biological communities.

Seminal fluid proteome and female post-mating responses in the black soldier fly *Hermetia illucens*

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Sperm competition occurs when the sperm of multiple males compete to fertilize the same oocytes. Winning sperm competition requires more than just a high number of gametes; the composition of a male's seminal fluid is also crucial. The latter comprises a cocktail of molecules that affect an individual's fitness. More specifically, seminal fluid proteins (SFPs) have been the focus of numerous studies due to the post-mating responses (PMRs) they trigger in females, including stimulation of egg-laying and a reduction of sexual receptivity. We investigated the seminal fluid proteome and the associated post-mating responses in the black soldier fly (BSF), a newly farmed species of growing economic importance. Through injections of organ extracts—testes, male accessory glands, and seminal vesicles—in virgin females, we observed an induction of egg-laying and a decrease in sexual receptivity. Additionally, we identified the organs containing the seminal fluid components responsible for these effects. We described the first BSF seminal proteome which consists of 827 proteins. Using the results of the injections and the proteomes of the male reproductive tract, we identified 6 SFPs which are certainly involved in PMRs. Among these were two SFPs from the serine protease family, which is known to play a crucial role in the PMRs of *D. melanogaster*. This first description of the seminal proteome in the black soldier fly highlights the various SFPs that may be involved in post-copulatory sexual selection and lays the foundation for future studies on sexual selection and sexual conflict in the black soldier fly.

Evaluation of the growth performance of BSF larvae on a polyethylene terephthalate (PET)-contaminated substrate

R

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The larvae of the black soldier fly (BSF), *Hermetia illucens*, have been recognized as one of the most promising candidates for insect-mediated bioconversion of organic waste into valuable biomass. Unfortunately, organic biomass, side streams, and by-products derived from food processing, agrifood chain, or municipal solid waste can exhibit high contamination of microplastics (MPs), which could hinder the bioconversion process. Nevertheless, the effects of MPs exposure on BSF larvae performance are scarcely known. To fill this gap of knowledge, BSF larvae were reared on the Organic Fraction of Municipal Solid Waste (OFMSW) spiked with different quantities of polyethylene terephthalate (PET) microparticles (4 and 20% w/w), and the impact of this contamination on larval growth, pupal survival, and adult emergence rate, as well as on gut physiology and morphology, were investigated.

PET exposure did not alter the larval growth performance, although 20% PET contamination demonstrated to interfere with the bioconversion efficiency. Moreover, a morphological analysis of the midgut revealed the integrity of the gut epithelium and associated peritrophic matrix, despite MPs transit. The functionality of this organ was confirmed by endo- and exopeptidase activities, which showed values comparable to controls. Finally, the analysis of oxidative stress and detoxification markers (i.e., Reactive Oxygen Species, Glutathione S-transferase, and Catalase) on the midgut, fat body, and Malpighian tubules, showed no variation between treatments and control, too.

The micro-PET contamination of the OFMSW demonstrated not to negatively affect the growth performance and physiology of BSF larvae, expanding our knowledge on the potential of insect-mediated valorisation of challenging substrates.

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Investigation of olfactory cues for host-location in European ecto-parasitoids *Sclerodermus cereicollis* and *Sclerodermus domesticus* (Hymenoptera: Bethylidae)

R

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Sclerodermus domesticus is a cosmopolitan ectoparasitoid of longhorn beetle larvae, also developing on other woodboring species in the family Ptinidae. *Sclerodermus cereicollis*, first described by Kieffer from specimens in Annobón Island (Equatorial Guinea) and Giglio Island (Italy), has been poorly studied in terms of taxonomy and biology. Investigating the chemical ecology of these species is essential for improving their effectiveness as biological control agents and understanding their role in human sting dermatitis. Bioassays in Y-tube olfactometer, still air olfactometer and open arena have been performed. Females of both species, in agreement with the typical behaviour of generalist parasitoids, revealed to be able to perceive and respond to different volatile chemicals produced by the host habitat (seasoned-wood sawdust from pine and beech) and host frass of the two longhorn beetles *Hylotrupes bajulus* and *Trichoferus holosericeus*. In addition, they can perceive non-volatile chemicals from the host cuticle (cuticular hydrocarbons of the two longhorn beetles). The two species revealed numerous similarities and some differences in their behaviour. Such differences highlight the importance of clarifying the chemical ecology of the different species of the genus *Sclerodermus* a taxon in which the taxonomy and biology of the different species are still unclear.

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Impact of pest control management of phytophagous and predatory mites in hazelnut orchards in Central Italy

R

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The big bud mite, *Phytoptus avellanae* Nalepa (Acari: Phytoptidae), is responsible for severe infestations of both vegetative and reproductive hazelnut buds, causing hypertrophic alterations in the tissues and the formation of pseudo-galls (big buds), which result in decreased productivity. Despite the damage it causes in various hazelnut-growing regions, several aspects of the biology and management of this pest still require further investigation and improvement.

Monitoring activities using different strategies were conducted in 2023 and 2024 in the Viterbo province (Lazio Region, Italy). During winter, the infestation level was assessed by counting big buds, both in a hazelnut variety collection to evaluate variations in susceptibility among the world's main cultivars and in hazelnut orchards managed under IPM, organic, and renaturalized practices (where cultivation ceased thirty years ago) to assess the impact of phytosanitary management on the mite. Additionally, during the vegetative season, in the same orchards with different management practices, the presence of vagrant forms of *Phytoptus avellanae* and predatory mites (Acari: Phytoseiidae) was evaluated.

The results of this study showed significant differences in susceptibility among cultivars. In the renaturalized sites, both big buds and phytophagous mites were less abundant, while predatory mites, as expected, were more abundant compared to other managed orchards.

This study provides valuable insights into how current agronomic practices influence pest infestations and serves as a foundation for future research on one of the primary pests affecting hazelnut cultivation.

Lifestyle switches in entomopathogenic fungi – from pest control to plant benefit and beyond

SENIOR LECTURE

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Fungi evolved more than 900 million years ago, but unprecedented radiation and diversification occurred ~480 million years ago due to interactions between fungi and terrestrial plants. This created a wide range of lifestyles, including fungi that functional primarily as saprophytes and saprobes in the soil (necessary for nutrient recycling and soil health) as well as pathogens and parasites that leech nutrients from other organisms. One of the most fascinating adaptable is the widespread entomopathogenic fungi, which can proliferate as independent saprophytes, plant-associated endophytes, or pathogens infecting arthropod hosts, switching between lifestyles according to need. These fungi are beneficial to plants at multiple levels including pest and disease reduction, plant induced resistance and growth stimulation and are therefore interesting both as model organisms and for their potential applications in agriculture. In agriculture, entomopathogenic fungi (EPF) are important players in integrated pest management aimed at minimizing the use of chemical pesticides that are harmful to both humans and the environment. The fungus *Metarhizium brunneum* has evolved a remarkable ability to switch between different lifestyles. It develops as a saprophyte, an endophyte establishing mutualistic relationships with plants, or a parasite, enabling its use for the control of insect pests of various taxonomical orders. In recent years we have been studying the interaction of *M. brunneum* with its environment at various levels. We tested our hypothesis that switches between lifestyles must be accompanied by fundamental transcriptional reprogramming, reflecting adaptations to different environmental settings.

Effects of plant-mediated soil microorganisms on the olfactory response of insect natural enemies in tomato

R

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Tomato crops are constantly threatened by numerous arthropod pests. Root-associated microorganisms, such as Plant Growth Promoting Fungi (PGPF) and Rhizobacteria (PGPR), are used to enhance plant defenses. However, the role of volatiles emitted by microbial-inoculated tomatoes on the olfactory response of biocontrol agents of tomato pests is often unknown. The olfactory responses of three natural enemies (*Cryptolaemus montrouzieri*, *Eretmocerus eremicus* and *Nesidiocoris tenuis*) of tomato key pests to volatiles emitted by tomato plants previously inoculated with PGPF and PGPR were assessed in a two-way olfactometer in laboratory conditions. Three fungal commercial strains (*Trichoderma asperellum*, *T. harzianum* and *Beauveria bassiana* ATCC 7404), and six bacterial species (commercial strains of *Bacillus subtilis* and *B. amyloliquefaciens*, and laboratory isolates of *B. spizizenii*, *Pseudomonas fluorescens*, *P. veronii* and *P. gessardii*) were tested 3 and 7 days after inoculation in comparison to untreated plants. In dual choice tests, *C. montrouzieri* showed significant attraction towards tomato plants inoculated with *B. spizizenii* and *T. asperellum*, 3 and 7 days after inoculation, respectively. By contrast, *C. montrouzieri* females were generally repelled by tomato emitted volatiles when inoculated with other microbials. A similar trend was observed with *E. eremicus*. Tomato plants inoculated with *B. bassiana* and *B. subtilis* significantly repelled *N. tenuis* both 3 and 7 days after treatment. This study represents pioneering research into how root-associated microorganisms influence tritrophic interactions in tomato crops system. Our initial findings suggest that future research into pest-natural enemy interactions may improve biocontrol strategies using biostimulants for Integrated Pest Management.

First report of *Leptoconops noei* in Tuscany inland and new morphological findings

S

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The genus *Leptoconops* (Diptera: Ceratopogonidae) is a taxon of blood-feeder biting-midges, with unknown vectorial role. In Italy the distribution is limited to non-urbanized coastal marshes and river mouths, with five species described. We investigated the presence of *Leptoconops* species in the land-locked hilly of Siena district, where these biting-midges had never been reported. We collected over 300 specimens of *Leptoconops* in 13 sites in July during the three years 2022-2024. To identify the species using taxonomical keys, the samples have been pictured with a high-precision digital stereomicroscope and with a scanning electron microscopy. Furthermore, we sequenced mitochondrial gene COI. As comparison, we conducted the same analysis with specimens collected during previously sampling campaigns in Grosseto district, where *Leptoconops* genus is well established and studied. All Siena specimens have been identified as *Leptoconops noei* (CLASTRIER&COLUZZI 1973) at morphological level. Molecular data and phylogenetic analysis confirmed the morphological identification. At SEM observation, Siena *L. noei* lacks the bulb-shaped sensilla in the palpal pit. These supposed olfactory organs have been descripted in every Italian *Leptoconops* species so far. For the first time, we attested the presence of *L. noei* in a new Italian district, with different geographical features, and described new morphological characteristics.

Humic substances-based nanoparticles as a novel nano-delivery system for an RNAi-mediated control strategy against *Spodoptera littoralis*

R

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To limit the use of chemical insecticides and develop bioinspired control strategies for phytophagous insects, some bioactive molecules have been already identified by exploiting insect-natural antagonist interactions. These include a target-specific dsRNA that, when orally delivered, is able to silence the immune gene *Sl102* (ds*Sl102*) in *Spodoptera littoralis* larvae, inducing immunosuppression and increasing the insect susceptibility to *Bacillus thuringiensis* toxins. Due to its extreme sensitivity to environmental degradation, ds*Sl102* must be protected by sustainable formulations when sprayed in the field. These formulations must have specific characteristics, such as (i) preserving the integrity of ds*Sl102* and increasing shelf-life and storage stability; (ii) enabling rapid and efficient penetration of ds*Sl102* within plant tissues, which are ingested by the chewing insect; (iii) ensuring the release of ds*Sl102* inside the insect's gut lumen.

In this context, the use of sustainable materials to produce nano-delivery systems can be potentially beneficial. Three formulations composed of plant-based materials and with different physical and chemical characteristics are presently under development and analysis.

Here we report the use of new nanocarriers based on humic substances derived from the degradation of vegetable and coffee husks, conjugated with chitosan (HS-Chi NPs). These NPs can encapsulate ds*Sl102* with an efficiency of over 90% and preserve ds*Sl102* from environmental degradation for up to 12 months. To study their fate within plants, we applied fluorescent-labelled HS-Chi NPs to tomato plants *via* foliar spraying. All the administrated HS-Chi NPs penetrate the cuticle of leaves within 3 hours and remain stable without degradation in the apoplast of the upper epidermal layer for at least 96 hours. Notably, the HS-Chi NPs did not alter the photosynthetic efficiency of treated plants indicating their compatibility with plant physiology. In addition, while 3rd instar *S. littoralis* larvae fed with tomato leaves sprayed with naked ds*Sl102* did not show any gene silencing in the haemocytes, those fed with leaves treated with ds*Sl102* encapsulated within HS-Chi NPs showed a significant reduction in *Sl102* gene expression, demonstrating the RNAi efficacy of the HS-Chi NPs-*dsSl102* complex. Overall, our results strongly suggest the potential use of HS-Chi NPs as efficient biocompatible carrier for the delivery and protection of silencing dsRNA molecules in the field. The proposed nano-delivery strategy might be profitably used also for other bioactive molecules, such as peptides and proteins.

The power of plants: The power of plants: insect manipulation in nectar-mediated plant-animal interactions

SENIOR LECTURE

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The essential role of nectar in mediating plant-pollinator relationships has been known since the 18th century. Until the last decade, nectar was considered a mediator of a perfect mutualism: a plant food reward for pollinators in exchange for the pollination service of an animal, generally an insect. Over the last ten years, our knowledge of the biology of nectar has increased to such an extent that today this classical vision is completely outdated. According to the most recent evidence, nectar can now be considered a plant interface for complex interactions with other organisms. In particular, the presentation will focus on the nectar-mediated partner manipulation hypothesis which has recently been proposed and which has already received numerous inputs from the latest research on nectar biology.

A humanistic approach to entomology: tracing the imprint of the past on modern challenges

S

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This study adopts a humanistic approach to entomology, exploring the historical and contemporary roles of insects in human practices and global challenges. It begins by examining entomophagy, highlighting how ancient cultures regarded insects as a valuable food resource, a perspective that now offers innovative, sustainable solutions to todays's food crises. The focus then shifts to the importance of entomological collections, which not only preserve the historical record of biodiversity but also provide essential data for analyzing the effects of climate change on ecosystems and species. Lastly, the study delves into the pioneering work of Maria Sibylla Merian, one of the first naturalists to investigate insects from both scientific and artistic perspectives, anticipating modern ecosystem studies. Through this overview, the study illustrates how historical insights can suggest solutions to contemporary challenges, highlighting the continued importance of insects in both science and society.

Sublethal Effects of Agricultural Chemicals on Pollinators: Insights from the POLYPOLL Project

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This research is part of the POLYPOLL project (https://site.unibo.it/polypoll/it), which investigates the sublethal chronic effects on pollinators of chemical pollutants with extensive use in agriculture. Although not designed to target pollinators, these substances, present at residual doses, can cause unpredicted interactions and adverse effects on non-target organisms. Accordingly, our objective was to assess the sublethal impacts of two fungicides (boscalid and copper chloride), as well as the veterinary drug ivermectin and the herbicide glyphosate, on pollinators. It is established that boscalid residues can impair the ability of pollinators to fly, to form and recall memories, to learn, to recognise their nests, and to maintain a healthy microbiota. Similarly, copper compounds have been shown to affect the activity of antioxidant enzymes. Ivermectin has been demonstrated to cause physiological disruptions, while glyphosate has been shown to impair physiological parameters. The present research examines the effects of sublethal doses of four chemicals on the survival of two bee species (*Osmia bicornis* and *Apis mellifera*) in laboratory trials. To simulate natural conditions, *Osmia* bees were tested individually, whereas honeybees were tested in groups. The doses were provided in syrup solution. Preliminary results showed that the combination of copper chloride and Boscalid significantly reduced *Osmia bicornis* survival, while in honeybees susceptibility was influenced by the family of origin. For both *Osmia bicornis* and *Apis mellifera*, ivermectin and ivermectin-glyphosate mixture, showed the greatest reduction in the period of survival.
These findings highlight the potential risks posed by sublethal doses of agricultural chemicals to both solitary and social pollinators.

Identification and Characterization of Membrane Transport Proteins in *Buchnera aphidicola*: Insights into Nutrient Exchange in Aphid Symbiosis

R

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Sap-feeding Hemipterans form partnerships with microbes to acquire essential nutrients for survival. One well-known example is the symbiosis between aphids and *Buchnera aphidicola*, an obligatory intracellular bacterium. *Buchnera* provides aphids with essential amino acids, allowing them to survive on the nutrient-poor phloem sap. These bacteria reside within specialized cells called bacteriocytes and have significantly reduced genomes compared to free-living bacteria. Despite this reduction, *Buchnera* retains a limited number of membrane transporters, likely crucial for maintaining the symbiotic relationship by exchanging nutrients like amino acids and metabolites between the bacterium and its host. This project aims to identify and characterize key membrane transport proteins in *Buchnera* and evaluate their role in nutrient sharing. By using *Escherichia coli* as an expression system and performing structural and functional analyses, along with computational modelling, we seek to understand the mechanisms of these transporters. Unravelling the structure, function, and regulation of these proteins is essential for comprehending how this symbiotic association is maintained and its broader implications for both *Buchnera* and the aphid.

Behavioural response of *Philaenus spumarius* to different compounds from essential oils

R

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The meadow spittlebug, *Philaenus spumarius* L. (Hemiptera: Auchenorrhyncha: Aphrophoridae), is the primary vector of *Xylella fastidiosa* subsp. *pauca* ST53, the pathogen responsible for Olive Quick Decline Syndrome (OQDS) in southern Italy. These insects feed on the xylem sap of olive plants using piercing-sucking mouthparts. During feeding they can acquire *Xylella fastidiosa* from the xylem vessels, promoting the spread of the disease. Nowadays, reducing the vector population is one of the most effective strategies to control the disease. Therefore, understanding the behaviour of *Philaenus spumarius* is crucial for developing targeted control measures. Essential oils are a potential source of bioactive volatile compounds that can disrupt insect physiology and behaviour. In this study, we evaluated the behavioural responses (repellence, attractive) of male and female *P. spumarius* to the most active components of *Lavandula officinalis* and *Rosmarinus officinalis* essential oils, as identified through gaschromatography coupled with mass spectrometry and electroantennography (GC-MS-EAD). Our preliminary results offer insights into the promising products for implementing sustainable strategies to reduce *P. spumarius* populations in the field.

Pollination networks in urban areas: a pathway to sustainable and biodiverse cities

R

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In recent decades, biodiversity has declined globally due to the significant impact of human activities. However, large cities still offer refuges for pollinators, making it essential from a One-Health and urban sustainability perspective. Understanding plant-pollinator interactions is crucial for evaluating pollination ecosystem services and creating green spaces that support ecosystems and biodiversity. My research focuses on analyzing the ecological components of pollination services across urban landscapes, as part of Spoke 5 “Urban Biodiversity” of the NBFC. To achieve this, monitoring has been conducted in six major Italian cities along an urbanization gradient, measuring the diversity and abundance of wild bees, flower visitation, and pollen transport. Integrative techniques and automated methodologies are employed to identify insect pollinators and their interactions. To evaluate the pollination ecosystem service, the pollen load of wild bees was quantified and correlated with the individuals' morphological and biological characteristics. Additionally, plant-pollinator networks were constructed to compare interactions along the urbanization gradient. This research aims to guide actions that enhance biodiversity, including selecting appropriate plant species and installing nests. The study highlights the connection between the environment and ecosystem services, offering valuable insights for developing policies that manage food and nesting resources for pollinators, ultimately supporting a healthier and more sustainable urban environment.

Developing innovative digital solutions for the automatic detection and discrimination of *Drosophila suzukii* and *Drosophila melanogaster*

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In an effort to develop a sustainable and integrated pest management (IPM) system aimed at reducing the use of synthetic insecticides, a novel digital optical sensor and its embedded algorithm are currently being tested to detect *Drosophila suzukii* in both lab and field conditions. This detection is achieved by analyzing the frequency of the adult wingbeats. The machine learning model is designed to distinguish *Drosophila suzukii*, the target species, from the closely related *Drosophila melanogaster*, as these species often co-occur together in infested orchards. Lab-training of the algorithm is currently underway, where environmental (temperature, photoperiod etc.) and physiological factors (species, sex, age etc.) are being established during subsequent training sections. The optical sensor, referred to as Lantern© (Irideon SL, Spain), is integrated into a specially designed cage to facilitate fly activity while minimizing sensor interference from repeated flights and shadowing, all with the aim of maximizing accurate frequency recordings. The attraction gradient within the trap, using light and attractants, has been optimized to further improve the detection stream. Moreover, the software used as test-bench has been adapted to incorporate specific classes relevant to this study. The first results suggest that using an incorporation box for the sensor is ideal as it provides a swarm flow gradient. However, multiple flights by individual flies remain a challenge for accurate detection. This work is supported by the project "Sustainable pest management through online monitoring and artificial intelligence for key pests of the South Tyrolean Agriculture (INSTINCT)" funded by the Fondo Europeo di Sviluppo Regionale (FESR) DRAFT-1029.

Exploring *Hermetia illucens* larval gut microbiota for innovative plastics biodegradation strategies

R

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Plastics are widely employed worldwide, and the accumulation of mismanaged plastics in the environment represents a global concern, as their physicochemical characteristics make them exceptionally persistent and pervasive. Indeed, microplastics and nanoplastics that results from their fragmentation represent emerging contaminants in soil, in water and even in food.

Extensive research efforts have focused on identifying microorganisms capable of degrading plastics polymers to implement novel approaches for plastics waste biodegradation and valorisation. Several plastics-degrading bacterial species have been isolated from disparate sources, and in recent years microorganisms inhabiting the alimentary canal of some insect species have shown to possess the capability to degrade plastics. Among promising insects, *Hermetia illucens* (Black Solder Fly, BSF) larvae (BSFL) displayed the ability to effectively degrade two types of plastics polymers (i.e., polyethylene and polystyrene) thanks to their gut bacterial community. Our research aims to unveil the potential of BSFL as a bioincubator for bacteria able to degrade PET, one of the most widely used plastics in food packaging. To this purpose we reared BSFL on a PET-based diet and i) monitored growth and development, ii) studied BSFL physiological responses to oxidative stress and damage triggered by PET exposure, and iii) characterized the composition of the microbial community in different gut regions.

Both BSFL and its associated gut microbiota significantly responded to PET exposure. These results indicate that BSFL can be considered a promising source for the isolation of PET-degrading microbial strains as well as PET-degrading enzymes, to widen the horizons of BSF biotechnological applications.

Using X-ray tomography to study ambrosia beetles

R

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In recent years, ambrosia beetles (Coleoptera; Curculionidae; Scolytinae) have been recognized as one of the most successful invasive species worldwide, primarily facilitated by the movement of wood-packaging materials through international trade. Their symbiotic trophic specialization with fungi, haplodiploid reproductive system, and sibling-mating behavior, enable small founder populations and facilitate their rapid establishment and spreading in non-native environments. The interest in ambrosia beetles has significantly increased in recent years; however, their diminutive size and cryptic lifestyle pose a critical challenge for research on these insects. Previous investigations have primarily employed manual dissection of infested wood samples, which provided limited quantitative data on gallery development. X-ray tomography, already used to study other insect taxa, facilitates the reconstruction of virtual models of the wood samples while preserving the integrity of the galleries. In a first study, we used this technique to investigate whether the colonization success of ambrosia beetles change among different plant species subjected to simulated flooding stress. In a second study, we investigated the occurrence and frequency of intra- and inter-specific gallery merging as a potential pathway for the exchange of symbiotic fungi between these insects. In both cases, we showed that X-ray tomography can provide novel insights into the ecology, biology and impact of ambrosia beetles.

Testing chitosan-based Fosetyl-Al nanocrystals in *Meloidogyne javanica* control

S

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Plant-parasitic nematodes pose a significant threat to global plant health, with root-knot nematodes, particularly *Meloidogyne* spp., causing severe yield losses in horticultural and field crops. Chemical nematicides, traditionally used for control, are now restricted due to their harmful environmental and health effects. This has driven the search for eco-friendly alternatives, leading to increased interest in innovative solutions such as nanotechnology. For this reason, we conducted a study to evaluate the efficacy of a novel Fosetyl-Al formulation in controlling *Meloidogyne javanica*. *In vitro* experiments were performed using chitosan-based Fosetyl-Al nanocrystals (CH-nanoFos), synthesized through a sonication-assisted method employing chitosan as a coating agent. Preliminary experiments established optimal CH-nanoFos concentrations for testing against *M. javanica*. Nematicidal, nematostatic, and hatching inhibition effects were assessed at concentrations of 1, 2, and 4 mg/ml over different exposure times. Results revealed that nematicidal activity increased with higher concentrations and longer exposure, while nematostatic activity decreased. Hatching inhibition was also concentration- and time-dependent, with hatching decreasing with both concentration and time.

The study also investigated the effects of CH-nanoFos on mature tomato plants and seeds germinated for up to 14 days, focusing on germination percentage, seedling growth, genotoxic effects, and the content of polyphenols, flavonoids, and photosynthetic pigments. Ongoing TEM observations are assessing CH-nanoFos uptake and translocation within plant tissues, while *in vivo* experiments are evaluating its efficacy against *M. javanica* in tomato plants.

Histological alterations in honeybee gut suggest caution in using sewage sludge as soil improver in agriculture

S

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Agriculture poses many threats to insect pollinators, including the use of synthetic products to improve crops. Recently, policies have been implemented to reduce the use of synthetic products and promote recycling, e.g. the use of sewage sludge as fertiliser. However, sludges can contain harmful substances such as trace elements. In agricultural environments, bees can be exposed to heavy metals through feeding. Their toxicity to bees is known but there is little information on the possible effects on their digestive system. We therefore investigated, using histology techniques, the effects of trace elements in sewage sludge on the gut epithelium of *A. mellifera* workers through bee sampling at agricultural areas treated or not with this soil improver. Soil levels of known toxic elements were also quantified. We hypothesised that bees collected in treated areas would show more alterations to the midgut epithelium. Soil levels significantly differed between the two areas, with higher concentration of Cr and Ni in sewage areas. Individuals from treated areas, showed alterations of the midgut epithelium as well as a high density of vacuoles containing spherites, a defence mechanism to isolate toxic metals. Semi-quantitative analysis revealed higher vacuolization and overall gut damage in bees from treated areas. This suggests that exposure to heavy metals represents a source of stress for the gut epithelium of bees and may impair its digestive functions, with a consequent impact on their health. This study lays the foundations for further research on the effects of apparently "eco-friendly" chemical treatments in agriculture on bees.

Characterization of the responses mounted by *Aedes albopictus* larvae exposed to the entomopathogen *Bacillus thuringiensis*

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Climate change and globalization have contributed to the spread of vector insects from tropical regions to temperate climates. Among these, the Asian tiger mosquito (*Aedes albopictus*) has rapidly expanded in the Mediterranean area, posing a threat due to the variety of viruses it can transmit to humans. While the immune system of adult mosquitoes has been extensively studied, little is known about the larvae's immune response. However, studying larval immunity is crucial not only for understanding how these aquatic organisms interact with the microorganisms and parasites in their environment but also for improving larval control using immune-suppressive strategies to enhance entomopathogen effectiveness. To explore these issues, we conducted transcriptomic analyses on mosquito larvae exposed to different doses of a *Bacillus thuringiensis var. israelensis* (*Bti*)-based bioinsecticide. Our results allowed the identification of key immune genes and pathways involved in humoral and cellular immune responses to *Bti* exposure, at both gut and systemic levels. Notably, we characterized the *Aea102* gene in *Ae. albopictus*, which plays a pivotal role in the larvae's immune defense against *Bti*, as found in lepidopteran species exposed to a specific *Bt* strain. This study provides a picture of genes and pathways involved in the immune defenses of mosquito larvae for the first time. Moreover, gaining insight into the responses mounted by *Ae. albopictus* larvae exposed to *Bti* offers useful information for improving the effectiveness of *Bti*-based insecticides, e.g., by co-administration with molecules affecting larvae responses to this bioinsecticide.

A regional plan for the management of the alien hornet *Vespa velutina* (HYMENOPTERA: VESPIDAE) in Tuscany

R

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Biological invasions are one of the leading causes of biodiversity loss all over the world. Here we present the regional plan adopted in Tuscany, in implementation of current national and European legislation, for the management of the alien invasive hornet *Vespa velutina nigrithorax* (Lepeletier, 1836). *V. velutina* has been accidentally introduced in Europe from South-East Asia in 2004. Since then, it has colonized numerous European countries and, from 2012, Italy, with the first reports in Tuscany in 2017. The presence of *V. velutina* on the European territory has generated particular concern because of its impact on the beekeeping sector, as it massively preys on honeybees; on biodiversity, as it also preys on other arthropods; and on public health, as it poses a risk to allergic people. Eradicating invasive social wasps and hornets is not an easy task and, as it is no longer an option, it is necessary to develop management projects aimed at limiting their spread and impact. Such management plan was implemented in the Tuscany region thanks to state funds allocated for invasive alien species of Union interest. The regional management plan (2023-2025) is being carried out thanks to the joint effort of universities and beekeeping associations. It involves a monitoring network of 368 stations spread over the entire regional territory and aims to contain the expansion of *V. velutina* through the neutralization of its nests. This contribution will present the regional plan aims, methods and first results, as well as it will raise awareness about this ongoing biological invasion.

Tiger mosquito, *Aedes albopictus*, larval salivary glands characterization

S

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Mosquito salivary glands (SGs) play a major role in the pathogen transmission. Despite knowledge about vector SGs is constantly expanding, larval SGs remain largely unexplored especially in *Aedes* mosquitoes. This study aims to better understand the structure, secretions, and gene expression of *Aedes albopictus* larval SGs.

We focused on the secretome of larval SGs using MS approach. Fourth-instar larvae were dissected to collect SGs, and the extracted proteins were analysed with a mass spectrometer. To further investigate previous mentioned results, the tissue specificity of the genes encoding the aforesaid proteins was determined.

Additionally, to overcome the gap in morphologic data about the *Aedes* mosquitoes larval SGs we provided in this work images, obtained via light microscopy, and 3D models basing on tomography obtained with X-ray microscopy; the tissue ultrastructure will be analysed via transmission electron microscopy (TEM).

Regarding morphological characterization, the different imaging techniques exploited in this work lead to new insight into the current knowledge of the larval salivary glands of *Aedes albopictus*. Furthermore, mass spectrometry-based characterization of larval salivary gland proteome led to the identification of about thirty proteins. According to their function, in mosquito larval salivary glands immune related proteins, enzymes involved in digestion or detoxification, transporters and others can be found.

Enhanced knowledge of larval SGs can provide insights into mosquito biology and identify potential targets for disrupting SG function, potentially leading to innovative vector control strategies.

The egg parasitoid *Ooctonus vulgatus* (Hymenoptera: Mymaridae) in Northwestern Italy

R

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Philaenus spumarius L. (Hemiptera: Aphrophoridae) is the main vector of *Xylella fastidiosa* in Europe. The management of vector population has a key role in contrasting the spread of the bacterium, and it is currently based on tillage and insecticide application. However, new sustainable control strategies are needed to cope with *X. fastidiosa* epidemic. Exploitation of egg parasitoids as biological control agents of *P. spumarius* is a promising tool that, in the context of IPM, could reduce vector abundance. The egg parasitoid *Ooctonus vulgatus* Haliday (Hymenoptera: Mymaridae) was recently reported associated to *P. spumarius* in Corsica, with parasitisation rate up to 69%, making it an interesting putative biocontrol agent. The aim of this work – still ongoing – is to evaluate the prevalence of *O. vulgatus* in Northwestern Italy and study its biological traits. The prevalence of parasitoid has been evaluated for three years using the sentinel egg technique. *Philaenus spumarius* egg batches were exposed in field conditions in eight sites from September to January, using different setups. About 700 egg parasitoids emerged in the first two years (third-year trial is ongoing), all identified as *O. vulgatus*. Parasitisation rate was very variable (0.5-66%), with an average of 21.7 ± 7.6%. This work provides methods for collecting the parasitoid species, together with preliminary data on its presence, prevalence, and biological traits. Further studies are needed to evaluate the possible of exploitation of *O. vulgatus* in biological control programmes against the *X. fastidiosa* vector.

Wolbachia: endosymbiotic bacteria of insects and their potential use for sustainable pest control

S

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Wolbachia are endosymbiotic bacteria that have gained considerable attention for its potential in pest control strategies. Found naturally in insects and other arthropod species, t*hese maternally inherited endosymbionts* can manipulate host reproductive mechanisms, such as cytoplasmic incompatibility (CI), parthenogenesis, and male-killing, with strong fitness effects to their hosts which make it a valuable tool for control pest insects. We focus on investigating the effects of *Wolbachia* on Tephritid fruit flies, which are important agricultural pests causing considerable economic losses in agriculture worldwide. The symbiotic relationship between *Wolbachia* and these insects offers the potential to suppress pest populations and reduce crop damage. In particular, *Wolbachia* induced CI could lead to reduced fertility in wild populations, thereby lowering their reproductive potential and thus suppressing the pest population. Here we provide insight into *Wolbachia* and their relationship with Tephritid fruit flies and discuss how it can be used to control these pests.

Monitoring of pollinators supported by GIS: territorial classification and ecological restoration based on bee and hoverfly diversity

R

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Climate change has been shown to significantly impact ecosystems, disturbing habitat communities and leading to biodiversity loss. This PhD project aims to investigate the effects of climate change on pollinator communities using two complementary approaches. The first approach focuses on the effects of rising temperatures and vegetation changes in a site located in the Bolognese Apennines, where historical biodiversity data are available. This peculiar habitat, although situated in the Mediterranean region, exhibits a climate more typical of northern Europe. Sampling activities in this area, ongoing since the late 1950s, have revealed the complete disappearance of species from the *Eumerus* genus, a group of Syrphid flies with phytophagous larvae. Additionally, satellite and climate data indicate landscape changes and significant temperature increases over the decades, which may have contributed to shifts in the hoverfly community. The second approach utilizes data from sampling efforts in a national park (Val Grande National Park, Piedmont). By analysing satellite imagery and ecological variables, this research aims to identify areas within the park that are more favourable for pollinator communities. The goal is to create an initial framework for mapping the park's pollinator-friendly zones. Preliminary results have already shown differences across the four sampling sites. The outcomes of this research will enhance our understanding of pollinator population dynamics and provide insights into potential future scenarios under climate change. Additionally, the findings may support the development and implementation of targeted conservation measures.