



European PhD Network "Insect Science"
XIV Annual Meeting

8 – 10 November 2023

PROGRAMME
&
BOOK OF ABSTRACTS

CREA – Centro di Ricerca per la Difesa e la Certificazione, Firenze

Organizing Secretariat Event Planet Srl Contact: Marina Morra - marina.morra@eventplanet.it

European PhD Network "Insect Science" - XIV Annual Meeting

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PROGRAMME

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Wednesday 8 November 2023

[ZOOM LINK](#)

- 14:00 **Registration**
- 14:25 **Welcome address**
- 14:30 **Senior scientist lecture:** [Francesca Romana Dani](#) - University of Firenze
Chemical communication in social insects
- 15:00 - 16:30 **Oral Presentations** (regular & short talks)
Chair: TBD onsite
- 15:00 - 15:15 **R** [Andrea Arpellino](#) - University of Torino
**Olfactive preference of *Drosophila suzukii* toward different strains of viticultural-
enological yeasts, from laboratory to vineyard trials**
- 15:15 - 15:30 **R** [Laura Besana](#) - University of Padova
Exploring the effect of visual cues to improve longhorn beetle catches in traps
- 15:30 - 15:45 **R** [Sara Boschi](#) - University of Siena
**Molecular tools to study historic and recent pathways of entry and spread for the invasive
Japanese beetle**
- 15:45 - 16:00 **R** [Aurora Bozzini](#) - University of Padova
**Early detection of *Ips typographus* infestation symptoms using multispectral images
acquired by drone**
- 16:00 - 16:15 **R** [Juan Carlos Cambronero-Heinrichs](#) - University of Padova
**Defensive and nutritional role of Erwiniaceae bacteria in two widespread ambrosia
beetles**
- 16:15 - 16:30 **R** [Sara Caramella](#) - Insubria University
Dietary Protein and Carbohydrate affect immune functions in *Hermetia illucens*
- 16:30 **Coffee break**
- 16:50 - 18:25 **Oral Presentations** (regular & short talks)
Chair: TBD onsite
- 16:50 - 16:55 **S** [Giacomo Ortis](#) - University of Verona
Detection of *Scaphoideus titanus* using electronic and sticky traps
- 16:55 - 17:10 **R** [Eleonora Cresta](#) - Tuscia University
**Monitoring of ambrosia and bark beetles species (Coleoptera: Curculionidae, Scolytinae
and Platypodinae) in beech forests with different management: a preliminary study**
- 17:10 - 17:25 **R** [Claudio Cucini](#) - University of Siena
**De novo assembly and annotation of the genome of *Popillia japonica* with initial clues to
its potential as an invasive pest**
- 17:25 - 17:40 **R** [Giovanni Dal Zotto](#) - University of Verona
**Efficacy of different mulching materials in preventing *Popillia japonica* oviposition in
potted plants within the nursery chain**

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

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- 17:40 - 17:55 **R** Flavia de Benedetta - University of Napoli "Federico II"
Characterization of "Cimiciato" damage and improvement of the management of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in hazelnut orchards
- 17:55 - 18:10 **R** Luca Deganutti - University of Padova
Winter survival in *Ips typographus* populations in relation to temperature and elevation in the South-Eastern Alps
- 18:10 - 18:25 **R** Matteo Dho - University of Torino
Development of a novel MLST approach for understanding the genetic variability of *Halyomorpha halys* and *Candidatus Pantoea carbekii*

Thursday 9 November 2023

[ZOOM LINK](#)

- 09:00 **Senior scientist lecture:** David Giron - IRBI - University of Tours (FR)
Unravelling the plant-insect interactions through the lens of microbial partners
- 09:30 - 11:00 **Oral Presentations** (regular & short talks)
Chair: TBD onsite
- 09:30 - 09:45 **R** Nicolò Di Sora - Tuscia University
Control of *Toumeyella parvicornis*: predation evidence by *Exochomus quadripustulatus* and *Cryptolaemus montrouzieri*
- 09:45 - 10:00 **R** Ilaria D'Isita - University of Foggia
Susceptibility of different *Triticum* spp. genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) and olfactory responses of adult beetles to kernel VOCs
- 10:00 - 10:05 **S** Aya Elsayed - University of Torino
Insect gut microbiome as a resource to improve insect mass rearing
- 10:05 - 10:20 **R** Martina Falagiarda - University of Torino
Stink bugs and their egg parasitoids in South Tyrol: distribution and diversity
- 10:20 - 10:25 **S** Marco Falasco - University of Padova
Effects of biodiversity of agricultural ecosystems on the insect's microbiota
- 10:25 - 10:40 **R** Priscilla Farina - University of Pisa
***Notonecta maculata*: more than just a model predator of mosquito larvae**
- 10:40 - 10:55 **R** Eleonora Vittoria Fontana - University of Torino
Ambrosia beetle monitoring on *Castanea sativa* Miller
- 10:55 - 11:00 **S** Lorenzo Fortini - Roma Tre University
Wild bees of Rome: biodiversity and pollination network in the natural reserves of the city
- 11:00 **Coffee Break**
- 11:20 - 13:00 **Oral Presentations** (regular & short talks)
Chair: TBD onsite
- 11:20 - 11:35 **R** Rebecca Funari - University of Siena
Whole genome resequencing reveals origins and worldwide invasion pathways of the

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Japanese beetle *Popillia japonica*

- 11:35 - 11:50 **R** [Elena Gazzea](#) - University of Padova
Effectiveness of forest restoration for the conservation of insect pollinators in agricultural landscapes
- 11:50 - 12:05 **R** [Vito Antonio Giannuzzi](#) - University of Perugia
Variables affecting infestation of the olive fruit fly, *Bactrocera oleae*, in Umbria
- 12:05 - 12:20 **R** [Camille Heisserer](#) - IRBI, Tours, France
Role of transposable elements and bracovirus in a parasitoid wasp ongoing speciation
- 12:20 - 12:25 **S** [Erica Holzer](#) - University of Milan
How the environment changes inside hives in relation to the material of construction: effects on temperature variability and honeybee biological parameters
- 12:25 - 12:30 **S** [Giovanni Iadarola](#) - University of Foggia
Identification and evaluation of the biological activity of semiochemicals for the monitoring and control of olive moth, *Prays oleae* (Bernard), and cotton bollworm, *Helicoverpa armigera* (Hübner)
- 12:30 - 12:45 **R** [Giovanni Jesu](#) - University of Napoli "Federico II"
Endophytic colonization of *Bacillus thuringiensis* on tomato reduces *Spodoptera littoralis* survival
- 12:45 - 13:00 **R** [Lucia Lenzi](#) - Università di Bologna
Evaluation of pollination deficit of *Pyrus communis* L. in Italy
- 13:00 **Lunch**
- 14:30 **Senior scientist lecture:** [Kahraman Ipedkal](#) - Hacettepe University, Ankara, TR
Hybridization in the pine processionary moth species complex: what we know and what we don't
- 15:00 - 16:05 **Oral Presentations** (regular & short talks)
Chair: TBD onsite
- 15:00 - 15:15 **R** [Giulia Magoga](#) - University of Napoli "Federico II"
Ecological and biological drivers of bacterial communities' diversity in the leaf beetles species (Coleoptera, Chrysomelidae)
- 15:15 - 15:30 **R** [Serena Malabusini](#) - University of Milan
Scaling-up the rearing system of *Sclerodermus brevicornis* (Hymenoptera, Bethyilidae) for behavioural studies
- 15:30 - 15:35 **S** [Paolo Masini](#) - University of Perugia
Natural enemies of xylophagous insect pests: host selection, morphology and behaviour
- 15:35 - 15:50 **R** [Aurora Montali](#) - Insubria University
Effects of the oral administration of *Trichoderma asperellum* chitinases and *Bacillus thuringiensis* on Lepidoptera
- 15:50 - 16:05 **R** [Agata Morelli](#) - University of Bologna
Drivers of pollinator assemblages in urban contexts: the effects of local-scale and landscape factors on pollinators
- 16:05 **Break**

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- 16:30 - 17:50 **Oral Presentations** (regular & short talks)
Chair: TBD onsite
- 16:30 - 16:45 **R** Elia Russo - University of Napoli "Federico II"
Host aphid immunosuppression by *Aphidius ervi* venom
- 16:45 - 17:00 **R** Giuseppe Morgante - University of Padova
Physiological, molecular, and behavioural investigations on *Ips typographus* vision (Coleoptera, Scolytinae)
- 17:00 - 17:15 **R** Livia Maria Negrini Ferreira - University of Catania
Foraging behavior of eusocial bee colonies (Hymenoptera, Apidae) towards food contaminated with pesticides
- 17:15 - 17:30 **R** Ahmed Oraby - University of Torino
Survey for '*Candidatus Liberibacter*' spp. and relative putative vectors in north-western Italy
- 17:30 - 17:35 **S** Francesca Casale - Roma Tre University
Diversity and distribution of Ixodids from Latium: data for a regional Atlas
- 17:35 - 17:50 **O** Chloe Start - University of Newcastle, UK
Developing novel RNAi-based biopesticides targeting the central nervous system of *Spodoptera littoralis*

Friday 10 November 2023

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- 09:00 **Senior scientist lecture:** Donato Grasso - University of Parma
Supercooperators: the biology of ant mutualisms from basic science to applications
- 09:30 - 11:55 **Oral Presentations** (regular & short talks)
Chair: TBD onsite
- 09:30 - 09:45 **R** Cecilia Parise - University of Torino
Exploring RNA interference (RNAi) for the control of hemipteran vectors of plant pathogens
- 09:45 - 10:00 **R** Laura Pasquier - IRBI, Tours, France
Do social behaviours reflect local adaptation in the European earwig?
- 10:00 - 10:15 **R** Onofrio Marco Pistillo - University of Foggia
Identification of the sex pheromone of the asparagus moth, *Parahypopta caestrum*: chemical, electrophysiological, and field investigations
- 10:15 - 10:20 **S** Adriana Poccia - University of Perugia
Efficacy of natural products and dusts on *Halyomorpha halys*
- 10:20 - 10:25 **S** Valeria Rossi - University of Perugia
Evaluation of lure-dispenser combinations for *Halyomorpha halys* traps in Italy
- 10:25 - 10:40 **R** Valerio Saitta - University of Perugia
Arrestment behavior of the egg parasitoid *Trissolcus basalus* towards the secretion of the tarsal gland of *Nezara viridula*

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- 10:40 - 10:55 **R** Giacomo Santoiemma - University of Padova
Host preference and chemical ecology of *Saperda tridentata*: preliminary results
- 10:55 - 11:10 **R** Evgenia Sarakatsani - University of Palermo
Nectar-inhabiting bacteria affect the performance of biological control agents
- 11:10 - 11:25 **R** Nachida Tadrent - IRBI, Tours, France
Exploring functional evolution of microbial communities in termites with comparative metagenomics: insight into diazotrophy
- 11:25 - 11:40 **O** Robert Calvert - University of Newcastle, UK
The Development of Novel Biopesticides Derived from the Venom of Insect Antagonists
- 11:40 - 11:55 **O** Gaetan Seimandi-Corda - Rothamsted Research, Harpenden, UK
Identification of the predators of oilseed rape pests using camera traps
- 11:55 - 12:05 **Discussion on PhD education and future careers and meeting closure**
- 12:05 **Brunch**

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REGULAR & SHORT PRESENTATIONS



ABSTRACTS

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

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Olfactive preference of *Drosophila suzukii* toward different strains of viticultural- enological yeasts, from laboratory to vineyard trials



A. Arpellino

University of Torino - Department of Agricultural, Forest and Food Sciences, Italy

Drosophila suzukii is a generalist species, introduced in Europe in 2008 from Asia. Female flies have a serrated ovipositor, which allows the deposition in berries before ripening, mainly soft red fruits. Damage is both direct, due to larval feeding on the grape, and indirect, following exposure of the grapes to rot. To avoid chemical residues in wine, the available management strategies are very limited and mostly rely on physical barriers, together with mass trapping and biological control. The aim of this work was to test the potential of several yeasts as attractants for *D. suzukii* females to develop a more selective and efficient mass trapping method. To avoid unwanted side effects on grape processing due to a yeast spillover from the traps, yeast strains were selected among non-*Saccharomyces* strains that are commonly used in enology or as biocontrol agents. Multi-choice trials and two-way olfactometer bioassays were performed to choose the most attractive yeast. The most attractive strain was *Starmerella bacillaris* FC54, which was then used to establish the most suitable bait composition, testing different concentrations of their components. Afterwards, the yeast-enriched baits were compared with the standard ones, testing their selectivity and attraction for *D. suzukii* in vineyard. Preliminary results of vineyard trial confirmed the attraction of the yeast-enriched bait for *D. suzukii*, additionally they suggest an increased selectivity with respect to other drosophilids, opening to the potential use of the selected strain as a bait additive in traps, both for monitoring purposes and for mass trapping.

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Exploring the effect of visual cues to improve longhorn beetle catches in traps



L. Besana¹, G. Cavaletto¹, G. Santoiemma¹, J. Spaethe², D. Rassati¹

¹University of Padova - Department of Agronomy Food Natural Resources Animals and Environment, Italy; ²Department of Behavioral Physiology & Sociobiology, Biozentrum, University of Würzburg, Germany

Longhorn beetles (Coleoptera, Cerambycidae) are among the most significant groups of invasive forest insects worldwide. Hidden within live plants and wood-packaging materials, these beetles can escape routine inspections at entry points and become major pests in the invaded environment. Traps baited with pheromones and kairomones and set up in and around entry points are commonly used to improve chances of intercepting exotic species soon after their arrival. Several factors known to influence catches have been extensively investigated over the years, such as lures, trap height, or trap position in the habitat. However, the role of visual cues in longhorn beetle traps is still unclear. In a trapping study carried out in 2023 at three forest sites in Veneto, northern Italy, we investigated the response of three target longhorn beetle species (i.e., *Xylotrechus antilope*, *Xylotrechus stebbingi*, *Neoclytus acuminatus*) and a number of non-target species to baited cross-vane traps characterized by five different visual stimuli. These stimuli combine black with three different yellow hues found in the elytral patterns of the target species. The five different traps correspond to: i) full black; ii) full yellow; iii) yellow dots on black background; iv) larger than life size yellow elytral pattern on black background; v) larger than life size pictures of the whole target beetle on black background.

Preliminary results clearly demonstrated a species-specific response of both the target and non-target longhorn beetles to the different visual stimuli, indicating that integrating them in baited traps can strongly improve trap efficacy.

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Molecular tools to study historic and recent pathways of entry and spread for the invasive Japanese beetle



S. Boschi, C. Cucini, R. Funari, F. Frati, A. Carapelli, F. Nardi

University of Siena, Department of Life Science, Italy

Popillia japonica Newman (Coleoptera: Scarabaeidae) is a beetle endemic to Japan unintentionally introduced in North America (1916), the Azores Islands (~1970), northern Italy (2014) and Swiss Ticino (2017). Since *P. japonica* is a pest of a wide range of cultivated and wild plants, including economically important crops, it is listed by EPPO as an A2 quarantine organism and by EFSA as a potential EU quarantine pest. Invasions of this species in different parts of the world have raised concerns and there is a need to better understand population dynamics and invasion modes. First, a genomic analysis was performed by *de novo* assembly of the entire beetle genome using Illumina NovaSeq and MinION technologies, resulting in a 578 Mb genome with an N50 value of 0.89 Mb. This new genome was then used as a reference for population genetic sequence remapping. The whole genomes of 86 individuals were sequenced to cover the distribution of the species, and the mitochondrial DNA data were used for population genetic analyses. These analyses revealed a significant split between southern and central/northern Japan, with all invasive samples coming from the latter. The USA invasive samples showed evidence of multiple introductions from different Japanese lineages, whereas the two European invasive populations are likely the result of separate invasions with minimal local differentiation, both ultimately derived from the USA population. Demographic assessments reveal a population expansion followed by a contraction phase before the invasive spread. This scenario agrees with previous findings and introduces valuable temporal and demographic dimensions to existing reconstructions.

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Early detection of *Ips typographus* infestation symptoms using multispectral images acquired by drone



A. Bozzini, M. Faccoli, A. Battisti

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

Increasing frequency and intensity of extreme weather events due to climate change determine greater stress to European conifer forests and create conditions suitable for pests' infestations. Since 2019, the south-eastern Alpine populations of the Eurasian spruce bark beetle (*Ips typographus* L.) shifted from an endemic to an epidemic phase, elicited by the dramatic windthrow occurred at the end of 2018 (Vaia). The successful management of outbreaks faces difficulty in detecting the infested trees at their early stages. Remote sensing techniques (such as time-series analysis of multispectral imagery acquired by satellites, airplanes, or drones) have a great potential to obtain useful information about damage due to bark beetles' infestations. Our study tested a method to detect the early symptoms of the infestations, employing multispectral images acquired by a drone to determine the optimal indices and the best timing for the early detection. An attack was induced on individual spruce trees in ten experimental sites, using an aggregation pheromone. Then, the trees were monitored once every two weeks for three months, to observe the development of the bark beetles under the bark while collecting multispectral images by drone. Two vegetation indices, NDRE (Normalized Difference Red Edge index) and SAVI (Soil Adjust Vegetation Index, with a correction factor of 0.44) allowed recognising the infested trees at least one month before the appearance of visible symptoms, *i.e.*, the colour change of the tree crowns. This early-detection tool could allow the automatic diagnosis of the bark beetles' infestations and provide a useful guidance for the management of areas suffering pest outbreaks.

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The Development of Novel Biopesticides Derived from the Venom of Insect Antagonists



R. Calvert, M. Edwards, A. M. R. Gatehouse

Newcastle University – School of Natural and Environmental Science

Over the past century the use of pesticides has changed agricultural practices, resulting in substantially increased yields without increasing the agricultural footprint. However, subsequent over-reliance on chemical pesticides is causing an increased prevalence of resistance in insect pests, challenging future global food security. This drives the need to develop novel and sustainable methods of pest insect control. Arthropods, such as spiders and parasitic wasps, remain a largely untapped resource of insecticidal compounds, compromising insect immune defences and causing paralysis by blocking insect ion channels. Such compounds could be highly effective if developed for use in agriculture. This study involves the expression of two previously identified insecticidal proteins, *PhVPr1* derived from the venom of the parasitoid wasp *Pimpla hypochondriaca* and *TnBVank1* derived from the bracovirus associated with the parasitoid wasp *Toxoneuron nigriceps*. Recombinant *PhVPr1* and *TnBVank1* have been expressed in the microbial expression system *Pichia pastoris* and purified by His-tag affinity chromatography. We have demonstrated that *PhVPr1* is able to enhance the efficacy of the entomopathogen, *Bacillus thuringiensis* against the cotton leafworm *Spodoptera littoralis*. In addition, this work demonstrates the oral toxicity of *TnBVank1* in aphids, *Myzus persicae* and *Sitobian avenae*.

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Defensive and nutritional role of Erwiniaceae bacteria in two widespread ambrosia beetles



J.C. Cambronero-Heinrichs¹, A. Battisti¹, P. Biedermann², G. Cavaletto¹, V. Castro-Gutierrez³, L. Favaro¹, G. Santoiemma¹, D. Rassati¹

¹Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università, 16, 35020, Legnaro (PD), Italy; ²Chair for Forest Entomology and Protection, University of Freiburg, Stegen, Germany; ³Center for Research on Environmental Pollution (CICA), University of Costa Rica, Montes de Oca, 11501, Costa Rica

Ambrosia beetles are fungal-growing insects excavating galleries deep inside the wood. Their success as invaders increased scientific interest towards them. However, most studies on their microbiota targeted their fungal associates whereas the role of bacterial associates is understudied. To explore the role of abundant microbial associates, we isolated bacteria from active galleries of two widespread ambrosia beetles, *Xylosandrus crassiusculus* and *X. germanus*. These isolates were classified within the Erwiniaceae family and through a phylogenetic analysis including isolates from other insects we showed that they clustered with isolates obtained from ambrosia and bark beetles, including *Erwinia typographi*. The whole genome analysis of the isolate from active galleries of *X. crassiusculus* suggested how this bacterium play both a nutritional role, by providing essential amino acids and enzymes for the hydrolysis of plant biomass, and a defensive role, by producing antibiotics. This defensive role was also tested *in vitro* against fungi, including mutualists, common associates, and parasites. The bacteria inhibited the growth of common associates and parasites but did not affect mutualists. Our study supported the hypothesis of a mutualist role of Erwiniaceae bacteria in ambrosia beetles and highlighted the importance of bacteria in maintaining the symbiosis with their nutritional fungi.

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Dietary Protein and Carbohydrate affect immune functions in *Hermetia illucens*



S. Caramella¹, D. Bruno¹, A. Montali¹, M. Casartelli^{2,3}, G. Tettamanti^{1,3}

¹University of Insubria - Department of Biotechnology and Life Sciences, Varese, Italy; ²University of Milan - Department of Bioscience, Milan, Italy; ³University of Napoli Federico II - Interuniversity Center for Studies on Bioinspired Agro-environmental Technology (BAT Center), Portici, Italy

Hermetia illucens (Diptera: Stratiomyidae), also known as Black Soldier Fly (BSF), is a saprophagous insect that can be used as a source of protein and lipids for producing innovative high nutritional value animal feeds. The larvae of this dipteran are able to grow on decaying organic waste, and therefore they are exposed to various microorganisms that may affect their health and development. In order to produce safer BSF-derived raw materials and biomolecules, it is important to improve the performance of the immune system by modifying nutritional parameters, such as the protein:carbohydrate (P:C) ratio.

The aim of this project is to evaluate the effect of a protein-rich and of a carbohydrate-rich diet on the immune response after challenging BSF larvae with a bacterial mix of *Escherichia coli* and *Micrococcus luteus*. Different markers, representative of the cellular and humoral branch of the immune system, were analyzed (i.e., number of circulating hemocytes, phagocytosis and encapsulation activity, lysozyme activity, proPO system activity, antimicrobial activity, and expression of antimicrobial peptides (AMPs)). The data collected showed a variation in the activity especially of cellular markers, a stronger antimicrobial activity against bacteria, and a higher expression of AMPs in larvae reared on the protein-rich diet, highlighting the capacity of the rearing substrate to influence the immune response.

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Diversity and distribution of Ixodids from Latium: data for a regional Atlas



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¹University of Studi Roma Tre – Department of Science, Italy; ²Istituto Superiore di Sanità – Department of Infectious Diseases, Italy

Ticks are obligate hematophagous ectoparasites feeding on vertebrate hosts, that are widely distributed around the World, especially in warm and humid habitats. From an ecological standpoint, ticks can influence a wide range of ecological parameters therefore playing a crucial role in population dynamics. To date, research regarding the ticks feeding on wildlife in Italy is scarce, especially concerning species with little or no medical or veterinary relevance. Aim of this three-year project is improving knowledge concerning tick species in Latium, Italy, for the creation of an Atlas of diversity, distribution and host range. In this study we present the data collected during the first two years of sampling, from May to September in 2022 and 2023. 23 Natural Reserves sited in Lazio and 3 CRAS (Centro Recupero Fauna Selvatica) were visited, and ticks were collected both from the field and directly on wildlife. Field sampling was carried out using a white wool blanket both on herbaceous vegetation (“*dragging method*”) and shrub vegetation (“*flagging method*”), collected tick specimen were put in 70% Ethanol. For the collection of specimens for this project, in addition to the sampling activity, we collaborated with the staff from the natural parks, CRAS, veterinary doctors, local farmers and hunters. Ticks were identified to species using dichotomous keys. In total of 400 specimens were collected (208 in 2022 and 192 in 2023), belonging to 5 genera, and 9 species: *Dermacentor marginatus*, *Hyalomma detritum*, *Hy. marginatum*, *Haemaphysalis punctata*, *Hae. Inermis*, *Rhipicephalus bursa*, *Rh. sanguineus*, *Rh. turanicus*, *Ixodes Ricinus*.

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Monitoring of ambrosia and bark beetles species (Coleoptera: Curculionidae, Scolytinae and Platypodinae) in beech forests with different management: a preliminary study



E. Cresta^{1,2}, M. Contarini¹, N. Di Sora¹, L. Rossini³, S. Speranza^{1,4}

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The recent introduction of new alien ambrosia beetles (AB) species (Coleoptera: Curculionidae, Scolytinae and Platypodinae) in the Mediterranean basin is currently considered an overriding concern. This is mainly given by their ability to target a wide range of tree species of agriculture and forest interest, affecting their distribution and abundance. This preliminary study is included in a National Biodiversity Future Center PhD project, which aims to explore the effects of non-native AB on ecosystems dominated by *Fagus sylvatica* L. in Italian Protected Areas (PAs), and to assess the efficiency of PAs zoning strategies in controlling invasive AB. The present study aimed to determine the species composition of bark and AB in two beech forests (BF) within the Mount Terminillo area (central Apennines) having different management, and to evaluate the efficiency of a high degree of naturalness BF in managing alien AB species, compared to one with lower naturalness. In June 2023, 18 multifunnel traps were placed in two sites, an old-growth BF and an old coppice BF. Traps were inspected every fortnight for samples collection and ethanol lure replacement. *Xylosandrus germanus* (Hoffman), was the only alien AB species found at both sites. Furthermore, the results show greater diversity and more even distribution of individuals among the various species in the community in old-growth BF, where *X. germanus* was captured in significantly lower numbers than in old coppice BF. Additional research will be carried out to investigate the relationship between *X. germanus* population density and the degree of naturalness in BF.

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De novo assembly and annotation of the genome of *Popillia japonica* with initial clues to its potential as an invasive pest



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The recent spread of *Popillia japonica* in non-native areas (USA, Canada, the Azores islands, Italy and Switzerland) poses a significant threat to agriculture and horticulture, as well as to endemic floral biodiversity, entailing that appropriate control measures must be taken to reduce its density and limit its further spread. In this context, the availability of a high-quality genomic sequence for the species is liable to foster basic research on the ecology and evolution of the species, as well as on possible biotechnologically-oriented and genetically-informed measures of control. The genomic sequence presented and described here improves over the available draft sequence in terms of completeness and contiguity, and includes structural and functional annotations. A comparative analysis of gene families of interest, related to the species ecology and potential for polyphagy and adaptability, revealed a contraction of gustatory receptor genes and a paralogous expansion of some subgroups/subfamilies of odorant receptors, ionotropic receptors and cytochrome P450s. The new genomic sequence as well as the comparative analyses data may provide a clue to explain the staggering invasive potential of the species and may serve to identify targets for potential biotechnological applications aimed at its control.

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Efficacy of different mulching materials in preventing *Popillia japonica* oviposition in potted plants within the nursery chain



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The Japanese beetle, *Popillia japonica* has been threatening Italy since it was first detected in the Ticino Valley in 2014. Being a quarantine organism, the movement of plants from infested areas is regulated and, in the nurseries, the absence of the pest must be guaranteed. In the potted plant's soil, the presence of eggs and larvae of *P. japonica* represents the main worry concerning the spread of the pest through trading.

This work aims to evaluate the efficacy of different mulching materials in preventing egg laying in potted plants in nursery production.

To assess *P. japonica* oviposition behaviour, both no-choice and choice tests were performed using potted winegrape plants mulched with different covering materials. Beetles were allowed to lay eggs and feed on the plants for two weeks and, afterwards, the number of preimaginal stages was assessed through the visual inspection of the soil in each pot.

Heavier materials (gravel and fine lapilli) and coconut fiber discs exhibited the largest significant reduction in egg deposition.

This work underlines the importance of adopting a holistic approach to guarantee potted plants free from eggs of *P. japonica*. Successful integrated control strategies should include, next to mulching, the cultivation of plants under protected conditions during adult flight and the use of insect-free commercial growing substrates.

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Chemical communication in social insects

SENIOR LECTURE

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Social insects encompass species displaying diverse social organization regarding colony cycle, colony size, polymorphism of castes and reproductive differences between colony members. According to traditional definitions by Batra (1962), Eusociality is defined by overlapping generations of adults within a colony, the presence of reproductive and non-reproductive individuals and cooperative brood care. Within this broad definition, primitive eusocial insects show reduced and sometimes reversible differences between reproductive and non-reproductive individuals and often small colonies, while highly eusocial species show remarkable differences between castes and often large colonies. In both cases, colony homeostasis, including within-colony caste demography, colony integrity and labour organization largely depend on communication that mainly relies on chemical signals or cues. However, while chemical communication is the main medium of social integration in highly eusocial species, the social integration of primitively eusocial colonies also depends on direct physical interactions. The richness of exocrine glands in social insects provides a plethora of volatile and semivolatile compounds that may act as cues or signals in intraspecific communication and interspecific interactions.

In this lecture, we will go through aspects of colony organization in social Hymenoptera where chemical communication is pivotal, such as caste differentiation, maintenance of colony integrity and defence, labour organization and the role of chemical cues in the interactions between social parasites and their hosts.

Finally, we will see the other side of chemical communication, i.e. cues detection and the molecular bases of olfactory and pheromonal perception.

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Characterization of “Cimiciato” damage and improvement of the management of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in hazelnut orchards



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Over the last decade, *Halyomorpha halys* has emerged as a major threat to Italian hazelnut production. Stink bug trophic activity causes different types of damage depending on the fruit development stage (blank, shriveled, and cimiciato). The cimiciato defect has strong detrimental effects on the kernel organoleptic quality. The Ph.D. project aims to characterize the *H. halys* cimiciato defect and collect data to improve long-term pest management strategies for hazelnuts. Two field trials were carried out in the Campania region (southern Italy). The cultivars San Giovanni (SG) and Tonda Romana (TR), which ripen early and late, were evaluated in 2022, along with two pest management techniques [No insecticide (NI) and Integrated pest management (IPM)]. This study included phenological analysis of fruits as well as stink bug populations monitoring using traps and plant beatings. The second trial, which began in 2022 and is still in progress, involves four cultivars (SG, TR, Tonda di Giffoni, and Mortarella). Adult stink bugs were introduced into cages attached to plant branches at various stages of fruit development. The resulting hazelnuts were subjected to physical, chemical, and visual examinations. Visual examination of the hazelnuts produced in the cages reveals that cimiciato damage occurs primarily during the kernel expansion stages. Moreover, results showed that SG cv exhibited a higher incidence of cimiciato in the untreated plots than the late TR cv (40% SG-NI vs. 23% TR-NI). Understanding the relationship between hazelnut growth stages and the brown marmorated stink bug is critical for developing effective pest control strategies.

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Winter survival in *Ips typographus* populations in relation to temperature and elevation in the South-Eastern Alps



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The spruce bark beetle *Ips typographus* is considered the most destructive pest in European coniferous forests, and it generally attacks weakened Norway spruce trees. A big outbreak, still ongoing, started following the Vaia storm occurred in October 2018 in the Central-Eastern Italian Alps. Climate change is affecting phenology in the spruce bark beetle, with higher temperatures allowing faster development and earlier start of spring activity. Mild winter temperatures may increase survival rates in the overwintering generation. The diapause in the spruce bark beetle is induced by photoperiod in late summer. In central and southern Europe beetles overwinter in the bark, excavating towards the cork which is drier and more thermal insulating than the phloem. During diapause part of the population dies depending on winter temperatures, development stage of overwintering beetles, colonization densities, predation and pathogen infection. Winter survival affects the extent of infestations in the following year. The aim of this study was to assess survival rates in overwintering *Ips typographus* populations in relation to temperature, elevation and stage of development of the beetle. Results show a mortality rate of 35%. Lower number of larvae and immature beetles were found in fall, probably died during winter as less resistant to winter conditions. High densities under the bark and mild temperatures may have favored bacterial and fungal infections. Regarding sex ratio we observed a decrease in male individuals after overwintering, while elevation is not significantly affecting survival. The low reduction in the overwintering populations is favoring outbreaks in the following year.

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Development of a novel MLST approach for understanding the genetic variability of *Halyomorpha halys* and *Candidatus Pantoea carbekii*



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Halyomorpha halys is a phytophagous and polyphagous insect native to Eastern Asia, now widespread in many regions of the Northern Hemisphere. To reconstruct the invasion patterns, most of the studies dealt with sequencing COI and COII mitochondrial regions. In some works, also the pseudogene $\Delta YbgF$ of the primary endosymbiont *Candidatus Pantoea carbekii* was sequenced. A deeper analysis of the genetic variability of the insect-symbiont system could reveal undiscovered genetic variants. One of the aims of my PhD is to develop a new, fast and cost effective MLST approach, focusing on *H. halys* nucleus and increasing the number of *P. carbekii* markers. For *H. halys*, 3 novel nuclear molecular markers have been identified. Each one of these markers showed to be at least as informative as COI, with no redundant information with mitochondrial markers and among each other. Regarding *P. carbekii*, 3 molecular markers were chosen, but the information resulted redundant. Five populations, sampled in Italy and Turkey, have been analysed, whilst 2 Chinese and 1 American populations are under analysis. The application of MLST approach for *H. halys* led to the identification of 13 different haplotypes, whilst the use of only mitochondrial markers displayed just 5 haplotypes. Moreover, highly informative markers have been selected for *P. carbekii*, providing information on the phylogenetic separation of “holobiont haplotypes”. The implemented method could be a tool for population genetic studies, but also for correlating genetic variability to biological features of different populations, like invasiveness or different susceptibility to symbiotic control treatments.

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Control of *Toumeyella parvicornis*: predation evidence by *Exochomus quadripustulatus* and *Cryptolaemus montrouzieri*



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Toumeyella parvicornis (Cockerell, 1897), the pine tortoise scale, is an invasive pest for the stone pine (*Pinus pinea* L.) in Italy and France, where it is responsible for severe damages. The pest is enlarging its distribution in Europe and it is rapidly changing the landscape shape of mediterranean vegetation in urban and coastal areas. Recent advances in the knowledge on this scale insect, indicate that the currently applied endotherapeutic treatments do not offer a coverage on the long term period. This fact underlines the necessity to explore alternative methods to control this pest, among which there is classical biological control. During the past two years, we tested the activity of two Coccinellidae predators in controlling *T. parvicornis*. Notably, we focused on two ladybugs' species: *Exochomus quadripustulatus* (Linnaeus, 1758) and *Cryptolaemus montrouzieri* Mulsant, 1850. Different tests were carried out under laboratory conditions. We investigated the: *i*) longevity, *ii*) attraction and timing response of the ladybugs' species fed with the pest, *iii*) effect given by the presence of another predator on the prey attraction, and *iv*) predation rate based on different prey densities. Our results showed the attractiveness of *T. parvicornis* to the ladybugs, given that the predators responded positively to the different prey densities proposed, and with a high predation rate. These findings represent a crucial milestone to set complete field experimentation and to confirm the encouraging laboratory results we obtained.

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Susceptibility of different *Triticum* spp. genotypes to *Sitophilus granarius* (L.) and *Rhyzoperta dominica* (F.) and olfactory responses of adult beetles to kernel VOCs



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The susceptibility of different *Triticum* spp. genotypes to *Sitophilus granarius* (L.) and *Rhyzoperta dominica* (F.) and the olfactory responses of adult beetles to the blend of volatile organic compounds (VOCs) emitted by the different genotypes have been studied. In susceptibility tests, 3 old (Saragolla Antica, Dauno III, Senatore Cappelli) and 3 modern (Ofanto, Svevo, Faridur) *T. durum* varieties, 1 modern (Mec) *T. aestivum* variety, 3 pigmented genotypes (*T. aestivum*: Sebesta I, Sebesta III; *T. durum*: T1303), and 2 emmers (*T. dicoccum*: Padre Pio; *T. spelta*: Benedetto) were assessed by evaluating mortality percentage during the oviposition period, F1 progeny production, number of offspring female, and mean development period. The olfactory responses of *R. dominica* and *S. granarius* adults to VOCs emitted by intact kernels of old and modern wheat varieties were evaluated using two-choice pitfall bioassays and Y-tube olfactometer bioassays, respectively. Furthermore, VOCs emitted by old and modern varieties, extracted by head-space solid-phase micro-extraction (HD-SPME), were identified by gas chromatography coupled with mass spectrometry (GC-MS). For both species, different levels of susceptibility and attractiveness were found among genotypes tested. The GC-MS analysis of HS-SPME kernel extracts highlighted the presence of 27 VOCs with some quantitative and qualitative differences among varieties. The identified compounds mainly included alcohols, aldehydes and alkanes, and to a lesser extent, alkenes, terpenes, organic acids, benzene derivatives, lactones and ketones. The physical and chemical factors possibly involved in different levels of susceptibility and attractiveness are discussed.

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Insect gut microbiome as a resource to improve insect mass rearing

S

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Insects' diversity and evolutionary effectiveness have relied on their many interactions with beneficial microorganisms. Microbial communities are prevalent in the digestive system of insects assisting them in maintaining their various lifestyles. This study aims in optimizing protocols for microbiome sampling, preservation, and genetic characterization from insect gut of selected pest species that are reared to implement control strategies, such as biological control or sterile insect technique. The final objective is to identify microbial consortia that are crucial for insect health and can be redelivered to improve the rearing success. Target insects include the fruit fly *Drosophila suzukii*. Different preservation methods are being tested for the gut microbiome of a lab-reared *D. suzukii* population, by assessing the consistency of microbiome composition overtime through high-throughput sequencing. Additionally, the microbiome sequences of the lab-reared insects will be compared with those from insects that came from the field, emerging from different fruits. The microbiome typical of flies from the preferred fruits will be delivered to the lab-reared population, and the survival rate, length of pre-adult stages, and adult fecundity will be compared between insects fed on the microbe-enriched diet and others fed on the standard diet. Finally, larvae of the population fed with the microbe-enriched diet will be offered to reared parasitoids, to assess their performance on these hosts. The outcome of these experiments will support the creation of an ameliorated protocol for parasitoid rearing to implement biocontrol, also providing information about how to preserve and use insect gut microbiome as a diet additive.

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Stink bugs and their egg parasitoids in South Tyrol: distribution and diversity



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The presence of phytophagous stink bugs (Hemiptera: Pentatomoidea) in South Tyrol has increased in recent years. These species are polyphagous and feed on cultivated, wild and ornamental plants. Egg parasitoids can favor the regulation of stink bug populations by reaching in some cases high levels of parasitization. To better understand the abundance and distribution of stink bug species and their parasitoids, an extensive monitoring program was carried out in the last two years in 27 sites, different in terms of habitat type and altitude range.

During field surveys, more than 1200 stink bug adults and nymphs were recorded, while almost 300 egg masses were collected. Greater diversity and abundance of species were found in forests and urban areas compared to orchards. Species abundance varied with both altitude and habitat type, with more individuals found between 500 and 800 m a.s.l. and in urban areas. The two predominant species found were *Pentatoma rufipes* and *Halyomorpha halys*. The first one was more abundant at higher altitudes, while the second one was more present in the valleys, at lower altitudes. Egg masses were mainly found in urban areas, while only a few were collected in orchards. Eleven species of parasitoids emerged from the collected eggs. Parasitization rate was higher in forests and urban areas, where the abundance of individuals was also higher.

The results of this study add important information on the distribution of stink bugs and their parasitoids and can be exploited for planning effective and sustainable crop management in the area.

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Effects of biodiversity of agricultural ecosystems on the insect's microbiota

S

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The intestinal bacterial community of insects plays a crucial role in their behavior, longevity, fertility, and overall health by aiding digestion, detoxification, nutrient provision, and defense against parasites and pathogens. The microbiota can exert positive, negative, or neutral effects on insects and is influenced by environmental factors.

The current project, employing a multidisciplinary approach, aims to investigate the influence of landscape (floral biodiversity) and climate (temperature) on the composition of bacterial communities associated with insects using meta-barcoding techniques. This research will focus on model species, including pollinators and insects harmful to agriculture, such as *Bactrocera oleae*.

Analyzing the bacterial community allows us to identify microbial associations and their functional roles within the host. Examining the correlation between the microbiota of pollinators and environmental conditions, including environmental type (sampling from areas dominated by intensive agriculture and semi-natural environments) and climate (exposing bees to high temperatures in the lab), helps us understand their influence on microbiota composition and, consequently, insect health. This information is valuable for strategies that increase both pollinator quantity and health by enhancing floral biodiversity in less complex environments.

Furthermore, this project collects targeted data to develop strategies for disrupting insect-bacterial symbiosis in harmful insects (e.g., *B. oleae*), such as exposing samples to different antimicrobial treatments.

Understanding insect microbiota and its environmental correlations is of paramount importance for promoting more environmentally sustainable strategies, with potential positive economic implications.

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***Notonecta maculata*: more than just a model predator of mosquito larvae**



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Notonecta species, commonly known as backswimmers, are often employed as model predators of rotifers, annelids, zooplankton, crustaceans, molluscs, juvenile fish, frog tadpoles, and all the developmental stages of water insects. Indeed, they have a relevant impact on the size, structure, and richness of aquatic communities. Relative to mosquito populations, juvenile and adult backswimmers are more than just predators of larvae: their sole presence can also discourage the oviposition of gravid females. In this study, we selected a backswimmer of Western Palearctic distribution, *Notonecta maculata* (Hemiptera: Notonectidae), as a predator and deterrent of the highly invasive and health threatening Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae). From our biological observations on *N. maculata*, we assumed that, in central Italy, the species is univoltine and overwinters as an adult. Females oviposit from the following February to the end of May, and the new adults emerge in late June after 44.5 ± 3.1 days of nymphal development. Through different predation evaluations, we observed that the number of *Ae. albopictus* larvae preyed on by *N. maculata* increases while growing, starting from an average of 0.40-3.36 larvae per day for first, second, and third instar nymphs up to about 20 for adults. In the oviposition deterrence trial, a single *N. maculata* nymph in a mosquito ovitrap was sufficient to significantly discourage *Ae. albopictus* oviposition by 66 to 88% for five days. Overall, limiting or suppressing mosquito populations involving a biological control agent such as *N. maculata* might sound promising.

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Ambrosia beetle monitoring on *Castanea sativa* Miller



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Ambrosia beetles (Coleoptera: Curculionidae, Scolytinae and Platypodinae) can represent a severe threat to plant health, especially for young plants and in presence of stressing biotic and abiotic factors, such as rising temperatures and prolonged drought conditions. These xylomicetophagous insects can penetrate the xylem and dig tunnels where mating, oviposition and larval development occur. Their behaviour could cause irreparable damage to the vascular system of the host plants, compromising their survival.

To identify the insect species, the level of infestation, and the flight period, a monitoring started in Piedmont in 2023, specifically in Cuneo and Turin provinces. For each province, two sites were selected, choosing an area with prevalence of chestnut and one mixed with other deciduous trees (mainly *Acer*, *Betula*, *Fraxinus*, *Prunus*). For monitoring the species, ethanol baited bottle traps were placed in each surveyed site, for a total of 24 traps. The field activity lasted from April to October. All the individuals were counted and identified at the DISAFA - Entomology laboratory.

A total of about 12,000 individuals was recorded, mainly belonging to five species, namely *Anisandrus dispar* F., *Cycloripidion bodoanus* Reitter, *Xyleborinus saxesenii* Ratzeburg, *Xylosandrus crassiusculus* Motschulsky, and *Xylosandrus germanus* Blandford. Moreover, a few specimens of the Asian ambrosia beetle *Anisandrus maiche* Kurentzov were found, and this finding represents the first record for the Piedmont region.

Further research is needed to assess the community composition and its changes over time.

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Wild bees of Rome: biodiversity and pollination network in the natural reserves of the city

S

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In the last few years, many urban ecology studies have been carried out to understand the effect of urbanization on the urban insect diversity, often focusing on pollinating insects. The results obtained are conflicting sometimes. The objectives of this study are: to describe the wild bee communities within the city of Rome, in terms of species richness and composition, in order to verify if a large and green city like Rome can represent a biodiversity hotspot for pollinators; to evaluate how urbanization and other environmental and anthropogenic factors, influence the structure of pollinator communities and pollination networks. From March 2022 to September 2023, we performed field samplings of wild bees within the urban area of Rome, delimited by the GRA road ring, using 2 standard methodologies for pollinator insects. In 2022 we collected wild bees in the Nature Reserves of the "RomaNatura" network, using entomological net along fixed transects. In addition to morphological identification, for each bee captured was recorded the flower species during foraging, to obtain information on food preferences. Bee identifications are still in progress, but more than 40 genera of wild bees and about 200 species, some of them not reported yet for the Italian fauna, were already identified. In 2023 samplings were conducted by using pan traps, arranged radially along the urban-rural gradient in the Appia Antica Regional Park, with the aim of evaluating the effects of the urbanization gradient on the wild bee communities. Samplings are just ended, and the analyses of these data are still in progress.

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Whole genome resequencing reveals origins and worldwide invasion pathways of the Japanese beetle *Popillia japonica*



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The Japanese beetle *Popillia japonica* is a pest of significant interest, as it feeds on hundreds of economically valuable plant species. Native to Japan, it expanded its range in the first decades of the 20th century, invading large areas of the USA, Canada, the Azores (Portugal), Italy, and Ticino (Switzerland) and has been considered a priority pest in the European Union since 2019. Population genomics analyses of nuclear single nucleotide polymorphisms, derived from samples covering the entire species distribution, were used to gain insights into the geographic differentiation of *P. japonica* and to infer the routes of expansion from its native area to the USA and Europe. The present study also investigates, for the first time in this species, possible signals of selection to better understand the fundamentals that lay under *P. japonica*'s invasion success. Main results showed six genetically distinguishable clusters, corresponding to the approximate areas of colonization at a continental scale, with extensive admixture within areas. Our analysis supported an ancestral genetic divergence between South and Central/North Japan, identified the latter as the source of global invasion, and sustained dual independent introductions in the Azores and in Italy from the USA with cross border introductions in Canada and Ticino from neighboring populations (i.e., USA and Italy). Our analysis allowed a detailed inference of *P. japonica*'s invasion process and may give the chance of identifying, with reasonable confidence, the origin of individuals in future invasion events.

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Effectiveness of forest restoration for the conservation of insect pollinators in agricultural landscapes



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Forests are critically important habitats for pollinators. Besides providing nesting and other non-floral resources, forests can host distinct pollinator species, which can greatly benefit adjacent crops. However, in intensively managed agricultural landscapes such as temperate lowlands, forests are scarce, and often small and fragmented. In our research, we focused on exploring the effectiveness of forest restoration as a conservation measure for forest-associated pollinators. We selected 17 mesophilic oak-hornbeam restored forests and compared them with 17 primary forest remnants in the Po Valley in Italy. Then, we investigated which local and landscape characteristics affect the diversity of pollinating bees, hoverflies, and butterflies within forests. Results suggest that restored forests have a great potential in conserving forest-associated pollinators. Furthermore, forest structural characteristics, local heterogeneity, and landscape connectivity between patches affect pollinating insects differently, based on the ecology and the dispersal ability of single pollinator taxa. Our research contributes to the knowledge of pollinating insects in forest interiors and provides evidence on the potential of forest restoration in supporting pollinators in intensively managed agricultural landscapes.

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Variables affecting infestation of the olive fruit fly, *Bactrocera oleae*, in Umbria



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The impact of climate change on insect populations can significantly affect future pest management strategies. This makes it likely that crop losses due to insect outbreaks will increase substantially. Optimizing the monitoring efforts is essential for site-specific Integrated Pest Management (IPM) over vast areas, and the demarcation of monitoring zones based on varying levels of risk over time can play a significant role in this regard. *Bactrocera oleae* Rossi (Diptera: Tephritidae) is a herbivorous pest associated with *Olea* spp. in Europe, Asia, Africa and North America. It is expected to expand further its range due to global warming, colonizing areas at higher latitudes and altitudes. In this study, we analysed *B. oleae* infestation over seven years (2015-2021) across 86 olive farms in Umbria, Italy. We evaluated the relationship between infestation indexes and a set of agroclimatic variables for modelling purposes. Given the strong relationship between olive fly development and weather data, the analysis was based on weather data (maximum and minimum temperature and rainfall), morphometric and environmental data, and on data obtained from infestation surveys. Our results would be helpful for the development of predictive models and for increasing the reliability of Decision Support Systems in olive orchards.

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Unravelling the plant-insect interactions through the lens of microbial partners

SENIOR LECTURE

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Sustainable agriculture is a promising solution for balancing food production and reducing the environmental impact of chemical products. Microorganisms such as arbuscular mycorrhizal fungi (AMF), plant growth-promoting fungi (PGPF), and plant growth-promoting rhizobacteria (PGPR) play a key role in this approach. They improve plant nutrition, promote growth, and protect them against aggressors.

Similarly, insects' symbionts contribute to host nutrition and resistance. However, the link between symbiotic microorganisms and their impact on caterpillar performance is a particularly controversial subject.

Due to the potential importance of symbiotic microbes for their hosts, we hypothesize that soil-borne beneficial microbes, through plant-mediated effects, modify the bacterial communities of foliar-feeding caterpillars, ultimately affecting larval performance.

Untargeted metabolomics was used to investigate potentially underlying mechanisms of microbe-induced resistance and a metabarcoding approach targeting the 16S rRNA gene was used to explore differences in the bacterial community of caterpillars.

A comparative analysis between different biological systems and under different ecological settings allowed us to confirm that plant-beneficial microbes are promising resources to reduce the use of chemicals for controlling insect pests and for optimizing crop productivity. This is confirmed when plant resistance can be consistently induced regardless of the feeding guilds and diet specialization of the attacking insect, host plant species and abiotic environmental fluctuations. Our results demonstrated that this occurs for the AMF *Funneliformis mosseae* pointing towards this microbe as a promising tool for sustainable agriculture.

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Supercooperators: the biology of ant mutualisms from basic science to applications

SENIOR LECTURE

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Interspecific mutualistic cooperation, being a major source of innovation, represents a leading force in evolution. In this context, ants offer an outstanding array of cases since they may establish a complex network of cooperative interactions with virtually every biotic component of their ecosystems from microorganisms to fungi, from other animals to plants. In fact, ants are dominant organisms in most of the terrestrial habitats and, within social insects, they have reached the most impressive adaptive radiation. Among the keys to their success there are the complexity they achieved in social living and the tendency to establish close relationships with other organisms. This allowed them to control their physical environment, exploit resources efficiently and overcome competitors. The long common story involving ants and their mutualistic partners has led to the development of many co-adaptations whose knowledge could be extremely important in many fields of basic and applied biology. In this context, ants and their mutualistic relationships offer a unique opportunity to open a window on phenomena whose study may help to develop important interpretative tools in sociobiology, behavioural ecology, evolutionary biology as well as new insights for solutions to practical problems such as pest control, conservation biology, and new drug development.

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Role of transposable elements and bracovirus in a parasitoid wasp ongoing speciation



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A recent study documented that two parasitoid wasp populations of *Cotesia congregata* have different lepidopteran hosts and different ecological niches. Moreover, these two populations present an inability to produce viable offspring in some crossings. Specific courtship songs and genetic differentiation have also been reported that suggest that speciation is in progress in these parasitoid wasp populations. *Cotesia* development which occurs at the larval stage within the body of parasitized caterpillars relies on highly regulated molecular host-parasite interactions involving an endogenous virus (bracovirus) used to circumvent host immune response, thus ensuring wasp reproductive success. The bracovirus is thus tightly adapted to the specific wasp lepidopteran host and likely contributes to the divergence of the two wasp populations. In addition, a hybrid fertility asymmetry in *Cotesia congregata* was described, which resembles the one described in *Drosophila* (hybrid dysgenesis) involving transposable elements (TE) and their piRNA repression system. We hypothesize that a TE could contribute to speciation by limiting genetic exchanges between the two wasp populations. The project aims to evaluate the contributions of the bracovirus and TEs to the speciation in progress, using comparative genomics. Here, we present preliminary results identifying TE candidates potentially involved in dysgenesis, the study of the piRNA repression system targeting these TEs in both populations using bioinformatics analyses, and the first results on bracovirus analysis.

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How the environment changes inside hives in relation to the material of construction: effects on temperature variability and honeybee biological parameters

S

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In recent years, the productivity and survival of honeybee populations are at risk due to anthropogenic and environmental factors. The effects of these inputs on the colony cause its progressive weakening, with consequences on queen fecundity, worker foraging capabilities, homeostasis (e.g. the ability to thermoregulate in summer). Therefore, the influence of hive construction material on bee survival needs consideration. The effect on honeybees of classical wooden Dadant Blatt hives was compared with that of an aluminium hive with cork insulation. Hives were placed in an experimental fruit farm in Montanaso Lombardo (Lodi province). Monitoring started in March 2022 and ended in September 2023, with periodical observations of biological parameters and productivity. At least fortnightly, honey, pollen and brood present in the combs were estimated, the presence/absence/replacement of the queen and mortality in the underbasket verified. Analysis of the results showed an effect of the hives on daily mortality trends: in the wooden hives, the highest mortality was observed in spring with a decline over the summer and a recovery at the end of August. In the aluminium hive, the average worker mortality was lower at the beginning of the season and peaked between July and August. Finally, considering the data of temperature inside the hives the aluminium hive presented lower fluctuation throughout the monitoring period. The results obtained from these first two years of research seem to confirm the effectiveness of the aluminium and cork hive (Dadant Blatt) in honeybee rearing and an alternative to classical ones.

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Identification and evaluation of the biological activity of semiochemicals for the monitoring and control of olive moth, *Prays oleae* (Bernard), and cotton bollworm, *Helicoverpa armigera* (Hübner)

S

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Semiochemicals are natural compounds regulating many aspects of insects' life including reproductive behaviour and selection of suitable oviposition and host food sites. The identification and application of insect semiochemicals is one of the most promising research areas for the development of sustainable control strategies of insect pests. In fact, insect semiochemicals can be applied both as indirect (monitoring) and direct control means (mass trapping, attract and kill, mating disruption, male disorientation). New sustainable control tools of olive moth, *Prays oleae* (Bernard), and cotton bollworm, *Helicoverpa armigera* (Hübner), respectively among the most damaging pests of olive trees and processed tomato, are urgently needed due to recent legislation limits on the use of synthetic insecticides. This research aims to identify and develop effective applications of *P. oleae* and *H. armigera* semiochemicals. Ph.D. research activity is carried out in collaboration with the partner company CBC Europe - Biogard division. During the first year of Ph.D. program, field trials, carried out to assess the effectiveness of new aerosol formulations for mating disruption (MD) of *P. oleae* and *H. armigera*, showed promising results. In fact, a significant reduction of male trap catches was found in MD-treated plots compared to untreated ones. Moreover, visual samplings showed lower infestation levels of leaves, flower clusters, and fruits by *P. oleae* larvae as well as of tomato fruits by *H. armigera* larvae in MD-treated plots compared to untreated plots. Further studies are in progress to confirm results of MD applications against these pests and to identify new bioactive plant-derived semiochemicals by chemical, electrophysiological, and behavioural investigations.

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Hybridization in the pine processionary moth species complex: what we know and what we don't

SENIOR LECTURE

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Anatolia harbours numerous hybrid zones owing to its diverse topography and its past as a glacial refugium. Pine processionary moth species complex has been added to the cases of hybridization events in Anatolia recently. With its two well-defined species, *Thaumetopoea pityocampa* and *T. wilkinsoni*, it has been known in Turkey for a long time but the ranges of these two species in Turkey was largely unknown. Ipekdal et al. (2015) described the distribution of the two species in Turkey by using molecular markers. While most of Turkish pine forests were found to be dominated by *T. wilkinsoni*, *T. pityocampa* was more prevalent in European Turkey. Although the two species are mostly distributed in separate areas in Turkey, both were found on adjacent trees at the same locality in the European part of İstanbul, northern Gelibolu, and İzmir (limits of the contact zone). This study also showed taxonomic incongruences between nuclear and mitochondrial markers in the contact zone (i.e., an asymmetric pattern of mitochondrial introgression from the northwestern *pityocampa* clade to the southeastern *wilkinsoni* clade). Petrucco-Toffolo et al. (2017) showed that *T. pityocampa* and *T. wilkinsoni* can hybridize to produce viable and fertile offsprings under laboratory conditions. Finally, Ipekdal et al. (2020), by using microsatellite and mitochondrial data, confirmed introgression between the two species in a restricted zone in northwestern Turkey without any F1 individuals.

We are still far from understanding the evolutionary and ecological outcomes of the hybridization between the two species which will be focused on in future studies.

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Endophytic colonization of *Bacillus thuringiensis* on tomato reduces *Spodoptera littoralis* survival

R

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Beneficial microorganisms can promote positive effects on plant biology, plant growth and defence barriers, offering a valuable alternative to synthetic agrochemicals. *Bacillus thuringiensis* (*Bt*) is an entomopathogen successfully applied as bioinsecticide worldwide and its ability to naturally colonize plants has been recently demonstrated. While the role of *Bt* as entomopathogen is well studied, its role as plant endophyte is still relatively less explored. Here we contribute to this research topic by studying the effect of tomato plants colonization by *Bt* on the survival and the development of the phytophagous pest *Spodoptera littoralis* (Lepidoptera: Noctuidae). Endophytic *Bt* negatively affected the survival of the first two larval instars that fed on detached leaves of colonized plants. The larvae which were able to reach the third instar developed normally and pupated, but exhibited higher pupal mortality, and the emerging adults exhibited lower fertility and longevity. In addition, *S. littoralis* larvae feeding on *Bt*-colonized plants were more susceptible to sublethal doses of Xentari, a commercial bioinsecticide containing the entomopathogen *Bt*. Further studies are underway to better characterize the mechanism underlying this tritrophic interaction between microorganism, plant and insect. Our results show a clear effect of *Bt*-colonized plants on *S. littoralis* larvae, which could contribute to a reduction in pesticide use in agriculture by directly suppressing this pest or enhancing its susceptibility to biocontrol agents.

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Evaluation of pollination deficit of *Pyrus communis* L. in Italy



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Pyrus communis L. is one of the most important crops for Italy, with a production of 3.703 Mt produced in 2023 across 24,106 hectares, of which 13,610 in Emilia-Romagna. Pear, like most Rosaceae species, is self-incompatible and thus relies on insect pollinators for a successful pollination. Pollination deficit, defined as the inadequate quantity and quality of pollen deposited on the stigma, can therefore affect the production of this crop. The aim of this study was to assess pollination deficit in pear orchards in the Emilia-Romagna region, as well as their pollination dependence and pollinator diversity and abundance, in relationship to landscape and crop management factors (e.g. percentage of agricultural area, cultivar distribution, pesticide load). To achieve these objectives, 16 pear orchards (Abate Fetel cultivar) across the Emilia-Romagna region were monitored and pollination treatments (pollinator exclusion, open pollination and hand pollination) were performed alongside with pollinator surveys at the peak of bloom. The results showed significant differences between pollination treatments, indicating a strong pollinator dependence and pollination deficit in this crop. Overall deficits across farms were significant and on average 21%, both for fruitlet set and fruit set. Seed set was significantly higher in fruits from hand pollination than those from open pollination and the latter was higher than those from exclusion. A total of 1,859 insects were observed on pear flowers; the most abundant flower visitors were honeybees, dipterans, and bumblebees.

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Ecological and biological drivers of bacterial communities' diversity in the leaf beetles species (Coleoptera, Chrysomelidae)



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Little is known about the factors shaping the phytophagous insects' microbiota diversity and the role of microorganisms in the host plants adaptation. In this project, we focus our attention on the bacteria associated with the phytophagous insects of the family Chrysomelidae and closely related ones. By performing a screening of the bacteria present in the abdomen of these non-model organisms, we aim to test which biotic and abiotic factors can shape the bacterial diversity, including the host plants spectrum breadth, the sex, and the living environment. The microbiota of 35 species has been characterized with a DNA metabarcoding approach targeting the bacterial 16S rRNA gene. From this screening, we observed that the species with the widest host plant spectrum (generalists) harbour a more diversified microbiota than those with a narrow one (specialists). Moreover, males were found to be more prone to acquire bacteria from the environment, while females' microbiota was generally dominated by reproductive manipulators (*Wolbachia* and *Rickettsia*) or symbionts with functional roles. Two putative primary symbionts belonging to the Enterobacteriales, previously isolated only from one Eumolpinae species, were detected in other two species suggesting the presence of a widespread symbiosis within this subfamily. Surprisingly, some possible new symbionts for *Orsodacne* and *Cryptocephalus* were also identified. At present, we are processing further Chrysomelidae species to better understand the influence of the host plant spectrum and geography on the microbiota diversity and identify the bacteria with a potential role in conferring to the insect the ability to exploit different plants.

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Scaling-up the rearing system of *Sclerodermus brevicornis* (Hymenoptera, Bethyridae) for behavioural studies



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The hymenoptera *Sclerodermus brevicornis* Kieffler (1906) appears to be a good candidate for the control of *Psacotha hilaris hilaris* (Pascoe) (Coleoptera, Cerambycidae, Lamiinae, Lamiini), an invasive xylophagous pest causing damage to mulberry and fig plants in Italy. In order to optimise its release, it is essential to deepen the knowledge of the bioethology. The study was first focused on biological aspects related to the developmental cycle and reproduction, along with the possibility of using a factitious host for a cheaper and easier mass rearing system. Then, considering the particular social aspect of this species, defined as quasi-social (multiple females cooperate to paralyze the host and care for the brood), studies turned to behaviour. Initial evidence has shown that kinship and the number of foundresses per host play a crucial role in parasitism efficiency. A progressive scaling up in the choice tests has been set up to simulate a major degree of interactions between hosts and parasitoids. The results showed that when many females emerge from the same brood they tend to segregate in groups to reach as many hosts as possible. It will remain necessary to evaluate also the kinship factor (thus considering more broods) to see possible changes in behaviour and to simulate better field conditions.

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Natural enemies of xylophagous insect pests: host selection, morphology and behaviour



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The arthropods of the genus *Sclerodermus* (Hymenoptera: Bethyridae) and *Pyemotes* (Acarina: Pyemotidae) are ectoparasitoids of many species of insects. These arthropods can be important biological control agents against insect pests. Additionally, they are reported as a common cause of dermatitis in humans. Until today we are breeding the species *S. brevicornis*, *S. domesticus*, and *P. tritici*. Our goals are: to investigate their host location process and the interactions with their hosts and humans; to deepen our knowledge regarding the morphology and taxonomy of the Italian species of the genus *Sclerodermus*; to study, in the case of the coexistence of *Sclerodermus* and *Pyemotes* on the host (frequent in nature), if there is some interference between the two species for host exploitation. In this context we have investigated the ultrastructure of the antennal sensilla of the species *S. brevicornis* using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) providing a detailed description of antennal sensory equipment. We are conducting olfactometer bioassays to investigate the main chemical cues involved in the host location. The preliminary results show that *S. brevicornis* is mainly attracted by host larval frass and by wood debris. Furthermore, extracts from the larval frass will be tested on *Sclerodermus* antennae with electroantennographic investigations. Concerning *Pyemotes*, a specifically adapted two-way olfactometer was made to test the mite towards different human VOCs (volatile organic compounds). The results of our investigations can be important to the sustainable control of insect pests and to understand the relationship between these arthropods and human dermatitis.

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Effects of the oral administration of *Trichoderma asperellum* chitinases and *Bacillus thuringiensis* on Lepidoptera



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The widespread use of synthetic pesticides has raised multiple concerns in last years due to their toxic effects on the environment and human health. Consequently, researchers are looking for alternative and more sustainable solutions for crop protection. The entomopathogenic bacterium *Bacillus thuringiensis* (*Bt*) is the most widely used tool in integrated pest management (IPM) but fungi, in particular from *Trichoderma* spp, could represent suitable alternatives. Indeed, they produce bioactive compounds and chitinases that negatively affect insect development and survival. In this context, the present study aims at investigating the biological effects of the oral administration of *T. asperellum* ICC012 chitinases, alone or in combination with a *Bt*-based product on two lepidopteran species (i.e., *Bombyx mori* and *Spodoptera littoralis*). Our results demonstrate that the multi-enzymatic cocktail produced by *T. asperellum* is able to alter the structure of the insect peritrophic matrix, consequently affecting the development of the larvae and leading them to death. Moreover, larval mortality increased after the co-administration of chitinases and sublethal doses of *Bt* toxins. This study may contribute to the development of new IPM strategies, increasing the efficacy of *Bt*-based products.

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Drivers of pollinator assemblages in urban contexts: the effects of local-scale and landscape factors on pollinators



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Insects are responsible for pollinating most wild flora and food crops worldwide along with contributing many ecosystem services. However, global declines in pollinator populations jeopardise the provisioning of these ecosystem services in both managed and natural ecosystems. Urbanisation is a notable driver of land cover change and has posed detrimental effects on biodiversity and ecosystem integrity as natural and semi-natural habitats are converted into landscapes dominated by built features and impervious ground cover. As such, it is imperative to determine the variables that drive pollinator communities in urban contexts to adopt adequate and sustainable conservation strategies that preserve ecosystems throughout future city developments. This study, conducted in Bologna, Italy, assesses the factors driving the pollinator assemblages present across the city. The objectives of this research are firstly, to monitor the populations of pollinators present and secondly, to compare the relative importance of local-scale factors (plant diversity) and landscape factors (surrounding land cover) in structuring these pollinator communities. Pollinators were surveyed across 15 sites, including parks, gardens and urban farms, using two practical methods; observational plots combined with hand netting, to determine the interactions and networks between plants and pollinators and pan traps, to gain a broader scope of the pollinator diversity in each site. The effect of the surrounding land cover of each site will also be evaluated. This is a two-year experiment, with the first year of sampling recently concluded. The preliminary results and trends of the first year of data are reported.

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Physiological, molecular, and behavioural investigations on *Ips typographus* vision (Coleoptera, Scolytinae)



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Aggressive bark beetle species such as the Eurasian spruce bark beetle *Ips typographus*, play a fundamental role in forest ecosystems but, along with their importance from a biodiversity and ecological point of view, they can also lead to extensive forest mortality and massive economic damage during outbreaks. Nowadays *Ips typographus*' eyes, visual perception of the reality and recognition of specific targets like host plants are understudied topics on which very little is known. Studying of such sense can open the way to new systems resulting in more efficient management methods, particularly important during the switch from an endemic to an epidemic condition. In addition, the integration of visual cues in trapping systems of *Ips typographus* may offer new opportunities for surveillance. Research reported here concerns morphology and physiology of the compound eyes of *I. typographus*, including an exploration of the opsin genes involved in light recognition and consequent behavioural tests. The number and size of ommatidia is reported and the light spectral sensitivity analysed through the electroretinography (ERG) technique. The results confirmed the capability of this bark beetle to perceive green light (530 nm) and showed a new, distinct response to UV light (370 nm), thanks to two different photoreceptors. Further evidence of such findings was obtained through the exploration of beetle genome, where the presence of two different opsins involved in green and UV light perception was found, confirming the ERG results. Finally, behavioural y-maze experiments were set up to confirm the response of *I. typographus* to different light stimuli and intensity. Overall, the results may explain the host selection of the beetle and its orientation and dispersal pattern.

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Foraging behavior of eusocial bee colonies (Hymenoptera, Apidae) towards food contaminated with pesticides



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While foraging, the exposure of bees to pesticides due to floral contamination depends on the behavior of the bee, which can be attracted, repelled or indifferent to these compounds. We conducted a semi-field study to test if bees prefer food contaminated with pesticides over non-contaminated food. Colonies of *Plebeia lucii* Moure, 2004 (Apidae, Meliponini) were placed in a greenhouse and submitted to a preference test, where bees were free to choose between the following honey syrup options: non-contaminated, contaminated with acephate (2 mg a.i./L), contaminated with glyphosate (31.3 mg a.i./L) or contaminated with a mixture of both (2 mg acephate/L and 31.3 mg glyphosate/L). To simulate a more realistic scenario, experiments were carried out under the environmental conditions of a greenhouse. We found that at higher temperatures (>25°C), bees displayed no preference for contaminated or non-contaminated food. Under lower temperatures (<25°C), bees preferred food contaminated with pesticides. Our results show that pesticide residues in nectar poses a threat to colonies of *P. lucii*, since foragers do not avoid contaminated food, despite detrimental effects caused by acephate and glyphosate to bees. Also, even though the behavior of bees towards contaminated food was influenced by temperature, they collected pesticide-contaminated food under all temperatures observed.

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Survey for '*Candidatus Liberibacter*' spp. and relative putative vectors in north-western Italy



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The gram-negative bacteria '*Candidatus Liberibacter*' spp. are phloem-limited plant pathogens causing diseases in several crops' families and are carried out by psyllids. In Europe, haplotypes C, D and E of '*Ca. Liberibacter solanacearum*' (CLso) are reported to be associated with disorders on carrot (*Daucus carota* L.), parsnip (*Pastinaca sativa* L.) and celery (*Apium graveolens* L.). On the contrary, '*Ca. Liberibacter europaeus*' (CLEu) is present in different rosaceous plants and apparently do not behave as a pathogen but as an endophyte, but not in New Zealand where it causes serious damage to *Cytisus scoparius* (L.) Link. To investigate the presence and dynamics of these pathogens in north-western Italy, field samplings were carried out for the presence of '*Candidatus Liberibacter*' spp. in seven localities with *C. scoparius* plants as well as in two fields of carrots, four of potatoes, surrounding weeds, and relative psyllids. Plant samples were regularly collected in conjunction with insect monitoring performed using the beat-tray technique and yellow sticky traps. CLEu was detected in broom samples, as well as in the two broom psyllids, *Arytainilla spartiophila* (Förster) and *Arytaina genistae* (Latreille). On potatoes, it was possible to catch psyllids, all belonging to *Heterotrioza* spp. and *Bactericera* spp., exclusively with traps. On the contrary, on carrot, *Heterotrioza chenopodii* (Reuter) and *Bactericera trigonica* Hodkinson were sampled both using yellow sticky traps and the beat-tray method. One of these latter specimens tested positive for CLso. Moreover, an individual of *Trioza urticae* (Linnaeus) collected from *Parietaria officinalis* (L.) was founded CLso positive.

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Detection of *Scaphoideus titanus* using electronic and sticky traps



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Monitoring tools typically adopted in agricultural fields are sticky and pitfall traps, often painted with a specific color and/or baited with pheromones and lures. In most cases, the operator must return in the fields at periodic intervals to identify and count captures and to replace the traps with new ones. These kinds of monitoring are often costly and labor intensive, and can produce a delay in the decisions due the time spent on gathering and analyzing data in multiple locations. The development of electronic traps represents a new way to improve the pest management process, allowing remote control of insect captures from distant locations, limiting costs and labor requirements during field visits. Currently, many insect pest species are monitored and detected automatically with different devices and sensors, however, very few models of electronic traps have been proposed to monitor insects of the suborder Auchenorrhyncha (Hemiptera), as cicadas, leafhoppers, treehoppers and planthoppers. Among these, the leafhopper *Scaphoideus titanus* Ball (Hemiptera, Cicadellidae) is the main vector of Grapevine flavescence dorée phytoplasma, a disease causing economic losses in the main viticultural areas of Europe. In this work we tested a new type of camera-equipped trap coupled with a vertical sticky surface and a self-cleaning system to monitor adults of *S. titanus*. The performance of the prototypes was evaluated in vineyards in comparison with commercial sticky traps. Results indicated that, when catches were standardized on the surface, no differences were detected between commercial and electronic traps.

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Exploring RNA interference (RNAi) for the control of hemipteran vectors of plant pathogens



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RNA interference (RNAi) is a double stranded RNA (dsRNA)-based gene silencing mechanism. Recently, exogenous application of dsRNAs to crops has been shown to be a powerful tool to control different pests, mainly Coleopterans. Several xylem and phloem feeder Hemipterans are vectors of important plant pathogens. Among them, *Philaenus spumarius* is known as the main European vector of *Xylella fastidiosa* (Xf). Here different dsRNA molecules targeting five spittlebugs' genes were tested with the aim of giving some insights on RNAi machinery and application methods for *P. spumarius*. Initially, eleven genes have been selected and the sequences of spittlebug homologue genes retrieved. For those genes, potential off-target effects on beneficials have been screened *in silico* by the analysis of sequence homology. Then, dsRNA molecules targeting five of the eleven selected genes have been produced by *in vitro* transcription and delivered to *P. spumarius* by feeding (to nymphs) and microinjection (to adults). Gene expression level of target transcripts was evaluated by qPCR. The preliminary results showed strong and effective silencing effects of corresponding specific transcripts in both dsRNAs-microinjected and -fed insects, in comparison with dsGFP controls. The significant reduction in transcript level was measured from 3 until 24 days post injection (dpi), the longest dpi tested. We can conclude that RNAi machinery works in *P. spumarius*, as target gene down-regulation was robust and quite long-lasting. Further steps will be the evaluation of mortality rate of treated insects and the development of an effective delivery method of dsRNAs to *P. spumarius*.

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Do social behaviours reflect local adaptation in the European earwig?



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The success of a species' invasion is generally based on its ability to adapt its life-history traits to the constraints of a new environment. To date, most studies on this issue have focused on the physiology or reproduction of species and have taken little account of a species' ability to adapt in terms of social behaviour. Here, we tested this capacity in the European earwig, an insect that is invasive in North America and whose mothers express care for eggs and juveniles. Using a common garden experiment, we measured 11 forms of maternal care and 14 physiological traits in 376 females collected from 7 populations 10 to 45 km apart in France. Our results show firstly that all the physiological traits measured differ between these 7 populations (weight, day of laying, number of eggs, speed of egg development, hatching rate, etc.). However, these differences were not found in behaviour (defence of eggs and juveniles against predators, transfer of food between mothers and juveniles, general activity of mothers and juveniles, etc.). These results suggest the presence of local adaptations of the earwig in terms of physiology/reproduction, but not in terms of maternal care. This raises the question of whether the selection pressures exerted on this care are common to all populations, or whether the expression of this care is a plastic phenomenon that depends mainly on the conditions in which the females find themselves when they express it.

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Identification of the sex pheromone of the asparagus moth, *Parahypopta caestrum*: chemical, electrophysiological, and field investigations



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The asparagus moth, *Parahypopta caestrum* (Hübner) (Lepidoptera, Cossidae), is one of the most damaging pest of *Asparagus* spp. in many Mediterranean areas, including Foggia province, the widest producing area of green asparagus in Italy. Due to the cryptic nature of the immature stages and the adult emergence during the harvest time, control of this pest is very difficult. To contribute to the knowledge on the sex pheromone of this pest, chemical, electrophysiological, and field tests were carried out. Observation on the calling activity of virgin females suggested that sex pheromone emission starts during the first hour of the first scotophase. Accordingly, hexane and SPME (Solid-Phase Microextraction) extracts from abdominal tips of calling females were prepared. Analyses of solvent and SPME extracts by gas chromatography coupled with mass spectrometry and electroantennography (GC-MS-EAD) revealed the presence of four EAG-active peaks whose mass spectra matched those of three monounsaturated C₁₄ aliphatic acetate and one monounsaturated C₁₄ aliphatic alcohol, respectively. Double-bond position and configuration of sex pheromone candidates were also confirmed by comparative EAG experiments with series of monounsaturated cis (*Z*) and trans (*E*) C₁₄ aliphatic acetates and alcohols. In field trapping trials, individual compounds were not attractive. Some binary and even more ternary mixtures of acetates were attractive to *P. caestrum* males. The addition of the monounsaturated C₁₄ alcohol to the attractive blends did not improve male trap catches. Studies are in progress to develop further pheromone-based control methods (i.e., mass trapping) for a sustainable management of this pest.

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Efficacy of natural products and dusts on *Halyomorpha halys*



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The brown marmorated stink bug, *Halyomorpha halys* (Stål), is an invasive pest that originates from Eastern Asia. It has a broad host range, affecting numerous cultivated plant species, resulting in significant economic losses worldwide. *Halyomorpha halys* has caused serious damage to many crops in several countries, including Italy. To contain the spread of *H. halys*, chemical pesticides have been used, but these insecticides have proven to be largely ineffective and unsustainable. Among the best alternatives, the use of natural pesticides or dusts showed some potential in field trials. The aim of this research was to evaluate in the laboratory the lethal effects of natural insecticides and particle films on *H. halys*. The insects were exposed to commercial products at field doses for a 7-day period, and mortality was evaluated at different times. The tested products included zeolite, diatomaceous earth, sulphur, potassium salts of fatty acids, and calcium polysulfide. Part of these substances were tested in mixture. Preliminary results will be provided and discussed also in relation to the side effects shown by some of these products towards the egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), which has been released in several Italian regions to implement classical biological control of *H. halys*.

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Evaluation of lure-dispenser combinations for *Halyomorpha halys* traps in Italy



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The brown marmorated stink bug *Halyomorpha halys* (Stål) is an invasive species native to Eastern Asia and it is currently representing a significant threat to the global agriculture production. Monitoring pest populations is the key point in the development of suitable IPM (Integrated Pest Management) strategies. To attract insects, chemical lures are commonly used. The most used cue for capturing *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 efficient combination among different concentrations of Murgantiol and MDT loaded on different types of dispensers (blister pack, wax tablets and biodegradable polymer). A 12-weeks field trial is still ongoing. The efficacy of the different lure-dispenser combinations will be evaluated based on the number of *H. halys* individuals captured by the sticky traps and their density in the surrounding area. The most efficient combination will be considered for improvement of mass capture of *H. halys* and to develop a push-pull strategy.

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Host aphid immunosuppression by *Aphidius ervi* venom



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The host immunosuppression by parasitic wasps is an important component of the host regulation strategy. The venom injected at the oviposition is one of the key-factors involved in this host alteration and, in some parasitoids, its immunosuppressive role is complemented by wasp's symbionts. Most of the available studies in this research area are related to hosts belonging to Lepidoptera and Diptera, for which a strong immune response is observed, while little is known for hemimetabolous host species, characterized by defense barriers apparently much weaker. To fill this research gap, here we focus on the host-parasitoid system *Acyrtosiphon pisum* – *Aphidius ervi*. We functionally characterized *in vivo* a serine protease homolog (AeSPH) protein identified in the venom of the aphid endoparasitoid *A. ervi*, generating AeSPH-depleted female wasps by RNA interference and evaluating their capacity to successfully parasitize the host. Parasitism success rate was negatively affected by AeSPH knockdown and associated with an increased phenoloxidase (PO) cascade activation in aphids, scored by measuring PO enzymatic activity and the expression of *phenoloxidase activating factor 2*, a proPO-activating gene upregulated in response to *A. ervi* parasitism. Our results indicate that AeSPH contributes to parasitism success by inhibiting the melanization response of the host, which is therefore an important component of the defense barriers involved in the parasitoid egg suppression. The undergoing studies on other virulence factors in *A. ervi* venom will allow to further characterize the immunosuppression strategy and its possible broader role in the host regulation through its action on aphid symbiont development.

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Arrestment behavior of the egg parasitoid *Trissolcus basalis* towards the secretion of the tarsal gland of *Nezara viridula*



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The Southern green stink bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) is a cosmopolitan harmful polyphagous species feeding on more than 30 plant families. This species causes serious damage to crops and, in nature, is controlled by many natural enemies such as predators and parasitoids. To locate their hosts, parasitoids, and in particular oophagous parasitoids, are attracted by plant synomones induced by egg deposition, from a distance. Instead, at short range, kairomones play a role to guide parasitoids to the host. The egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) is a key natural enemy of *N. viridula* and is widely used in biological control programs. It is known that the chemical residues left by green stink bugs during walking induce an arrestment behavior in *T. basalis*, but little is known regarding the nature and the origin (i.e. from which part of the body is released) of these chemical traces. We hypothesize that these chemical residues consist of fluid typically released during walking by insects to better adhere on smooth and rough surfaces and that they are released by the tarsal gland. The morphology of the tarsal gland was previously investigated in a similar heteropteran species, *Coreus marginatus* (L.) (Hemiptera: Coreidae), throughout ultrastructural investigations. Our preliminary results indicate that kairomones could be present both in glandular fluid and hemolymph and its chemical nature is polar because parasitoid responses are stronger when glandular fluid and hemolymph are dissolved in distilled water than in hexane.

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Host preference and chemical ecology of *Saperda tridentata*: preliminary results



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Saperda tridentata (Olivier, 1795) (Coleoptera: Cerambycidae) is a longhorn beetle species which has gained importance after recent interceptions at Italian ports on rough wood elm logs imported from North America. This beetle feeds on leaves and bark of young twigs of elms, laying eggs on weakened stems in which larvae develop. However, its potential host range in Europe is unknown. Given the likelihood of further introductions of *S. tridentata* in Europe, there is an urgent need to characterize its behavior and potential impact on European trees, as well as to develop efficient monitoring strategies. To address this knowledge gap, we carried out choice and no-choice feeding and oviposition bioassays on ten species of European broadleaved trees. Our findings indicate that *S. tridentata* feeds readily on foliage of European elm species, but less so on European species of *Acer* and *Populus*. Results from no-choice bioassays testing oviposition and development from egg to adult on stem sections of European trees are pending. In addition, preliminary trapping experiments showed that adults, particularly females, were significantly attracted to stressed elms vs. healthy elms. Chemical analyses are ongoing to determine which host volatiles elicit attraction and whether males emit sex/aggregation pheromones. Our efforts aim to provide a more comprehensive understanding of *S. tridentata* host preference and chemical ecology, offering valuable insights for the development of effective surveillance strategies.

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Nectar-inhabiting bacteria affect the performance of biological control agents

R

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Parasitoids commonly exploit flowering plants for food resources, such as nectar, which is a sugar-rich solution with energetic and nutritional benefits. Nectar is also inhabited by microbial communities, such as bacteria from the phyla Actinomycetota, Bacillota, and Pseudomonadota. Bacteria belonging to these phyla have been isolated from the nectar of buckwheat *Fagopyrum esculentum* Moench (Polygonaceae: Polygonales), which has been proven beneficial to enhance the performance of several species of insect parasitoids. Yet, bacterial-mediated effects on the parasitoids' longevity, which is of relevance for biological control efficiency against economically important insect pests, are currently overlooked. In this study the effect of 14 bacterial isolates from buckwheat nectar was examined on the longevity of the adult parasitoids. Bacterial isolates were added to synthetic nectar, which had been prepared by filter-sterilizing sucrose solution mixed with casamino acid. The bacterial-fermented and non-fermented (control) synthetic nectars were provided *ad libitum* to the parasitoids *Trissolcus basalus* (Wollaston) (Hymenoptera: Scelionidae), *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae), *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae), *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae), *Anagyrus vladimiri* Triapitsyn sp. n (Hymenoptera: Encyrtidae) and *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae) as well as the predator *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). The total number of days alive for each female (longevity days) was used for the statistical analysis, which revealed species-specific positive, negative or neutral effects.

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Identification of the predators of oilseed rape pests using camera traps



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Cameras are becoming common tools for ecologists studying species abundance, richness, and interactions. They are mainly used to study large animals, but with their increasing availability they are increasingly used to study invertebrates: e.g., to record insect-flower and predator-prey interactions. Cameras can be powerful tools to identify and quantify the predators of specific agricultural pests. This knowledge can then be applied to adapt farming practices to favour the species of the predators identified. We used cameras to monitor the predation of two important pests of oilseed rape, the pollen beetle (*Brassicogethes aeneus*) and the brassica pod midge (*Dasineura brassicae*). Both species are vulnerable to predation by ground dwelling invertebrates in spring when larval stages drop from the plant canopy to the ground before pupation. Over 2 years of sampling and 240 days of cumulated observation, we identified the main ground predators of these species in the UK. Our observations showed that *Carabidae* and *Staphylinidae* larvae are key predators of pollen beetles and brassica pod midge larvae. Only adults of the Genus *Bembidions* or *Trechus* were frequently observed feeding on these preys. Other predators such as Acari and ants were also observed in predatory behaviours. These results show that neglected species and development stages can be important contributors to the biocontrol services provided in agroecosystems. This knowledge can be used to guide farmer's management practices, with the aim to support important predator populations in their fields.

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Developing novel RNAi-based biopesticides targeting the central nervous system of *Spodoptera littoralis*



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The polyphagous lepidopteran *Spodoptera littoralis* damages > 80 crop species. Most commercial insecticides targeting *S. littoralis* threaten non-target organisms and the environment. RNA interference (RNAi), a natural immune defence mechanism that regulates endogenous gene expression, can be triggered by dsRNA, causing insect mortality. Novel sprayable biopesticides incorporating dsRNA or genetically engineered plants expressing dsRNA offer highly specific pest control methods. Three genes essential to central nervous system functionality, acetylcholinesterase 1 (*ace-1*), nicotinic acetylcholine receptor (*nAChR*) and voltage-gated sodium channel (*para*) were targeted. Oral dsRNA delivery to 4th instar larvae via artificial diet and to 5th instar via gavage feeding did not significantly reduce ($P > 0.05$) gene expression, survival, pupation or adult emergence likely because dsRNA is rapidly degraded by nucleases in *S. littoralis* midgut juice, as revealed by *ex vivo* dsRNA incubation. Continuous feeding of dsRNA to neonates significantly reduced larval weight ($P < 0.05$), suggesting reduced nuclease activity in earlier instars, supported by the lack of significant effect on gene expression and survival upon direct dsRNA haemolymph injection into 4th instar larvae compared to significant developmental delays upon 3rd instar injection. Formulation with chitosan, a nanoparticle capable of protecting dsRNA and increasing cellular uptake, did not enhance dsRNA stability in pure midgut juice nor significantly reduce ($P > 0.05$) survival of 4th instar or neonate larvae. Bioinformatic analyses suggested the safety of these molecules against non-target organisms, confirmed by bioassay against *Bombus terrestris*. This work highlights the efficacy of targeting these genes whilst also indicating the need for optimised delivery techniques.

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Exploring functional evolution of microbial communities in termites with comparative metagenomics: insight into diazotrophy



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Most animals harbor gut microorganisms that often play important functions for the hosts. In termites, a complex gut microbiota composed by bacteria, archaea and, in certain species, flagellate protists, is known to be essential for the hosts to feed on diverse dead plant materials such as wood. While termite gut microbiota is primarily involved in the breakdown of cellulose and hemicellulose chains, some bacteria and archaea can fix atmospheric nitrogen N_2 and convert it into ammonia NH_3 , which can be metabolized by most organisms. The objective of our study is to identify the different taxa playing a role in nitrogen fixation, see their distribution within the community as well as their evolution. Metagenomics is the most relevant and efficient method for carrying out community-scale studies, then, we used termite metagenomes as well as termite-associated prokaryotic genomes to construct a global catalogue of genomes and protein sequences from termite microbiomes. Thanks to this substantial resource we will be able to identify all the *nif* genes involved in nitrogen fixation with bioinformatics methods and to associate these genes with the taxa that carry them. This will allow us to provide a clear picture of the diazotroph diversity across termite phylogeny and diet. Thanks to our genome-centric approach, we will also be able to decipher the metabolic potential of these diverse diazotroph to predict their role in termites.

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