European PhD Network **"Insect Science"**

XI Annual Meeting

30 November - 4 December 2020

ZOOM MEETING

SCIENTIFIC PROGRAM
&
BOOK OF ABSTRACTS

European PhD Network "Insect Science"

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PROGRAMME

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Segreteria Organizzativa Event Planet Srl Contact: Marina Morra - marina.morra@eventplanet.it

Monday 30 November 2020

14:25 **Welcome address**

Senior scientist lecture

14:30 Eric Conti – University of Perugia
Sustainable management of invasive species: an evaluation using herbivorous Pentatomidae as models

Oral Presentations

Chair: Enrico Ruzzier – University of Padova

15:00 R Tugcan Alinc – University of Palermo
The impact of *Trichoderma harzianum* strain T22 on tomato plant defenses in response to stink bugs feeding

15:20 R Annalisa Andreani – University of Firenze
Evolutionary adaptations in hippoboscid flies, parasites of different hosts

15:40 S Mokhtar Abdulsattar Arif – University of Palermo
The chemical ecology of host gender discrimination in the samurai wasp *Trissolcus japonicus* Ashmed

15:45 R Sabina Avosani – University of Trento
Use of vibrations to study and manipulate the behaviour of the meadow spittlebug *Philaenus spumarius*

16:05 R Eleonora Barra – University of Napoli
Induced immunosuppression as a strategy to enhance insect biocontrol

16:25 **Break**

Chair: Mizuki Uemura – University of Padova

16:55 R Andrea Becchimanzi – University of Napoli
A salivary chitinase of *Varroa destructor* influences honey bee immunity and mite's survival

17:15 S Alessio Bellini – University of Torino
A compost treatment confers suppresiveness in *Phytophthora capsici – Cucurbita pepo* pathosystem modifying the rhizosphere mycobiota

17:20 S Zineb Bennani – CIHEAM-IAMB Bari
Side effect of new pesticides on natural enemies in organic citrus: a case study

17:25 R Matteo Brunetti – University of Milano
The microbiota of Chrysomelidae: composition, typical symbioses and patterns of diversity

17:45 R Andree Cappellari – University of Padova
Contrasting effects of exotic plant invasions and managed honeybees on plant–flower visitor interactions

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Tuesday 1 December 2020

Senior scientist lecture

14:30 Antonino Cusumano – University of Palermo
Influence of parasitoid-associated viral symbionts on plant-insect interactions

Oral Presentations

Chair: Milos Sevarika – University of Perugia

15:00 S Elena Chierici – University of Perugia
Development and application of a PCR-based analysis of carabid beetle gut-contents reveal seasonal weed predation in wheat field

15:05 S Alex Cussigh – University of Bologna
Molecular systematics and phylogenetics of the Australian stick insect genus *Candovia* Stål, 1875 (Phasmida, Necrosciinae)

15:10 R Priscilla Farina – University of Pisa
Bioactivity of the Andean aromatic plants *Aloysia citrodora* and *Bursera graveolens* essential oils against the blowfly *Calliphora vomitoria*

15:30 R Elena Eustacchio – University of Milano
Plants and flower-visiting arthropods in mountain ecosystems: the case study of the alpine species *Androsace brevis* (Primulaceae)

15:50 S Alessio Minici – University of Milano
Who are my guests? Investigating the arthropod activity on high-altitude flowers by video observations, the example of *Androsace brevis* (Primulaceae)

15:55 S Elissa Daher – University of Perugia
Laboratory and field evaluation of new commercial products against *Bactrocera oleae*

16:00 **Break**

Chair: Michele Ricupero – University of Catania

16:45 S Valeria Fattoruso – University of Trento
Effectiveness of mineral oil alternatives and vibrational disturbance against *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae)

16:50 R Giobbe Forni – University of Bologna
Macroevolutionary analyses provide new evidences of phasmids wings evolution as a reversible process

17:10 S Antonio Franco – University of Basilicata
Innovative processes for lipid extraction from bioconverter insects, qualitative and quantitative evaluation and industrial applications for the formulation of personal care products

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17:15 R Mizuki Uemura – University of Padova
Locomotion of social caterpillars is influenced by polarised light, detected by a single pair of stemmata

17:35 R Lorenzo Goglia – University of Molise
Lepidoptera Tortricidae: zoogeography of the Matese Massif and Gargano Peninsula (Central-South Italy)

17:55 S Antonio Gugliuzzo – University of Catania
Antagonistic and mycoparasitic microorganisms as potential tools for controlling the invasive ambrosia beetle *Xylosandrus compactus*

Wednesday 2 December 2020

Senior scientist lecture

14:30 Elena Gonella – University of Torino
Microbial symbioses in Hemiptera, a cutting-edge target for pest control

Oral Presentations

Chair: Antonio Gugliuzzo – University of Catania

15:00 S Claire Hoarau – Harper Adams University
The use of entomopathogenic nematodes to control the cabbage stem flea beetle (*Psylliodes chrysocephala)*

15:05 S Badr-eddine Jabri – CIHEAM-IAMB Bari
Development of smart detection tools for some honey bee pathogens and preliminary survey on the sanitary status of honey bees in Morocco

15:10 R Giovanni Jesu – University of Napoli
Effects of Smoke Waters on *Bactrocera oleae*

15:30 S Ilaria Laterza – University of Bari
Pentatomoidea (Hemiptera: Heteroptera) of "Alta Murgia" National Park, Southern Italy

15:35 R Serena Malabusini – University of Milano
Inter- and intra-specific competition in Bethylid wasps

15:55 R Marco Malfacini – University of Bologna
Development of sexing systems functional to mass production of *Aedes albopictus* Skuse sterile males

16:15 R Matteo Marchioro – University of Padova
Improving longhorn and jewel beetles trapping protocols for maximizing bark and ambrosia beetle (Coleoptera: Curculionidae, Scolytinae) catches

16:35 **Break**

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Chair: Giovanni Jesu – University of Napoli

17:05 S Alessandro Mariani – University of Perugia
Effect of soil microplastics on the behaviour of fungus gnats (Diptera: Sciaridae)

17:10 S Jacopo Martelossi – University of Bologna
Uncertainty in molecular phylogeny and incongruence of morphological taxonomy throughout the systematics of four Euphasmatodea families

17:15 S Ilaria Martino – University of Torino
Stem blight and dieback of blueberry in Northern Italy

17:20 R Cátia Martins – University of Bologna
Impacts of pesticides on non-*Apis* pollinators

17:40 R Rihem Moujahed – University of Palermo
How to escape an evolutionary trap: fitness-related effects and behavioral responses of the egg parasitoid *Trissolcus basalis* developing on *Halyomorpha halys*

Thursday 3 December 2020

Senior scientist lecture

14:30 Lorenzo Marini – University of Padova
Species-habitat networks: a promising tool in applied entomology

Oral Presentations

Chair: Cátia Martins – University of Bologna

15:00 S Alberto Mele – University of Padova
Riparian vegetation influences *Halyomorpha halys* egg parasitoids impact in kiwifruit orchards

15:05 S Antonio Moretta – University of Basilicata
Characterization of antimicrobial peptides deriving from insects and their application in the biomedical field

15:10 S Francesca Napoli – University of Perugia
Sub-lethal effects of industrial crops on honeybee behavior

15:15 R Davide Nardi – University of Padova
Arthropod response to forest landscape dynamics

15:35 S Bianca Orrù – University of Torino
Symbiotic control of the brown marmorated stink bug *Halyomorpha halys*

15:40 S Giacomo Ortis – University of Padova
Effect of temperatures on embryonic development of the forest pest *Barbitistes vicetinus* (Orthoptera, Tettigoniidae)

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15:45 R Marta Panella – University of Milano
Effects of the plant beneficial microorganism *Trichoderma harzianum* on the microbiota of *Spodoptera littoralis* larvae

16:05 R Martina Parrilli – University of Bologna
Use of sugar dispensers to disrupt ant attendance and improve biological control against Pseudococcidae mealybugs in vineyard

16:25 S Onofrio Marco Pistillo – University of Foggia
Chemical and electrophysiological investigations on the sex pheromone of the asparagus moth, *Parahypopta caestrum*

16:30 **Break**

Chair: Andrea Becchimanzi – University of Napoli

17:00 S Sofía Victoria Prieto – University of Torino
Study of the gut microbiota in the green stink bug *Nezara viridula* for symbiont-targeted biological control

17:05 S Jovana Raičević – University of Belgrade
Structure of the bee-plant visitation network in the gradient of landscape complexity

17:10 R Michele Ricupero – University of Catania
Parasitism and phylogeny of *Dinocampus coccinellae* through native and exotic hosts in different continents

17:30 S Valeria Rossi – University of Perugia
Odorant receptor expression-related modulation in behavior during larval development in African cotton leaf worm, *Spodoptera littoralis*

17:35 S Elia Russo – University of Napoli
***In vivo* functional analysis of *Aphidus ervi* venom**

17:40 R Enrico Ruzzier – University of Padova
Jewels on the go: exotic buprestid around the world (Coleoptera: Buprestidae)

Friday 4 December 2020

Senior scientist lecture

14:30 Lucia Zappalà – University of Catania
Interactions among plants, essential oils and the omnivorous mirid *Nesidiocoris tenuis*

Oral Presentations

Chair: Sofía Victoria Prieto – University of Torino

15:00 S Valerio Saitta – University of Perugia
Mechanical ecology in plant-herbivore interaction: role of insect claws of different shape in

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the attachment to hairy plant surfaces

15:05 R Giacomo Santoiemma – University of Padova
Chemical control of *Popillia japonica* in the management of invasive populations in northern Italy

15:25 R Davide Scaccini – University of Padova
Egg parasitoids complex of *Halyomorpha halys* (Stål) in Veneto with particular emphasis on adventive populations of Asian species

15:45 S Carmen Scieuzo – University of Basilicata
Use of organic by-products from the agri-food chain to obtain valuable products through bioconversion process

15:50 R Milos Sevarika – University of Perugia
First evidence of a female-produced pheromone in *Philaenus spumarius*

16:10 R Chloe Start – University of Newcastle
RNAi mediated knockdown of essential Central Nervous System genes in two Lepidopteran pest insects

16:30 **Break**

Chair: Michele Ricupero – University of Catania

17:00 S Elena Tafi – University of Basilicata
Use of the Black soldier fly (*Hermetia illucens* L.) as an alternative source of chitin and chitosan for the production of biopolymeric films for agro-food applications

17:05 R Simona Tortorici – University of Catania
Herbivore induced VOCs, secondary metabolites and enzymatic activity in *Solanum* spp.

17:25 S Micaela Triunfo – University of Basilicata
The black soldier fly *Hermetia illucens*: an innovative and alternative source of chitin and chitosan

17:30 R Costanza Geppert – University of Padova
Contrasting response of native and non-native plants to disturbance and herbivory in mountain environments

17:50 S Gennaro Volpe – University of Napoli
RNAi silencing of an immune gene disrupts the embryonic development of *Spodoptera littoralis*

17:55 **Discussion on PhD education and future careers**

18:00 **Meeting closure**

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ABSTRACTS

Sustainable management of invasive species: an evaluation using herbivorous Pentatomidae as models

SENIOR

E. Conti

University of Perugia, Department of Agricultural, Food and Environmental Sciences

The ecological and economic impact of alien invasive herbivores is a serious issue. Unfortunately, in spite of the efforts to prevent accidental pest introductions, these are expected to increase in the future, mostly due to market globalization and climate change. It is therefore necessary to evaluate sustainable control strategies that can be effective in reducing the impact of invasive species. Herbivorous stink bugs (Hemiptera: Pentatomidae) may serve as a good model for this, as many species are agricultural pests, at least five species are invasive and three of them are almost globally distributed. Several sustainable control strategies are under investigation, starting from classical, augmentative and conservation biological control. However, biological control of stink bugs faces a complex set of challenges that must be addressed to maintain pest populations below the economic injury level. Therefore, additional sustainable control methods are being investigated by many scientists globally. These include semiochemical-based approaches to increase the efficacy of natural enemies or to manipulate the behaviour of stink bug pests. Further methods contemplate plant resistance, entomopathogens, physical barriers, sterile insect technique and treatments affecting the pest symbionts. All together, these tactics offer interesting perspectives for the integrated management of invasive stinkbugs and are worthy of further investigations, both under laboratory conditions and in the field.

The impact of *Trichoderma harzianum* strain T22 on tomato plant defenses in response to stink bugs feeding

R

T. Alinc

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

Plant growth promoting fungi belonging to genus *Trichoderma* are known to help plants by inducing systemic resistance against plant pathogens. In recent years, it is increasingly recognized that these beneficial soil microbes also have role in mediating plant defense mechanisms (direct and indirect) against insect herbivores. However, no data are up to now available regarding how *Trichoderma* can enhance direct and/or indirect plant defenses to such piercing-sucking herbivores. To address this, here we investigated the impact of a commercial *Trichoderma* strain, *Trichoderma harzianum* T22, on tomato plants against two polyphagous pest species, *Nezara viridula* and *Halyomorpha halys*. We have determined 3rd instar stink bug nymphs performance in terms of relative growth rate and survival on tomato plants inoculated by beneficial soil microbe. The results represented similar outcome for both stink bugs species in which *T. harzianum* T22 affected tomato direct defense against nymphs leading to reduction on relative growth rate. Nonetheless, we did not find any significant evidence showing the differences on survival of nymphs compared to uninoculated plants. Overall, these findings indicate that *T. harzianum* T22 can limit the feeding activity of stink bugs and induce direct defense of tomato plants. Further on this aspect, the role of *T. harzianum* T22 in tomato indirect plant defense such as recruitment egg parasitoids of the stink bugs is planned to be investigated.

Evolutionary adaptations in hippoboscid flies, parasites of different hosts

R

A. Andreani, A. Belcari, P. Sacchetti

Department of Agri-Food Production and Environmental Sciences, University of Florence, Piazzale delle Cascine, 18 - 50144 Firenze

Hippoboscidae family counts three subfamilies (Ornythomyinae, Hippoboscinae and Lipopteninae) of hematophagous ectoparasites living into the coat of different animals used as suitable hosts. Although hippoboscids are able to feed also on occasional hosts, included humans, they are strictly adapted to the parasitic life and have evolved morphological features to co-exist together with their victims. Parasites belonging to different subfamilies infest various species with different characteristics in terms of coat, skin or life environment, so they need peculiar structures to efficiently exploit their hosts. In order to evaluate if these flies have evolved some adaptations in response to the habitat and the features of their hosts, we carried out a morphological study on four hippoboscids (*Lipoptena cervi, Lipoptena fortisetosa, Hippobosca equina, Pseudolynchia canariensis*) analyzing also ungulate furs and pigeon feathers through Scanning Electron Microscopy. These observations show that the external surface of the antenna, the wings and the adhesion organs of the legs have differently evolved in the ectoparasites we analyzed, while the feeding apparatus is a convergent feature. Results clarify that the morphological characteristics of the host coats and the environment in which they live affected the structures of the flies remarking the close association between insects and hosts. For example, the relationship between *L. fortisetosa* and *Cervus nippon*, its original host, is particularly interesting because it may help to clarify the spread and the possible route covered by the parasite from its native area to Europe.

The chemical ecology of host gender discrimination in the samurai wasp *Trissolcus japonicus* Ashmed

S

M.A. Arif[1,2]

[1]*Department of Agricultural, Food and Forest Sciences (SAAF)- University of Palermo, Viale delle Scienze, Building 5, 90128 Palermo, Italy;*
[2]*Plant Protection Directorate- Ministry of Agriculture, Abu-Ghraib 10081, Baghdad, Iraq*

Egg parasitoids rely mainly on chemical cues while foraging for suitable host eggs, which are scattered in the environment. The chemistry of the active compounds eliciting egg parasitoid responses can be related to the host bug cuticle traces left on the substrate, which may give information about the presence and the gender of the host. However, the chemical bases of host sex discrimination in egg parasitoids have been rarely characterized. Here we carried out behavioral and chemical bioassays to investigate chemical cues exploited in host searching behavior and gender discrimination of the samurai wasp, *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae), the main egg parasitoid of *Halyomorpha halys* Stål (Heteroptera: Pentatomidae). A first set of bioassays showed that parasitoids spent more time exploring patches contaminated with chemical cues *H. halys* females compared with males. Similar responses were displayed by *T. japonicus* when hexane extracts of *H. halys* were tested suggesting the non-polar nature of the chemical compounds responsible of host gender discrimination. Chemical analysis of hexane extracts from *H. halys* adults revealed quantitative differences in the cuticular compounds of the two sexes, with 1-hexadecene (more abundant in males) being the most important component in determining these differences. Addition of 1-hexadecane to hexane extracts of *H. halys* females significantly reduced the wasps' arrestment responses compared to crude *H. halys* female extracts. The role of cuticular hydrocarbons as chemical cues mediating host gender discrimination in *T. japonicus* is discussed in the context of conservation biological control.

Use of vibrations to study and manipulate the behaviour of the meadow spittlebug *Philaenus spumarius*

R

S. Avosani[1,3], V. Verrastro[2], V. Mazzoni[3]

[1]DICAM-Department of Civil, Environmental and Mechanical Engineering-University of Trento; [2]CIHEAM BARI-International Centre for Advanced Mediterranean Agronomic Studies; [3]Research and Innovation Centre- Fondazione Edmund Mach

Philaenus spumarius is a widespread insect that uses vibrations to achieve mating. Due to its ability to transmit the bacterium *Xylella fastidiosa* to olive and other plants, this insect became a threat to the European agriculture. As recently demonstrated, vibrational stimuli can be used to acquire a deeper knowledge regarding an insect pest sexual behaviour in order to develop control strategies. Therefore, to assess when females are sexually mature, and whether the female receptivity to mating is correlated to the ovarian development, playback trials and morphological inspections were conducted on single females and male-female pairs of *P. spumarius*. In addition, to evaluate whether a vibrational stimulus could disrupt mating, pairs were treated with a continuous broad-band noise with a frequency range of 150-1200 Hz. Females emitted mating signals from the end of the summer onwards, and their signalling activity and responsiveness to mating were correlated with the ovarian development. Although both females and males emitted mating signals, the noise could disrupt the pair formation process, likely by preventing mate finding. Further research on a larger scale should therefore be conducted in order to develop a future control mating disruption technique, which should be applied at the end of the summer, when both sexes are sexually mature.

Induced immunosuppression as a strategy to enhance insect biocontrol

R

I. Di Lelio, E. Barra, M. Coppola, R. Rao, S. Caccia

University of Naples "Federico II" - Department of Agricultural Sciences, Naples, Italy

The identification of new bioinsecticides and of their appropriate delivery way is one of the approaches currently being pursued to reduce the use of chemical insecticides. Among these strategies, the use of natural antagonists as a source of virulence factors or of molecular technologies that mimic the negative effect of these latter on the host insects pave the way toward the development of new bio-inspired tools of pest suppression. The use of RNA interference (RNAi) to artificially down-regulate host genes negatively targeted by virulence factors of natural antagonists appears to be particularly promising. In fact, we have recently shown that the RNAi mediated silencing of a gene (*Sl 102)* controlling the cellular immune response (encapsulation and nodulation) in *Spodoptera littoralis* (Lepidoptera: Noctuidae) enhances the killing activity of the entomopathogen *Bacillus thuringiensis.* Here we explore two delivery strategies of dsRNAs targeting *Sl 102* gene, based on sonicated heat-killed *E. coli* and transgenic tobacco plant expressing *Sl 102* dsRNA. The experimental larvae showed marked immunosuppression associated with a significant transcriptional down-regulation of the target immune gene. The resulting immunosuppressed phenotype showed a very high mortality when exposed to sub-letal doses of *Bt* (Xentari). Therefore, the ingestion of dsRNA, delivered under realistic field conditions, has the potential to enhance the *Bt* insecticide activity on *S. littoralis* larvae. Moreover, the possible occurrence of a synergistic effect of a gene silencing strategy concurrently impairing two complementary arms of the cellular immune response (*Sl 102* and *gasmin* genes) in *S. littoralis* was performed. From a theoretical point of view, the induction of a reduced immune competence in the target pest appears to be ecologically more sustainable as it can enhance the ecological services provided by natural antagonists.

A salivary chitinase of *Varroa destructor* influences honey bee immunity and mite's survival

R

A. Becchimanzi[1], R. Tatè[2], E.M. Campbell[3], E. Caprio[1], S. Gigliotti[4], A.S. Bowman[3], F. Pennacchio[1]

[1]*University of Napoli "Federico II" - Department of Agricultural Sciences, Naples, Italy;*
[2]*Institute of Genetics and Biophysics "Adriano Buzzati Traverso"- CNR, Naples, Italy;*
[3]*University of Aberdeen - School of Biological Sciences (Zoology), Aberdeen, Scotland;*
[4]*Institute of Biosciences and Bioresources – CNR, Naples, Italy*

Varroa destructor creates a wound in the host's cuticle through which it feeds on haemolymph and fat body, representing an important stress factor that weakens honeybee colonies and promotes the spreading of diseases. In order to facilitate feeding, this ectoparasitic mite delivers a complex of factors with its salivary secretions. The characterization of these factors is still largely elusive and any progress in this area will offer new insights into the molecular basis of *Varroa*-honeybee interactions, on which to develop new sustainable strategies of mite control. Here, we have used a functional genomics pipeline to identify *V. destructor* salivary proteins putatively involved in the regulation of host physiology, and their expression in salivary glands has been assessed by qRT-PCR and *in situ* hybridization. This approach allowed the identification of a salivary chitinase, which was subsequently studied from a functional point of view. *In vivo* studies were based on gene knockdown followed by artificial infestation of honeybee pupae. The effectiveness and the time course of the silencing were assessed and associated with the observed level of mortality on experimental *Varroa* mites. To study the effect of the salivary chitinase of *V. destructor* on honeybee gene expression, we analyzed the transcriptome of worker pupae in response to parasitism by silenced mites. The results obtained indicate an important role of this enzyme in the modulation of host immune response.

A compost treatment confers suppresiveness in *Phytophthora capsici – Cucurbita pepo* pathosystem modifying the rhizosphere mycobiota

S

A. Bellini[1,2], I. Ferrocino[2], M. Pugliese[1,2,3], A. Garibaldi[1] and M. L. Gullino[1,2]

[1]*AGROINNOVA – Centre of Competence for the Innovation in the Agro-Environmental Sector, University of Turin, Turin, Italy;*
[2]*Agricultural, Forestry and Food Sciences Department (DISAFA), University of Turin, Turin, Italy;*
[3]*AgriNewTech s.r.l., Turin, Italy*

Phytophthora capsici Leonian (PHC) is a filamentous oomycete that causes losses on many important horticultural crop, including summer squash (*Cucurbita pepo* var. cylindrica L.). PHC chemical control strategies are difficult to adopt, due to the limited number of registered chemicals that are permitted and the scalar harvest system of *Cucurbita pepo*. For these reasons, other control strategies such as the use of waste-based composts have been studied intensively. In this study four composts were tested at different concentrations (1–10–20% v/v), to establish their ability to confer suppressiveness to the *Phytophthora capsici* (PHC) – *Cucurbita pepo* pathosystem in controlled greenhouse pot trials. A total of 12 compost mixtures were considered and of these, one (*Trichoderma*-enriched compost at 10% v/v) was able to statistically reduce the disease incidence caused by PHC (by 50% compared to the untreated control). Hence, the mycobiota composition of the most effective compost treatment was investigated through 26s gene amplicon based Illumina sequencing and compared with untreated and chemical (metalaxyl) controls. Mycobiota sequencing showed genera differences between the three treatments, with relative abundances of several fungal genera that were significantly different among the samples. Moreover, PCA analyses clustered the compost treatment differently from the chemical and the untreated controls. These findings suggest that the suppressive activity of a compost is strictly influenced by its ability to induce a shaping in the rhizosphere microbial composition.

Side effect of new pesticides on natural enemies in organic citrus: a case study

S

Z. Bennani, S. Keciri, K. Djelouah, V. Verrastro, D. Cornara

Centre International des Hautes Etudes Agronomiques Méditerranéennes - Istituto Agronomico Mediterraneo di Bari

Pesticides allowed in organic agriculture may disrupt ecosystem services provided by beneficial insects. Here we tested the lethal and sub-lethal effects possibly induced by Mineral Oil (MO), Sweet Orange Essential Oil (EO), extract of Clitoria ternatea (CT), combination EO+CT, and Spinosad, on *Planococcus citri* main biological control agents, the predator *Cryptolaemus montrouzieri* and the parasitoid *Leptomastix dactylopii*. Overall, MO residuals caused a significant mortality of parasitoid females, approaching 80% of the exposed individuals. On the contrary, once moved to infested plants, *L. dactylopii* females previously exposed to EO+CT showed a significantly reduced parasitization ability. On the contrary, no side-effect was observed upon toxicants topical application on cocooned pupae. The tested products did not induce a significant mortality in predators exposed to treated preys. On the other side, adults predation was significantly reduced during direct exposure to CT and EO+CT, and by EO+CT once the individuals were moved to untreated plants. Preys consumption for larvae was instead severely reduced upon direct exposure to CT, and to a minor extent to MO, SP, and EO+CT, while a predatory ability collapse was observed for the EO+CT treatment once larvae were offered untreated preys following toxicants exposure.

The microbiota of *Chrysomelidae*: composition, typical symbioses and patterns of diversity

R

M. Brunetti, G. Magoga, M. Montagna

University of Milan – Department of Agricultural and Environmental Sciences

Most of the insects are colonised by a multitude of microorganisms, prevalently living as commensals, but often able to confer either beneficial or detrimental effects. Leaf beetles (Coleoptera: Chrysomelidae) constitute one of the most diverse insect groups in the world. They are exclusively phytophagous with a wide trophic specialization spectrum, ranging from species able to exploit only one plant species, to species that can feed on hundreds of plant species belonging to different families. Some Chrysomelidae subfamilies are known to have evolved specialized organs, associated to gut and genitalia, hosting vertically transmitted bacterial symbionts that play an important role in supporting insect nutrition. The present study aims to characterize the microbiota associated to thirty selected species of leaf beetle, using a metabarcoding approach targeting two hypervariable regions of the bacterial 16S rRNA. The main bacterial classes found in the microbiota of Chrysomelidae are Alphaproteobacteria (~39%), Gammaproteobacteria (~45%) and Bacilli (~14%). In several leaf beetles the presence of reproductive manipulators (e.g., *Wolbachia*) was detected. The vertically transmitted bacterial symbiont present in Donacinae and Cassidinae have been detected in three species previously not reported to be infected. Furthermore, the presence of a bacterial symbiont, known to be associated with only one species of Eumolpinae, has been reported also in two other species, thus suggesting that it is probably widespread in this subfamily. Alpha-diversity metrics comparison support the hypothesis that the microbiotas associated to specialist species (i.e., feeding only on one plant genus/family) are less rich that those hosted by more generalist species.

Contrasting effects of exotic plant invasions and managed honeybees on plant–flower visitor interactions

R

A. Cappellari[1], D. Corcos[2], M. Mei[2], D. Paniccia[3], P. Cerretti[2], L. Marini[1]

[1]*University of Padua – Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), Padua, Italy;*
[2]*Sapienza University of Rome – Department of Biology and Biotechnology "Charles Darwin", Rome, Italy;*
[3]*Via Colle 13, Frosinone, Italy*

Buddleja davidii is an exotic shrub native from China that has invaded several riparian habitats across the European Alps. Being an excellent source of nectar, it is very attractive for both managed and wild pollinators, including the honeybee (*Apis mellifera*). Here, we observed plant–flower visitor interactions during the full flowering season of *B. davidii* in the Italian Alps and quantified how *B. davidii* presence, honeybee abundance, and flower diversity affected ecological network topology and foraging behaviour of flower visitors. High density of honeybees had positive effects on network stability, but it also impacted non-bee flower visitors, causing a shift in their diets and an increase in their specialization. In contrast, the presence of *B. davidii* facilitated the visits to neighbouring plants, decreasing flower visitor specialization. Our study highlights the importance of considering multiple concomitant factors when evaluating the potential impacts of exotic plant invasions on native pollinators.

Influence of parasitoid-associated viral symbionts on plant-insect interactions

SENIOR

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Insect parasitoids have evolved symbiotic interactions with several viruses and thousands of parasitoid species have established mutualistic associations with polydnaviruses (PDVs). While PDVs have often been described as virulence factors allowing development of immature parasitoids inside their herbivore hosts, there is increasing awareness that PDVs can affect plant-insect interactions. We show that PDVs alter not only host physiology, but also the feeding patterns and composition of oral secretions in herbivores, which are often used by plants to recognize the identity of the herbivore attacker. In turn PDV-induced changes in herbivore phenotype affect plant responses to herbivory with consequences ranging from differential expression of plant defense-related genes to wider ecological effects across multiple trophic levels. We conclude highlighting important missing gaps to fully understand the role of PDVs and other parasitoid-associated viral symbionts in a plant-insect interaction perspective.

Development and application of a PCR-based analysis of carabid beetle gut-contents reveal seasonal weed predation in wheat field

S

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As generalist predators, carabid beetles may have a key role in the biological control of weed seeds in the agroecosystems. Weeds are a serious issue in agriculture as their management requires high costs and efforts. Improving the knowledge of carabid beetles feeding behaviour and seasonal effectiveness in seed predation is crucial for future enhancement of weed biological control strategies. We designed and evaluated species specific primers, targeting chloroplast gene fragments, to detect predation upon three economically relevant weeds: *Lolium multiflorum* Lam., *Papaver rhoeas* L. and *Sinapis arvensis* L. Amplicon sizes ranged from 244 to 287 bp, allowing detection of recent feeding events. In May - October 2018, we sampled carabid beetles in a wheat field at the DSA3 Experiment Station, using pitfall traps filled with EtOH. Samples of *Pseudoophonus rufipes* (De Geer) and *Pterostichus melanarius* (Illiger) were dissected and the gut processed for DNA purification. PCR reactions were conducted to evaluate the entity of field predation upon weed seeds. The predation by *P. melanarius* towards the three species was higher than that of *P. rufipes*. In particular, overall predation by *P. melanarius* upon *L. multiflorum* was 27.2% compared to 2.9% by *P. rufipes.* Our results highlight a positive overall predation towards weeds, confirming the importance of the two carabid species in reducing the amount of seeds in the soil. The enhancement of soil functions and ecosystem services, aiming at promoting carabid beetle diversity and foraging activity, might contribute to the reduction of herbicide use while maintaining a reasonable crop yield.

Molecular systematics and phylogenetics of the Australian stick insect genus *Candovia* Stål, 1875 (Phasmida, Necrosciinae)

S

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The phasmid genus Candovia counts nine recognized species endemic to Australia. However, much of its species diversity has gone undetected, mainly due to the highly convergent morphological evolution related to crypsis in the Phasmida order. In this study, we unravel the diversity of the Candovia genus through three different molecular species delimitation approaches using the Folmer region of Cytochrome Oxidase subunit I gene, along with phylogenetic analyses on seven additional mitochondrial and nuclear markers. Our analysis confirmed the already described species and led to the delineation of twelve putative new taxa. The phylogenetic analyses reinforced our understanding of the systematic relationships among Candovia species clarifying interspecific divergences and suggested the monophyly of the taxon. Moreover, mapping the presence of egg capitulum on the phylogenetic tree implied the possible multiple origin of this structure in this genus.
Our analysis provided a framework for systematics studies on Candovia and suggested carefulness when reconsidering diagnostic characters for taxonomy.

Bioactivity of the Andean aromatic plants *Aloysia citrodora* and *Bursera graveolens* essential oils against the blowfly *Calliphora vomitoria*

R

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Tropical Andes is a biodiversity hot spot rich in aromatic plant species, whose potential as a source of active compounds for insect pests' control is still largely underexploited. Here, the Essential Oils (EOs) extracted from two Ecuadorian plants, the shrub *Aloysia citrodora* (Verbenaceae) and the tree *Bursera graveolens* (Burseraceae), were chemically analysed and tested for their bioactivity against the blue blowfly *Calliphora vomitoria* (Linnaeus, 1758) (Diptera: Calliphoridae). This fly is a vector of pathogenic microorganisms, dangerous in factories and stores where fresh meat is processed, stored, and sold. The main components of the *A. citrodora* EO are geranial and limonene, while in the *B. graveolens* EO they are limonene and α-terpineol. The effects of the two EOs were evaluated against *C. vomitoria* by a behavioural assay in a two-choice olfactometer (concentrations range 0.07-2.8 µL L^{-1} air). Besides, the insecticidal properties of the two EOs were tested by fumigation (EOs concentrations from 6.06 to 36.36 µL L^{-1} air), by contact with topical applications using a Burkard micro dispenser (EOs dose from 0.10 to 0.60 µl EO/fly), and by ingestion of a mucilage containing EOs (from 15 to 75 µl EO mL^{-1} mucilage), sucrose, and agarose. Furthermore, the ovicidal activity was assessed (concentrations from 0.006 to 0.075 µL EO cm^{-2} of filter paper). The results of the behavioural assay showed a stronger repellent effect exerted by the *A. citrodora* EO, while all the toxicity tests revealed dose-dependent mortality of the blowflies and eggs.

Plants and flower-visiting arthropods in mountain ecosystems: the case study of the alpine species *Androsace brevis* (Primulaceae)

R

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Climate change is expected to strongly modify fundamental plant-pollination networks of high-mountain ecosystems, but very little is still known about this topic. We investigated the complex interactions between flower-visiting arthropods and plants on Alps using *Androsace brevis* as model species, a narrow endemic plant which flowers in early season, immediately after snowmelt, when few trophic resources are available for those few active arthropods. We worked in Lepontine Alps (Como. Italy) and Orobic Alps (Bergamo, Italy) using an integrated approach involving manual sampling and in-field video observations of *A. brevis* flowers-visiting arthropods. These approaches allowed to obtain both an accurate identification of *A. brevis* flower-visiting arthropods and a characterization of their behavior, revealing variability among taxa and allowing to hypothesize their ecological roles. In particular, manual sampling method allowed to identify early-season active arthropods at low taxonomic level and to determine which taxa actually can pollinate *A. brevis* by quali-quantitative palynological analysis of pollen carried by sampled flower-visiting arthropods. In four years of manual sampling, we sampled specimens belonging to 10 orders and to 50 families. Diptera Anthomyiidae and Hymenoptera Apoidea turn out to be the main pollinators of *A. brevis* as well as the taxa at the base of high-mountain plant-pollinator networks. Our results give insight into early-season plant-pollinator interactions in the Alps, which represent a fundamental component of high-mountain ecosystems, strongly threatened by climate change. Manual sampling, together with videorecording method, could allow to develop conservation plans for ecological networks in high-mountain ecosystems.

Who are my guests? Investigating the arthropod activity on high-altitude flowers by video observations, the example of *Androsace brevis* (Primulaceae)

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Little is known about high-mountain arthropod communities and their interaction with flowering plants. We investigated flower-visiting arthropods behavior with the innovative approach of video observations, focusing on *Androsace brevis*, a vulnerable endemic plant of the Central Alps, living above 2000 m asl, and characterized by a very short and early flowering. The work was conducted in the Lepontine Alps (Como, Italy) and in the Orobic Alps (Bergamo, Italy), where we recorded about 300 videos (for a total of about 87 hours) of arthropod activity on the flowering plants in two years of sampling. Environmental parameters (i.e., wind, light and temperature) were also recorded to unravel any correlation with the abundance and diversity of flower-visiting arthropods. The videos were analyzed using BORIS, a software that allows building an ethogram of displays of interest and tracking the behaviour of multiple subjects on the basis of the codified displays. We observed a high variability in behaviour among taxa in terms of mean number of flowers visited, corolla tubes entered, time spent on flower and inside the corolla tube. Our results lead us to hypothesize the possible ecological role of flower-visiting arthropods, highlighting the role of Diptera Brachycera and Hymenoptera Apoidea in pollination. Moreover, we observed that some arthropods use *A. brevis* as a refuge (i.e., Thysanoptera) or hunting ground. This innovative approach, less invasive than direct observation and manual sampling, represents a powerful tool to describe the behavior of arthropods and their relationship with plants in this vulnerable environment.

Laboratory and field evaluation of new commercial products against *Bactrocera oleae*

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The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most important olive pest worldwide. Pest management has been extensively studied for this pest, however, the application of pesticides both as bait sprays and cover sprays is still the most common control method despite recognized drawbacks. Therefore, given the needs for sustainable and eco-friendly methods, preventive tools must be considered. Here, we tested the efficacy of two commercial products, i.e. “Propolis” and “Polvere di roccia” manufactured by Cifo Srl. A field trial was conducted from July to November 2020 in an olive orchard in Spello (PG). Three varieties (for olive oil or table olive production) were subjected to four treatments (Polvere di roccia, Propolis, the mixture of both, and copper) in addition to the control. Compared to control, the percentage of attacks on table fruits was significantly lower with Polvere di roccia alone, and even lower when Polvere di roccia was mixed with Propolis. The difference remained significant after 11 weeks of the trial. Data for olive oil varieties are still under inspection. Furthermore, laboratory no-choice and choice tests are ongoing to clarify the effects of the commercial products on female behaviour.

Effectiveness of mineral oil alternatives and vibrational disturbance against *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae)

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The greenhouse whitefly (GW), *Trialeurodes vaporariorum* is considered one of the most harmful insect pests in greenhouses worldwide. We evaluated the effectiveness of two essential oils, namely CT (Serox, *Clitoria ternatea* extract) and EO (Prev-am, Sweet Orange Essential Oil), that were tested alone and in combination with the playback of a vibrational disturbance signal that was synthesised on the basis of previous research on the GW vibrational communication. Trials were carried out on tomato and zucchini potted plants. In the first phase of the study we determined which was the life stage (eggs, nymphs or adults) more vulnerable to the action of the two formulations. We observed that nymphs appeared to be the more vulnerable, although EO affected the GW population density only on zucchini, while CT was more effective on tomatoes.
In a second set of experiments, where nymphs were chosen as target life stage, we treated the plants with a mixture of EO and CT in combination with the specific vibrational disturbance signal. Our scope was to manipulate the behaviour, i.e. to interfere with the female-male communication in order to affect the mating process of the individuals that survived to the adult stage after the essential oils treatment. The vibrations were transmitted to the plant through a vibrational device, which consisted of a plate provided with a mini-shaker on which the pots were positioned. A significantly higher reduction of whitefly population was observed for the combination of the mixture of EO+CT with the vibrational disturbance signal in comparison to the positive (a commercial pesticide) and negative (water) controls and to the other treatments (essential oils and vibrations, separately).

Macroevolutionary analyses provide new evidences of phasmids wings evolution as a reversible process

R

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The concept that complex ancestral traits can never be re-acquired after their loss has grown popular since its initial formulation and it's often referred to as Dollo's law. Nonetheless, several macroevolutionary evidences - along with molecular ones - suggest instances where complex phenotypes could have been lost throughout a clade evolutionary history and subsequently reverted to their former state in derived lineages. One of the first and most notable rejection of Dollo's law is represented by wing evolution in phasmids: this polyneopteran order of insects - which comprises stick and leaf insects - has played a central role in initiating a long-standing debate on the topic. In this study, a new and comprehensive molecular phylogeny of over 300 Phasmatodea species is used as a framework for investigating wing's evolutionary patterns in the clade, taking into consideration several sources of uncertainty and all the methodological recommendations which have been proposed to test Dollo's law rejection. Macroevolutionary analyses support a dynamic and reversible evolution of wings, with multiple transitions to ancestral states taking place after their loss. Our findings suggest that neither wings or flight have acted as drivers of Phasmatodea species diversification and that brachyptery is an unstable state, when not co-opted for non-aerodynamic adaptations. Our findings demonstrate that wings evolution can be a reversible and dynamic process in phasmids and contribute to shape our understanding of complex phenotypes evolution.

Innovative processes for lipid extraction from bioconverter insects, qualitative and quantitative evaluation and industrial applications for the formulation of personal care products

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The use of insects as a new source of lipids and proteins is a topic of great interest from both environmental and economic point of view. The bioconversion process mediated by the saprophagous insect *Hermetia illucens* is very advantageous because from substrates of low economic and biological value, as for example agri-food by-products or zootechnical waste and catering waste, only for scientific purpose, it is possible to obtain products of high commercial value. My PhD project proposes the extraction of lipids and the use of certain fatty acids, principally lauric acid, for the formulation of new products for personal care (shampoo, detergent, soap) from *H. illucens*. Different types of organic by-products will be used to feed the insect by exploiting its incredible bioconversion performances. *H. illucens* ability to bioconvert organic substrates into valuable products represents a valid solution to the request for the implementation of sustainable processes in a circular economy perspective. The project involves the construction of a biofabric (bio-farm) for the large-scale breeding of *H. illucens*, suitable for the bioconversion of agri-food by-products. A massive production of larvae reared on the "best diets", in terms of both lipid component and lauric acid content of larval biomass, will be carried out. Extracted fats will be used for experimental trials on formulation of products for personal hygiene.

Locomotion of social caterpillars is influenced by polarised light, detected by a single pair of stemmata

R

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Processionary caterpillars of *Thaumetopoea pityocampa* (in Europe) and *Ochrogaster lunifer* (in Australia) (Lepidoptera: Notodontidae) form single files of larvae crawling head-to-tail when moving to feeding and pupation sites. We investigated whether the processions are guided with polarisation vision. The orientation of processions could be manipulated with linear polarising filters held above the leading caterpillar. Orthogonal rotations of the filter resulted in orthogonal changes in procession heading angles. Anatomical analysis indicated specialisations for polarisation vision of stemma I in both species. Stemma I has a rhabdom with orthogonal and aligned microvilli, and an opaque and rugged surface; which are optimisations for skylight polarisation vision, similar to the dorsal rim of adult insects. Stemmata II-VI have a smooth and shiny surface and lobed rhabdoms with non-orthogonal and non-aligned microvilli; thus, optimised for general vision with minimal polarisation sensitivity. Behavioural and anatomical evidence reveal that polarised light cues are important for larval orientation and can be robustly detected with a simple visual system.

Lepidoptera Tortricidae: zoogeography of the Matese Massif and Gargano Peninsula (Central-South Italy)

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A zoogeographic study on Lepidoptera Tortricidae found in Matese Massif and Gargano Peninsula, using chorological analysis, was carried out. The Matese Massif is characterized by a precise altimetric zoning, with deciduous broad-leaved trees, oak and chestnut groves in the lower areas and beech woods and pastures in the higher areas. Gargano Peninsula, instead, features a thermo-Mediterranean vegetation in the northern sector with deciduous forests and evergreen vegetation in the southern area. The tortricid specimens were caught mainly by light during night-time, mostly using UV-LED light traps, but also by using butterfly nets during day-time. The material was primarily identified morphologically, supplemented by molecular data of the COI barcode region. As for the flora, Gargano Peninsula may be considered as a strip of land in the Balkans. Flora of eastern origin has landed on Gargano Peninsula undergoing speciation processes. Fauna is closely related to flora and this is shown also by our zoogeographic study, where the species with Turanic-European chorotype on Gargano Peninsula (10.9%) are more than on Matese Massif (7.8%); while *taxa* with European distribution are less on Gargano Peninsula (2%) than on Matese Massif (17%). What has been said before was also confirmed by the finding of *Ancylis minimana* (Caradja, 1916), new record for Italian fauna, previously known only in Eastern Europe, Ciscaucasia, and Ural Mountains.

Antagonistic and mycoparasitic microorganisms as potential tools for controlling the invasive ambrosia beetle *Xylosandrus compactus*

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Ambrosia beetles (Coleoptera, Curculionidae, Scolytinae) are among the most damaging pests of woody plants. *Xylosandrus compactus* (Eichhoff), also known as the black twig borer, is a subtropical species native to Asia that was recently recorded as a serious pest of Mediterranean trees and shrubs. The beetle performs most of its life cycle within the host wood, feeding on *Ambrosiella* spp. mutualistic fungi. The cryptic behaviour and the beetle broad host range negatively affect the efficacy of conventional control strategies. In this context, biopesticides (e.g. mycoparasitic fungi and antagonistic bacteria) could represent sustainable alternatives. Thus, we evaluated, in laboratory conditions, the antagonistic activity of five *Trichoderma* spp. and two *Bacillus* spp. based biofungicides against the mutualist *Ambrosiella xylebori* and their impact on beetle brood production. Microbial experiments were carried out *in vitro*, while *in vivo* beetle bioassays were conducted by using carob twigs previously treated with the tested biopesticides. The impact in terms of gallery establishment, mutualist growth and beetle brood production was thus evaluated as a proxy of pest control efficacy. Results demonstrate the ability of all biopesticides to strongly reduce the beetle progeny by outcompeting the mutualist. Specifically, a significant reduction of the mutualist mycelial growth was observed *in vitro* for both *Bacillus* and *Trichoderma* biofungicides. Moreover, *in vivo* bioassays showed a beetle mean brood size ranging from 23.1 (control) to 0.8 (*T. atroviride* SC1). Overall, we got evidences for a potential of the tested microbials to be included as innovative tools in sustainable IPM programs targeting *X. compactus*.

Microbial symbioses in Hemiptera, a cutting-edge target for pest control

SENIOR

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Symbiotic interactions with microbes are widespread in many insect orders, resulting in a diversity of effects on the hosts' life cycle. In the Hemiptera, obligate or facultative symbioses with bacteria have particular evolutionary significance in consideration of their nutritionally restricted diets, which are mostly based on plant saps, lacking of many essential nutrients. Moreover, the Hemiptera often posses a reduced immune repertoire, hence relying on symbiotic interactions to integrate their defenses from adversities. The huge diversity of roles played by bacteria in this order can be exploited for developing insecticide-free pest control strategies, defined as symbiotic control. Such an approach can be applied either by establishing heterologous associations, by genetical modification of symbionts, or by suppressing obligate symbionts. Several strategies have been proposed so far, mainly to block the transmission of insect-vectored plant pathogens by delivering antagonistic symbiotic strains, either natural or engineered. However, a critical aspect is the systemic administration of symbionts to vectors. In contrast, pest management strategies based on the suppression of essential symbionts may be much more easily applied by manipulating symbiont vertical transmission. Such an approach has been successfully proposed against the family Pentomidae, where obligate gut symbionts are smeared on the egg surface by the mother, and subsequently orally acquired by newborns. Treatment of egg masses with antimicrobial substances results in quick mortality of emerging nymphs in many pentatomid species. The novel way of action proposed for this strategy allows the integration of a sustainable tool in IPM protocols against pentatomid pests.

The use of entomopathogenic nematodes to control the cabbage stem flea beetle (*Psylliodes chrysocephala*)

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The cabbage stem flea beetle is economically the most important flea beetle species affecting winter oilseed rape in the UK. Options to control the cabbage stem flea beetle using synthetic insecticides have been reduced in recent years. The European Commission restricted the use of neonicotinoid insecticides in December 2013 before banning them completely in April 2018. From 2015 onward the only available foliar applied insecticides used for cabbage stem flea beetle control have been pyrethroids, but since 2014, widespread resistance to this class of insecticide in the UK has been reported. New approaches to the control of this pest are, therefore, urgently needed.
Entomopathogenic nematodes may offer one alternative to the control of cabbage stem flea beetles. Indeed, the nematode species *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* have previously been shown to be capable of killing cabbage stem flea beetles and other related species of flea beetles in the genus *Phyllotreta*.
In the present study, we investigated the potential of *Steinernema feltiae*, *Steinernema carpocapsae*, *Steinernema kraussei* and *Heterorhabditis bacteriophora* to control the adults of cabbage stem flea beetle in a laboratory setting. We carried out three consecutive bioassays to measure the cumulative mortality of cabbage stem flea beetles every two days for a week after inoculation of nematode solutions in three doses: 4,000 nematodes/ml, 10,000 nematodes/ml and 40,000 nematodes/ml. All nematode species showed encouraging results, and this was particularly true for *S. feltiae* and *H. bacteriophora* for which we recorded a maximum mortality of 100% at the highest doses.

Development of smart detection tools for some honey bee pathogens and preliminary survey on the sanitary status of honey bees in Morocco

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In the last decade, the loss of the honey-bees population has been continuously increasing worldwide; this phenomenon is referred to as colony collapse disorder. Several causes are associated with this situation, including *inter alia* honey-bee infections by various pathogens. In the present study, protocols for rapid and sensitive diagnosis of some pathogens were set up and tested during a preliminary survey in three Moroccan regions. In this context, new primer pairs were designed and validated in qPCR for *Nosema ceranae*, *Aspergillus flavus*, *Paenibacillus larvae* and Black queen cell virus (BQCV). Preliminary monitoring of the main honey-bee pathogens in Moroccan apiaries was performed using the newly developed protocols and the already reported RT-PCR for the detection of Bee macula-like virus (BeeMLV), Deformed wing virus (DWV) and Slow bee paralysis virus (SBPV).

Effects of Smoke Waters on *Bactrocera oleae*

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The pyrolysis of organic feedstock yields the biochar, bio-oils, and a volatile fraction that can be reused for technological applications in agro-ecosystems in the form of smoke-water (SW). This study examined the effects on Olive Fruit Fly (OFF - *Bactrocera oleae*) of 10 different SWs obtained at 300 °C and 500 °C (pyrolysis temperatures) from cellulose, wood sawdust, olive mill residues, maize, and alfalfa litter. The assays were conducted at two different concentration using a dynamic airflow glass Y-tube olfactometer and the results were compared with a positive control of fresh green olives. Data concerning the selective choices of *B. oleae* were statistically analyzed using the χ^2 test ($P < 0.05$). The analysis of the data show that the volatile compounds of SW significantly modified the behavior of *B. oleae*. A strong repulsive effect has been recorded with the two dilutions of SW from cellulose obtained at 300 °C (50% for males and 60% towards females) and wood sawdust at both temperatures ranging from 50% up to 70% depending on sex and SW concentrations. Further studies are ongoing to confirm the repulsive effect in semi-field and open-air tests, and to complete the chemical and biological characterization of the active volatile organic compounds (VOCs).

Pentatomoidea (Hemiptera: Heteroptera) of "Alta Murgia" National Park, Southern Italy

S

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The Pentatomoidea fauna was investigated in the "Alta Murgia" National Park, a Special Protection Area (SPA) and Site of Community Importance (SCI) IT9120007, located in the SE-Italy, extending on Apulia and Basilicata Districts (Southern Italy). The study was aimed at detecting their abundance in rural and agricultural areas, and monitoring the presence of *Halyomorpha halys*, already reported in Apulia District in the recent years. The sampled area is characterized by a Mediterranean climate and a variety of habitats. The sampling was carried out from May to October 2020, but is still in progress, and it was performed collecting specimens in four different habitats: forest both "Eastern white oak woods" of *Quercus pubescens* and pine forest mainly composed by *Pinus halepensis*, dry grassland characterized by "Eastern sub-Mediterranean dry grasslands (*Scorzoneratalia villosae*)" and "Pseudo-steppe with grasses and annuals of the *Thero-Brachypodietea*", as well as cereals and olive orchards. The abundance and richness of the species have been recorded for each habitat. Fifty-one species of Pentatomoidea, belonging to 35 genera, have been currently identified, recording the higher abundance in dry grassland habitats, with the prevalence of Pentatomidae and Scutelleridae. Among them, *Ellipscocoris* spp. represents a new record for Italy (paper *in prep.*), and *Holcogaster fibulata, Cyphostethus tristriatus, Acrosternum millierei* are new records for Apulia. Additionally, also the rare species *Ventocoris falcatus* and *Vilpianus galii* were collected. In contrast no individual of *H. halys* was collected.

Inter- and intra-specific competition in Bethylid wasps

R

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Intraspecific and interspecific competition is poorly studied in Bethylid wasps, a very complex family of parasitoids that includes genus with different degrees of sociality. For examples, *Goniozus* species are sub-social and aggressively excludes conspecific females from the brood, showing an increasing competition when multiple foundress are present on the same victim. Conversely, *Sclerodermus* species are quasi-social parasitoids, with foundress that share the oviposition site and take care of the brood all together. However, until now the information on the competition among *Sclerodermus* sp. foundress is poor. A major degree of competition can occur in interspecific rivalry. In this case the competition can be influenced by the prior ownership of the host as well as by the parasitoid and host size, the biology of the parasitoid, the oviposition time, etc. No study about interspecific competition in *Sclerodermus* sp. is present in literature so far. The present study aims at evaluating both inter- and intra-specific competition in *Sclerodermus brevicornis.* Tests have been set up to evaluate 1) the behavior of *S. brevicornis* in case of overexploitation of the host, forcing intraspecific competition with increasing number of foundress on the same victim (10-55 foundress/victim); 2) the effect of the degree of kinship in intraspecific competition (none, two siblings, all siblings) on different behavioral traits (cooperation, ability to parasitize, number of eggs, number of offspring., etc.); 3) interspecific competition occurring in the case of an encounter on the same host between a more aggressive species (*Goniozus legneri*) and a more cooperative one (*S. brevicornis*).

Development of sexing systems functional to mass production of Aedes albopictus Skuse sterile males

R

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Methods currently used to control *Aedes albopictus* Skuse are not only insufficient to ensure its reduction under acceptable sanitary threshold, but they are also sources of environmental pollution. For these reasons, over years many studies have been carried out to find alternative methods to control this insect vector able to transmit Dengue, Chikungunya, Zika and Yellow Fever viruses.
One of the possible environmentally friendly alternatives for the control of *Ae. albopictus* is the sterile insect technique (SIT) which involves the release of adult males, previously treated with ionizing radiations in order to induce sterility into the wild population. Continuous releases of sterile males, in combination with traditional control strategies could be a valuable tool to suppress or eradicate the wild population, especially in those areas where *Ae. albopictus* represents a newly imported invasive species that is not yet heavily spread into the territory.
However, the presence of females in the SIT release campaigns must be avoided or strongly reduced. In fact, even if completely sterilised, the females maintain their feeding activity and vectorial capacity. Moreover, their presence in the released insects can affect the dispersal and mating rates of the males, thus reducing the cost effectiveness of the SIT.
Sex separation of *Ae. albopictus* can be effectively achieved by exploiting different biological traits between males and females at either pupal or adult stage such as protandry, dimorphism or by investigating classical non transgenic genetic sexing strains.
Currently the available sexing methods are not mechanized and are based on manual procedures, therefore they are not capable to assure an effective separation and are strongly affected by operator procedures.
My PhD project, here presented, has the purpose to investigate new methods for an effective and reliable sex separation of *Ae. albopictus* at different stages in accordance with the European regulations, which limits the creation and use of genetic modified strains.
The implementation of a hyper-proterandry strain through a classical cross-breeding method and the production of an optical sexing system integrated with an artificial intelligence able to sort sexes at adult stage are the main lines of investigation during my current studies within the PhD in Health, Safety and Green Systems.

Improving longhorn and jewel beetles trapping protocols for maximizing bark and ambrosia beetle (Coleoptera: Curculionidae, Scolytinae) catches

R

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Wood-boring beetles (Coleoptera: Curculionidae, Cerambycidae, Buprestidae) are commonly moved among continents within wood-packaging materials, fresh timber, and woody plants. Visual inspections of goods at points-of-entry are often complemented with baited traps set up in surrounding natural areas. Given the high costs of these activties and the impossibility to predict the incoming species, trapping protocols that simultaneously attract multiple taxa are needed. We investigated whether trapping protocols commonly used to detect longhorn and jewel beetles can be exploited also for detecting bark and ambrosia beetles (Coleoptera; Scolytinae). In factorial experiments conducted in 2016 in Italy (9 semi-natural and 8 reforested forests in Veneto and Friuli-Venezia Giulia regions) we tested the effect of trap color (green vs purple), trap height (understory vs canopy), and attractive blend (hardwood-blend for broadleaf-associated wood-boring beetles vs ethanol) on the total number of species and individuals collected, as well as on individual species. In semi-natural forests understory traps performed better than canopy traps both in terms of individuals and number of species. Purple traps were generally more attractive than green traps. In particular, purple traps caught more individuals in semi-natural forests, and more individuals and species in reforested forests, although only in the understory. Hardwood-blend and ethanol performed almost equally in attracting ambrosia beetles, even if ethanol outperformed the hardwood-blend in reforested forests. In general, we showed that protocols for generic surveillance of longhorn and jewel beetles may also be exploited for survey of ambrosia beetles, even if some adjustments are necessary depending on the forest type.

Effect of soil microplastics on the behaviour of fungus gnats (Diptera: Sciaridae)

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Microplastics are recognized as pollutants of global interest. In agricultural soils, major incomes are represented by the run-off of particles from the surrounding environment or by direct contamination from agricultural plastic materials. Microplastic pollution can negatively affect the soil microorganisms, invertebrate biodiversity, and the related biological processes.
We investigated whether high-density polyethylene (HDPE) microplastics can interfere with the oviposition behaviour of a herbivorous insect. We used economically important insect pests, i.e. fungus gnats (Diptera: Sciaridae), and lentil (*Lens culinaris* Medik.) as model system.
Using one-way olfactometer, we recorded the female's attraction when exposed to odours from plants subjected to different concentrations of HDPE in the substrate (0-5%) and different watering regimes. Attraction of females was lower toward plants maintained with 5% HDPE compared to 0% HDPE. However, the response changed with different watering conditions. We demonstrated that HDPE interferes with the plant-herbivore communication and the effect is similar to that induced by a moderate water stress. More investigations are ongoing to clarify the mechanisms in this and other trophic systems.

Uncertainty in molecular phylogeny and incongruence of morphological taxonomy throughout the systematics of four Euphasmatodea families

S

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In the past two decades molecular phylogenetics challenged the traditional taxonomy of Phasmatodea based on morphological observation; yet, the different molecular phylogenetic hypotheses which have been proposed are conflicting in nature and contributed to the difficulty in interpreting morphological observation. Here we explored the uncertainty in molecular phylogeny and the incongruence of morphological taxonomy in a phylogenetic framework for the subfamilies Cladomorphinae, Diapheromerinae, Necrosciinae and Lonchodinae, along with Phylliinae and Aschiphasmatinae. We generated roughly 8 kb of DNA sequences from seven nuclear and mitochondrial loci for 94 new species and analyzed them along with 55 species coming from previous molecular studies, leveraging the most comprehensive taxon samplings for these clades so far. Our results show how both molecular and morphological approaches cannot disregard each other and should be combined to build a solid taxonomy and phylogenetic framework for phasmids. Each approach has specific limitations: molecular phylogenetics incurs in an extremely scarce phylogenetic signal, with topology being very sensitive to model misspecification and traditional nodal support metrics concealing uncertainty; morphological taxonomy is instead often affected by the convergent evolution and extreme plasticity of several features. Using an integrative approach, we present strongly supported and necessary changes to the taxonomy and systematic of the clade, particularly in the traditional subfamilies Cladomorphinae and Diapheromerinae; moreover, we uncover and correct discordances that have recently occurred in the naming and definitions of clades caused by the defiance of taxonomic rules and type-taxa.

Stem blight and dieback of blueberry in Northern Italy

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Highbush blueberry represents an important crop due to its relevant commercial value and fruit health benefits. The emerging cultivation in new areas and the increasing global trade of berries led to the spread and major incidence of pathogens affecting to this crop. Stem blight and dieback associated with different fungal pathogens are the most common symptoms observed, and represent serious threats to blueberry production. Surveys were conducted in highbush blueberry orchards in Cuneo province, Northern Italy, to assess the fungal species diversity associated with stem blight and dieback. A total of 38 isolates were collected from symptomatic plants of the cultivars 'Last Call', 'Blue Ribbon' and 'Top Shelf'. Four fungal species were identified through a multi-locus phylogeny and morphological characters: *Neofusicoccum parvum*, *Diaporthe rudis*, *Cadophora luteo-olivacea* and *Peroneutypa scoparia*. This is the first report worldwide of *P. scoparia* and *C. luteo-olivacea* on *Vaccinium corymbosum*, as well as the first report of *D. rudis* on blueberry in Italy. Molecular analyses were based on three different genomic regions: ITS, *tub2*, and *tef1*. Pathogenicity tests were conducted on plants of the representative cultivar 'Duke'. All the tested species were pathogenic to blueberry plants. *Neofusicoccum parvum* was the most aggressive species, followed by *Diaporthe rudis*. *Peroneutypa scoparia* and *Cadophora luteo-olivacea* were the least aggressive. The present study increases understanding of the fungal species diversity associated with blueberry stem blight and dieback, providing preliminary knowledge for further studies on disease epidemiology and management strategies.

Impacts of pesticides on non-*Apis* pollinators

R

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The western honeybee, *Apis mellifera*, is the model species for pesticide risk assessment on pollinators with the assumption that worst-case scenarios for honeybees are sufficiently conservative to protect other pollinator species. However, there is increasing evidence that due to differences in life history traits and sensitivity, other species may be more vulnerable to plant protection products. For this reason, it is important to extend the risk assessment to other pollinator species and to develop standard protocols for them.
To cover these gaps, we aimed to test pesticides in alternative insect pollinators at different development stages and to develop new methodologies that can be used in risk assessment. We used larvae of the solitary bee *Osmia bicornis*, and adults of the tachinid *Exorista larvarum,* as study species. In nature, *E. larvarum* plays a role as pollinator, besides its well-known activity as a larval parasitoid of Lepidoptera.
Field-realistic doses of a neonicotinoid and a fungicide alone and in mixture were applied to the food provisions of the *O. bicornis* larvae. Preliminary results showed no significant effects on larval development or mortality but we expect possible effects on adult post-emergence performance. In *E. larvarum* we assessed lethal and sublethal effects following an acute contact exposure to different doses of a neonicotinoid. When considering the median lethal dose (LD_{50}) our results showed a lower sensitivity of *E. larvarum* compared to the honeybee (520.96 and 81 ng/insect, respectively). However, sublethal effects on fecundity and fertility were observed at doses as low as 40 ng/insect.

How to escape an evolutionary trap: fitness-related effects and behavioral responses of the egg parasitoid *Trissolcus basalis* developing on *Halyomorpha halys*

R

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Accidental introduction of invasive species in non-native environments is a common by-product of globalization. The brown marmorated stink bug *Halyomorpha halys*, is a polyphagous stink bug pest of Asian origin that has invaded both North America and Europe. This species can act as an "evolutionary trap" for resident egg parasitoids because *H. halys* eggs are accepted, yet most of the parasitoid eggs fail to develop. This is the case of *Trissolcus basalis*, the main egg parasitoid associated with the green stink bug *Nezara viridula*. Interestingly we found that *T. basalis* females that completed development on *H. halys* eggs are about 25% larger than regular *T. basalis* that emerged from the associated host *N. viridula*. As size is an important component of the wasp fitness, we evaluated, under laboratory conditions, the behavioral response of *T. basalis* females that developed on *N. viridula* and on *H. halys* and compared wasp foraging behavior in open arena contaminated with stink bug contact kairomones. We found that wasp residence time was significantly affected by the size of the wasps, indicating that the bigger the size of the wasps lesser is the time they spent on the open arena. We discuss our findings in the light of the evolutionary trap framework.

Species-habitat networks: a promising tool in applied entomology

SENIOR

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Understanding how arthropod species use multiple habitats across agricultural landscapes is essential for the design of effective management strategies to control pests or to support beneficial species. We propose adapting existing bipartite network tools to create species-habitat networks that explicitly describe the associations between species and habitat resources. This approach can describe not only single habitat and species roles across the landscape, but also emerging properties of whole habitat networks. The versatility, visualization tools, and easy interpretation of bipartite networks enable its application to a wide range of applied entomological problems. Here, I will describe the framework and then exemplify its application in two case studies. First, I will apply the method to determine how specialization of pollinators and natural enemies change along gradients in landscape structure, focusing on the role of semi-natural habitats to support their populations. Second, I will describe the spatio-temporal dynamics of *Xylella fastidiosa* vectors across heterogeneous landscapes to identify the role of different patches as source or sink habitats of vectors that can colonize olive groves. One key advantage of the application of the framework is that the scale of the derived ecological information matches the scale of landscape management. In particular, species-habitat networks can help identifying optimal landscape compositions and configurations to design effective interventions to manage both ecosystem services and disservices delivered by arthropods.

Riparian vegetation influences *Halyomorpha halys* egg parasitoids impact in kiwifruit orchards

S

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In north-eastern Italy, the invasive alien species *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is recognized as major pest of kiwifruit and other fruit crops since its first establishment in 2012. The discovery in northern Italy of populations of Asian egg parasitoids *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae) in 2016 and *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) in 2018 set the basis for investigations on their biological control potential on *H. halys*. This study was conducted in 2019 and 2020 collecting naturally laid *H. halys* egg masses in 13 kiwifruit orchards in north-eastern Italy. Fruit orchards were categorized for the presence of ecological infrastructures on their proximity (i.e., hedgerows) and the distance from riparian vegetation buffers. The distance from the riparian vegetation explained most of the variation in parasitoid impact detected in kiwifruit orchards. Results show a negative relationship between the distance from the riparian vegetation and the egg parasitoids impact on *H. halys* egg masses. Indeed, parasitism rates on *H. halys* were higher in kiwifruit orchards close to rivers as respect to those far away.

Characterization of antimicrobial peptides deriving from insects and their application in the biomedical field

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The still current problem of antibiotic resistance has led research to find new solutions to fight infections by pathogens, alternatives to modern antibiotics. Antimicrobial peptides (AMPs) are excellent candidates because they are less prone to trigger resistance and are specific toward bacterial cells. AMPs are small molecules produced by all living organism but insects, in particular the Black Soldier Fly (BSF) *Hermetia illucens* (Diptera: Stratiomyidae), represent one of the richest and most innovative sources of these molecules. Following the transcriptome analysis and the functional annotation with the Blast2go software, it was possible to identify 82 putative antimicrobial peptides deriving from the BSF. All the sequences coding for AMPs were translated into the amino acid corresponding sequences verifying the completeness at 3' and 5' ends and the presence of the Signal Peptide was evaluated. The 57 sequences that have met these requirements, were then bioinformatically analyzed in order to predict their antimicrobial, anticancer antiviral and antifungal activity; the physical-chemical properties were calculated and the 3D structures were *ab initio* modelled. Then, the production of these peptides was started through the recombinant way for the larger peptides (more than 50 amino acids) and via chemical synthesis on the solid phase, exploiting the chemistry of the Fmoc protecting group, for the smaller peptides (less than 50 amino acids). Moreover, the identification of the AMPs was also performed through the direct extraction of the BSF larvae hemolymph, which were infected with *Escherichia coli* and *Micrococcus luteus* in order to induce the AMPs production.

Sub-lethal effects of industrial crops on honeybee behaviour

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The use of pesticides in industrial crops may cause negative impact on non-target insects, notably pollinators including honeybees. Besides lethal effects, pesticides often show significant sub-lethal effects, e.g. on insect behaviour, that are more difficult to assess. A well established procedure to evaluate pesticide effect on bee behaviour is the *proboscis extension response assay* (PER), which is based on the temporal paired association of a conditioned stimulus (CS) and an unconditioned stimulus (US). We collected honeybees from beehives located close to fields that were cultivated according to IPM strategies, and from beehive slocated in uncultivated fields. Laboratory bioassays are ongoing to reveal the effects of insecticide treatments in industrial crops on the honeybee behaviour. Results will be presented during the meeting.

Arthropod response to forest landscape dynamics

R

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Forests are important semi-natural habitats because they deliver many ecosystem services, such as wood, water balance, soil protection, biodiversity and recreation. However, abiotic (e.g. wind, fire) and biotic (e.g. pest outbreaks) disturbances, as well as management (e.g. timber production) contribute to quickly change forest cover. On the other hand, meadow abandonment slowly leads to an increment of wood. Here, we focused on two processes: the suspension of meadow management and the uprooting following a massive windstorm in NE Italy. First, we carried out an observational experiment, and we focused on spider community response to abandoned habitat. In Veneto Prealps, suspension of human management led to shrubland expansion in the last decades. We aimed at understanding how patches interact among them by sharing species at landscape scale. To address our purpose, we applied a recent landscape ecology approach (i.e. species-habitat network). As second experiment, we focused on wind disturbance outcomes on arthropod communities and pest population dynamics. At the end of October 2018, an intense windstorm called Vaia occurred in NE Italy causing massive damages on spruce forests. During summer 2020, we carried out a multi-taxa sampling in the protected area of Paneveggio-Pale di San Martino to understand how spiders and ground beetles reacted to this windstorm event. As conclusion, we showed that both long-term and short-term dynamics might contribute to improve landscape heterogeneity and to deliver habitats for species support. However, arthropod response could be different according to taxonomic group and environmental conditions.

Symbiotic control of the brown marmorated stink bug *Halyomorpha halys*

S

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Obligate bacteria symbioses are common in the order Hemiptera, which includes many important pests in agriculture, such as stink bugs. In the family Pentatomidae, bacteria are often related to the genus *Pantoea*. These symbionts are orally acquired by newborns from maternal secretions smeared on the egg surface, and they essential for growth, development, and survival of the insect. Aposymbiotic stink bugs usually display reduced survival or fitness. The most studied pentatomid species is *Halyomorpha halys* (Stål), one of the major damaging agriculture pests, due to its invasive potential and high polyphagy. This research project aims at characterizing the biological and molecular mechanisms regulating the anti-symbiont activity of different antimicrobial compounds and microbiological agents, capable to prevent nymph survival by avoiding symbiont acquisition from the egg surface. The effect on the gut bacterial community after the products' application will be evaluated on nymphs emerging from treated eggs as well. Furthermore, studies on the interaction of these treatments and the activity of natural enemies, such as indigenous and exotic egg parasitoids and different orders of predators, will be carried out. The results achieved will permit to significantly increase the current understanding of interactions between stink bugs and their primary symbionts. Additionally, this work will support the design of multi-target solutions against the main stink bug species threatening different crops, providing an effective reduction of insecticide treatments, protecting agricultural productions in terms of yields and quality.

Effect of temperatures on embryonic development of the forest pest *Barbitistes vicetinus* (Orthoptera, Tettigoniidae)

S

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Barbitistes vicetinus is an endemic forest pest recently described and spread almost exclusively in the Veneto region. Since 2008, severe outbreaks occurred in Euganean Hills, affecting the whole hillside area.
In order to understand the role of environmental factors that could lead to outbreaks, we investigate how different temperature regimes could affect the development of eggs of *B. vicetinus* during the seasons. An egg can hatch in the year following the oviposition reaching the final diapause or remain in the soil for many years in initial diapause.
We selected 30 different sites with different types of exposition and elevation: 18 in the Euganean Hills and 12 at Monte Cavalletto. In each site, we buried in the ground 200 newly laid eggs of *B. vicetinus* divided in 5 plastic cups. For each site we buried a datalogger to register the data of the temperature under the ground.
Furthermore, to clarify the effect of different temperatures on diapause, we placed 240 eggs in each of 6 climatic chambers along a thermal gradient (19°C to 24°C).
We found that high number of eggs reach final diapause in sites that show higher mean temperatures during the summer. Similar percentages of diapause were found in the field and at constant temperatures in the chambers.

Effects of the plant beneficial microorganism *Trichoderma harzianum* on the microbiota of *Spodoptera littoralis* larvae

R

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The microbial communities associated with insects play important roles in insect nutrition, development and reproduction. We evaluated the impact of plant treated with the beneficial microorganism *Trichoderma harzianum* (strain T22) on the midgut microbiota of *Spodoptera littoralis* larvae using a culture independent approach targeting a fragment of the bacterial 16S rRNA gene. The larvae were fed with leaves from tomato plants treated with *T. harzianum* T22 or with leaves from control plants (i.e. treated with water), and two instars were examined (IV and VI instars). Taxa usually found in insects, such as representatives of Firmicutes, Bacteroidia and Proteobacteria, resulted abundant in all samples as well as *Enterococcus mundtii*, a well-known symbiont of *S. littoralis.* Fourth instar larvae had microbiotas with the higher richness, with no dominant taxa, while in the VI instar, the richness was lower and different bacterial taxa became dominant. In particular, in the control group, the dominant taxa were Erysipelotrichaceae and *Tyzzerella*; while in the treated group the most represented taxa were Comamonadaceae, *Alicycliphilus* and *Erysipelatoclostridium*. The treatment was most effective on the VI instar, where it determined a significant taxonomic shift in the dominant bacterial taxa present. These results suggest that the detrimental effect experienced by phytophagous larvae feeding on plant treated with *T. harzianum* could be mediated, at least in part, by the insect microbiota.

Use of sugar dispensers to disrupt ant attendance and improve biological control against Pseudococcidae mealybugs in vineyard

R

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Nowadays, Pseudococcidae mealybugs represent one of the most damaging pests in Italian vineyards. Besides *Planococcus ficus*, *Pseudococcus comstocki*, a mealybug species native to Eastern Asia, is causing several damages on vine plants, resulting in important economic losses for growers.
The relative inefficacy of insecticides, and their high impact on environment as well as human health, have led to the research of alternative and sustainable control methods, including biological control. Natural enemy activity may be hindered by tending ants, which are known to create a mutualistic relationship with mealybugs, resulting extremely aggressive against beneficial insects. Consequently, this study explored a method to mitigate ant attendance by means of sugar dispensers in order to improve ecosystem services, as well as mealybug infestation, in vineyard ecosystem. Field trials were employed in four vineyards of Northern Italy, in which *Anagyrus sp*. near *pseudococci* and *Cryptolaemus montrouzieri* were released as biological control agents against Pseudococcidae mealybugs. Ant activity was estimated counting the number of ants crossing an imaginary line placed on vine trunk during 1 minute period. The rate of infested grapes was assessed by a visual sampling, whereas clusters were collected and analysed in laboratory to estimate parasitization and predation rates.
The use of sugar dispensers reduced ant activity and enhanced ecosystem services, showing great potentiality in boosting biological control against mealybugs.

Chemical and electrophysiological investigations on the sex pheromone of the asparagus moth, *Parahypopta caestrum*

S

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The Asparagus moth, *Parahypopta caestrum* (Hübner) (Lepidoptera, Cossidae), is one of the most damaging pest of *Asparagus* spp. in Europe. The soil-borne larvae of this pest bore galleries into the roots and the shoots causing the destruction of plantations after 2-3 years. The cryptic habitats of larvae makes it difficult to control this species even using synthetic pesticides. The identification of chemical cues involved in interspecific interactions of *P. caestrum* could greatly contribute to the development of effective monitoring tools and sustainable control means. To date, no studies have attempted to identify semiochemicals of this species, therefore *preliminary investigations on* the presence of a female sex pheromone in *P. caestrum were carried out during* the first year of the Ph.D. programme. Virgin females were obtained from pupae collected in an highly infested plantation in Foggia (Italy) during May. Observation on the calling activity (extrusion of the last urites) of virgin females suggested that sex pheromone emission starts during the first hour of the scotophase. Accordingly, hexane and SPME (100 µm polydimethylsiloxane fibre) extracts from terminal abdominal segments of calling females were prepared. Analyses of solvent and SPME extracts by gaschromatography coupled with mass spectrometry and electroantennographic detection (GC-MS-EAD) revealed four peaks eliciting EAG responses from male antennae. Fragmentation patterns of three active peaks matched those of 12-carbon monounsutured acetates while the remaining EAG-active peak matched those of 12-carbon monounsutured alcohols. Micro-derivatization and EAG experiments are ongoing to define isomerism and double bond position in the carbon chain of active compounds.

Study of the gut microbiota in the green stink bug *Nezara viridula* for symbiont-targeted biological control

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Green stinkbug *Nezara viridula* L. (Hemiptera: Pentatomidae), is one of the most important pentatomid insect pests in the world, that feeds on more than 30 plant families, preferentially legumes. Lately, it has attracted considerable attention through being a pest that is apparently responding to the climate change by a range expansion in many regions, including Europe. Evolutionary success of insects has depended in part on relationships with beneficial microorganisms. Regarding *N. viridula*, studies have focused on an obligate symbiont resident in the V4 ventricle, which has been identified in Brazil, Hawaii and Japan. Egg-surface sterilization disrupts nymphal infection with the symbiont. In some cases, eliminating the symbiont resulted in severe nymphal mortality, while in others it caused few fitness defects in the host. Control measures based on the disruption of symbiont acquisition by nymphs are under development against the pentatomid *Halyomorpha halys* Stål. The objective of this research is to describe the interaction between *N. viridula* and its symbiont in different zoogeographic regions, and to evaluate the use of different products admitted by the UE to remove the symbiont from the egg surface, as a biological control strategy. To that end, both laboratory and field trials will be conducted. This research will reveal the main characteristics of the interaction between *N. viridula* and its associated symbiont in previously undescribed populations, adding information to the worldwide study of the stink bug gut microbiota. Finally, by determining the effects of symbiont's removal, it may open the way to new biological control strategies.

Structure of the bee-plant visitation network in the gradient of landscape complexity

S

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The bees (Anthophila) are considered one of the most beneficial insects due to their role in pollination. The great proportion of wild flowering plants and vast majority of flowering crops need insects for pollination, and the bees are one of the most efficient pollinators among insects. There are examples of a positive relationship between diversity of bee pollinator community and enhancement of pollination ecosystem service. Loss of natural habitats due to agriculture leads to landscape simplification and decrease in diversity of wild bees which is recognized as one of the major issues worldwide. Therefore, it is important to maintain high proportion and diversity of remaining natural habitats in simplified agricultural landscapes. It is of the great importance to evaluate the interaction of bees and plants in different landscape complexity gradients to estimate specialization and vulnerability of the community. During summer season of 2020 we have sampled wild bees on flowering plants in semi-natural habitats in three different types of landscapes based on their complexity defined as percentage of non-arable land cover (Simple, Semi-complex and Complex landscapes). We aim to construct bee-plant visitation network and analyze its complexity and structure in regard to landscape complexity. Preliminary analyses showed that there are no statistically significant differences in average number of wild bee species per plant species between different landscape types. However, more detailed inspection of several plant species common to all three landscape types indicated that there might be some differences in wild bee species composition on the level of individual plants.

Parasitism and phylogeny of *Dinocampus coccinellae* through native and exotic hosts in different continents

R

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The cosmopolitan parasitoid *Dinocampus coccinellae* (Hymenoptera: Braconidae) parasitizes coccinellid beetles but little is known about its parasitism among areas invaded in different times by the invasive *Harmonia axyridis* (Coleoptera: Coccinellidae). Genetic differences among parasitoid populations from different geographical areas could help to determine its origin as well as the possible impact on the *H. axyridis* invasion processes. We compared parasitism by *D. coccinellae* on native and alien coccinellids in the native range of *H. axyridis* (China) as well as areas where *H. axyridis* invaded over the last 100 years (USA), more recently (Chile) and very recently (Sicily). About 6,000 specimens of *H. axyridis* and indigenous coccinellids were collected in several sites located in the four continents. We also investigated the phylogeny of *D. coccinellae* specimens sampled over this geographical distribution using mitochondrial COI and 16S rRNA sequences. The parasitism rates of *D. coccinellae* varied between native and non-native hosts and among the different sampled areas. The geographic area influenced the parasitism rate on *H. axyridis*, ranging from 0 to 8% in Sicily and Chile, respectively. European and American native coccinellids were highly attacked by this parasitoid independently by the sampled area. Parasitoid phylogenetic analyses highlighted variations in parasitoid COI sequences, while no differences in 16S rRNA region were recorded, making it difficult to pose hypotheses regarding the provenance of this species. Further studies on host-parasitoid interactions and on parasitoid genetic structure at narrower scale are needed to elucidate *D. coccinellae* phylogeography and its ecological role in coccinellid population suppression worldwide.

Odorant receptor expression-related modulation in behavior during larval development in African cotton leaf worm, *Spodoptera littoralis*

S

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In insect larvae, the olfactory system is crucial for locating food sources. In several lepidopteran species, food preferences change across instars and we hypothesized that this might be due to changes in the olfactory system, and precisely to instar-specific expression of odorant receptors (ORs). We therefore investigated the expression patterns of ORs in larvae of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) between the first and fourth instar. Among few ORs differentially expressed, one receptor expression was significantly different in RT-qPCR analysis. Using electrophysiological recordings, we detected significant response to β-caryophyllene and α-humulene. Correspondingly, we noticed that first instar larvae were attracted towards β-caryophyllene, while fourth instars were not. Furthermore, we successfully disrupted the function of this receptor using CRISPR-cas9 system that induced a targeted heritable mutagenesis. In the behavioral experiments, the mutant *S. littoralis* larvae were not attracted anymore to β-caryophyllene and α-humulene compared to wild type larvae. Electroantennography recordings on mutant and wild type male and female moths corroborate larval results, as mutant adults responded significantly less to β-caryophyllene and α-humulene than wild type adults. Hence, we demonstrated that the receptor we identified in *S. littoralis* is essential for host plant location in larvae and adults.

In vivo functional analysis of *Aphidus ervi* venom

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The parasitoid *Aphidus ervi* (Hymenoptera, Braconidae) regulates the physiology and reproduction of the host *Acyrthosiphon pisum* (Homoptera, Aphididae) in order to enhance its suitability for the developing progeny. The study of the underlying molecular mechanisms sheds light on the strategies used by the wasp to subdue and exploit its victim, paving the way towards the development of novel bio-inspired strategies for pest control. Here, we investigate, using RNA interference (RNAi), the functional role of the most abundant component of *A. ervi* venom, *Ae*-γ-glutamyl transpeptidase (*Ae*-γ-GT1), known to cause host castration. The silencing of *Ae-γ-GT1* gene expression in *A. ervi* adult females was successfully obtained, through the microinjection into the female pupae the double-stranded RNA (dsRNA) targeting *Ae-γ-GT1*. Parasitism by these wasps allowed to score the phenotypic changes of the host and of wasp's progeny as affected by a venom blend nearly lacking *Ae*-γ-GT1. Surprisingly, this experimental condition induced an increase in size of parasitized aphids 2 days post-parasitization and, 5 days post-parasitization, was found associated with larger wasp larvae and teratocytes (cells deriving from the embryonic membrane of the wasp), as well as with a higher *Buchnera* load. This suggests that Ae-γ-GT1 plays a crucial role in the regulation of the intricate network of interactions among the host, its primary symbiont and parasitoid pre-imaginal stages, which are worth of further research efforts. The methodology developed is *per se* an interesting novel tool that allows to perform an *in vivo* functional analysis *A. ervi* venom component, under very realistic physiological conditions.

Jewels on the go: exotic buprestid around the world (Coleoptera: Buprestidae)

R

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Buprestidae is a beetle family including more than 15.000 species worldwide. All buprestids are phytophagous, having wood boring and leaf mining larvae. Several species can act as pests, causing serious damage to the environment or to human activities such as agriculture and forestry. Due to their long larval development buprestids have been repeatedly introduced by human mean in several countries, where in some cases they become invasive. What do we know about buprestids introduction pattern and how we can prevent invasive and potentially invasive species to spread?

Interactions among plants, essential oils and the omnivorous mirid *Nesidiocoris tenuis*

SENIOR

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Generalist predators are often highly efficient biocontrol agents of various crop pests. Besides, they also feed on various non-pest food substrates. *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is largely employed in biocontrol programs in tomato crops through augmentative releases and conservative strategies. Under certain circumstances, it can cause economic losses by its repeated feeding on tomato plants. However, it has also been proved to induce defensive plant responses. Experiments were conducted: (i) to test the susceptibility of ten tomato cultivars, under different temperatures, to *N. tenuis*; (ii) to evaluate the influence of alternative plants (i.e. *Dittrichia viscosa* and *Sesamum indicum*) on the predator biological performances, damage on tomato plants and its biocontrol services; (iii) to evaluate the potential of plant essential oils to be used in the presence of *N. tenuis* in terms of side effects; (iv) to assess the role of various instars in inducing defensive plant responses.
The mirid damage was different among tomato cultivars although not significantly; while temperature significantly influenced the number of necrotic rings per plant. *Nesidiocoris tenuis* damage on tomato was proved to be mitigated by the presence of selected alternative plants. No significant acute toxicity of most essential oils was recorded, however overall the fertility was negatively affected. All stages of *N. tenuis* can trigger defensive responses in tomato plants, although these responses may be different depending on the stage considered. These results provide further elements on the ecological role of zoophytophagous mirids.

Mechanical ecology in plant-herbivore interaction: role of insect claws of different shape in the attachment to hairy plant surfaces

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During the long period of coevolution between insects and plants, these last developed a wide diversity of features not only to attract pollinators but also to defense against herbivores, such as chemical and physical barriers affecting their performance. In this context, the evolution of plant surfaces and insect pads is an interesting example of competition between insect attachment systems and plant anti-attachment surfaces. Among mechanical defenses, plants developed impenetrable barriers, such as bark and waxy cuticles or thorns and spines, to protect them from herbivores. Non glandular trichomes may impale, entangle or impede the locomotion of insects by physical interactions but some insect species developed adaptations to use them to better adhere to the plant surface. In particular, claws are adapted to interlock with rough surfaces, when the distances between adjacent asperities as well as their heights are larger than the claw tip diameter. The aim of the present study is to clarify the role of claws of different shapes (single claw, single claw with basal tooth, double claws with basal tooth) in the locomotion/adhesion to hairy plant surfaces. In particular the attachment ability of three species of Coleoptera, two Coccinellidae, *Harmonia axyridis*, (Pallas) *Chnootriba elaterii* (Rossi) and one Chrysomelidae *Chrysolina herbacea* (Duftschmid) to natural and artificial substrates characterised by hairy and smooth surfaces will be evaluated. Preliminary results reveal a high attachment ability of Coccinellidae claws (single claw with basal tooth, double claws with basal tooth) to natural and artificial hairy surfaces.

Chemical control of *Popillia japonica* in the management of invasive populations in northern Italy

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Popillia japonica Newman (Coleoptera: Rutelidae) is a beetle native to Japan, recently recorded in Europe (2014, between Lombardy and Piedmont Italian Regions). The insect is currently spreading from the Ticino River Park, initial infested area, to the neighboring areas causing considerable damage to crop, nursery and ornamental sectors due to its high polyphagy and adaptability. The pest is on the list of priority quarantine organisms (delegated regulation (UE) 2019/1702). Therefore, the involved Regions activated all the mandatory control measures in order to eradicate and/or contain the insect. Nowadays, the pest outbreak affected more than 9,653 km^2, in continuous expansion. The appearance of an alien organism requires the integration of agronomic, biological and chemical actions to protect the crops. However, during the early stages of infestation, the use of readily effective insecticides is essential to sustain the immediate needs of the agricultural production system. The current absence of registered products against the species requires experimental tests to evaluate the effectiveness of the active ingredients currently available on the market. Here, we presented the effects of different insecticides registered for adult beetle management in agricultural crops and ornamental plants, evaluated by field trials carried out in 2019 and 2020. Almost all the tested active ingredients, especially acetamiprid and deltamethrin, were effective in killing the adult insects. Other tests against the larvae in the soil are currently performed using both chemical and biological control methods.

Egg parasitoids complex of *Halyomorpha halys* (Stål) in Veneto with particular emphasis on adventive populations of Asian species

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Sustainable strategies such as classical or augmentative biological control are currently being evaluated for the long-term management of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). *Halyomorpha halys* is a pest able to cause economic damage to many crops, in particular in fruit orchards. In this four-year study carried out in Veneto region (northern Italy), we studied the parasitoid complex by collecting naturally laid *H. halys* egg masses. Emerged parasitoids belonged to three Hymenoptera families. *Halyomorpha halys* eggs resulted parasitized by *Trissolcus basalis* (Wollaston), *Trissolcus kozlovi* Rjachovskij (Scelionidae), and *Anastatus bifasciatus* (Geoffroy) (Eupelmidae). Besides these species, already known in the European fauna, *Trissolcus mitsukurii* (Ashmead) and *Trissolcus japonicus* (Ashmead) (Scelionidae) were recorded since 2016 and 2019 respectively. The hyperparasitoid *Acroclisoides sinicus* (Huang & Liao) (Pteromalidae) was also observed. The presence of *T. mitsukurii* in the study area represents the earliest detection of this parasitoid for Europe. Furthermore, this egg parasitoid was the most abundant and exhibited better performances in *H. halys* control than the other parasitoid species. The phylogenetic tree for *T. mitsukurii* distinguished a clade covering samples from Italy, Japan and China, and another one referred to insects collected in South Korea. Phylogenetic results also suggest a single introduction event of *T. mitsukurii* in Italy, which possibly followed invasion pathways of its invasive host.

Use of organic by-products from the agri-food chain to obtain valuable products through bioconversion process

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Bioconversion is a biological process by which organic materials are converted in products with high biological and economic value. During its larval stage, *Hermetia illucens* (Diptera: Stratiomyidae), is able to feed on a wide range of organic materials, including fruits and vegetables. The bioconversion process is highly efficient, as the organic substance the larva feeds on is reduced up to 65%-70% and converted into larval biomass. At the end of bioconversion process, larvae are suitable for pet food as they are rich in proteins and lipids, high energy content flours, deriving from appropriately dried and ground larvae can be used as feed for the aquaculture industry according to European regulation, and the resulting larval frass can be used as soil conditioner for crop fertilization. In order to study the effect of different substrates from the agri-food chain on specific parameters (insect growth, final larval yield, protein and lipid content, substrate reduction), 10,000 larvae were reared on 7.0 kg of strawberries, tangerines, oranges and on a standard diet, used as control. The results highlight that *H. illucens* is able to successfully feed on all the tested diets, although the analysed parameters are impacted by different substrates. Even though the standard diet, balanced in all nutrients, is the best diet for *H. illucens* development, larvae consumed all the by-products, confirming their extraordinary ability to feed and reduce different kinds of substrates. Bioconversion process opens new perspectives for a sustainable and environmentally friendly disposal and recovery of by-products from the agri-food chain.

First evidence of a female-produced pheromone in *Philaenus spumarius*

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Xylella fastidiosa subsp. *pauca* CoDiRO strain is a xylem-limited vector-borne bacterium recently introduced in Italy that is causing significant losses of olive trees. The meadow spittlebug, *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), is currently recognized as the main vector of this bacterium. *P. spumarius* and other Auchenorrhyncha are known to communicate via vibration, however the occurrence of semiochemical communication has been little investigated. Here, through a multidisciplinary investigation involving behavioural assays, chemical analysis and electroantennographic tests, we provide evidence of a female-produced pheromone in *P. spumarius*. In Y-tube olfactometer bioassays, 2-3 weeks old insects of each sex were exposed to the volatiles emitted by virgin males or females of the same age and to their head-space volatile extracts. Males were significantly attracted to females as well as toward the head-space volatile extracts collected from females. Conversely, no female attraction was observed when they were exposed to male volatiles or their extracts. Males and females seem not to respond to volatiles from individuals of the same gender, although these data need confirmation. GC-MS analysis of male and female head-space extracts showed qualitative differences in their GC profiles. Moreover, female extracts elicited a measurable EAG response in male antennae, suggesting the presence of compounds which are perceived by the male's olfactory system. Ongoing studies aim to clarify whether this is a sex pheromone, identify and describe the pheromone glands and elucidate the chemical composition of the pheromone.

RNAi mediated knockdown of essential Central Nervous System genes in two Lepidopteran pest insects

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Currently over 10% of people worldwide are chronically undernourished and with a predicted population of more than 9 billion by 2050, crop yield must be optimised without increasing land use. Unfortunately, crop productivity is decreased by many biotic and abiotic factors such as insect pests. *Tuta absoluta* and *Spodoptera littoralis* are both highly polyphagous Lepidopteran pests that damage a plethora of important crop plants including *Solanum lycopersicum*, one of the most nutritionally important fruits in LMIC. RNA interference (RNAi) is a revolutionary technique for insect pest management which offers greater species-specificity and consequently reduced damage to the environment compared to current control strategies. Four genes essential to the normal functionality of the insect nervous system, three of which are targets of commercial insecticides, were chosen for RNAi experiments. The *ace-1* gene encodes an acetylcholinesterase essential to the breakdown of the neurotransmitter acetylcholine, *para* encodes a voltage-gated sodium channel essential for action potential propagation, nAchR encodes a nicotinic receptor on the postsynaptic muscle cell and NADPH cytochrome P450 reductase is considered essential to the metabolic detoxification of insecticides. The expression of each gene was determined via qPCR at various life stages prior to the synthesis of dsRNA specific to each gene which will be administered in future experiments via (i) direct injection into the haemocoel and (ii) oral feeding, followed by subsequent feeding assays which will (i) test for negative effects on non-target insects and (ii) aim to optimise the efficiency of RNAi in Lepidoptera through the use of nanocarriers.

Use of the Black soldier fly (*Hermetia illucens* L.) as an alternative source of chitin and chitosan for the production of biopolymeric films for agro-food applications

S

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Chitin is one of the most abundant biopolymers in nature, being a structural component of the arthropods' exoskeleton and the fungal cell wall. Its main derivative, chitosan, is non-toxic, biodegradable, biocompatible and has antimicrobial activity. Thus, chitosan is widely used for several applications, including tissue engineering, drug delivery, wound dressing, waste-water treatment, and as antimicrobial coating for the preservation of fresh food.
The current main commercial source of chitin and chitosan comprises waste from the fishing industry, mainly crustacean exoskeletons. This source is no longer sustainable in view of the huge increase expected in the chitosan market in the next years. Among the potential alternatives, insects are getting great attention.
The Black soldier fly, *Hermetia illucens* L., is the most bred insect species in Europe for the production of animal feed, thanks to its ability to efficiently convert organic waste into valuable products (i.e. protein, lipids, frass with soil-conditioning properties). *H. illucens* farming also generates a substantial amount of side streams, such as larval exoskeletons, pupal exuviae and dead adults, rich in chitin.
The present project focus on the production of chitosan from side streams from the *H. illucens* breeding and its application as preservative biodegradable coating for fruit and vegetables. Chitin was extracted with chemical method from *H. illucens* pupal exuviae, obtaining a product comparable to the commercial one. Chitin was then converted into chitosan by different types of chemical deacetylation. The obtained chitosan was characterized in order to investigate its suitability for its application as food coating.

Herbivore induced VOCs, secondary metabolites and enzymatic activity in *Solanum* spp.

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Plants are able to respond to abiotic and biotic stresses from the external environment, including insect herbivores, with complex defence responses which involve resistance and adaptation to survive. Metabolites, mechanical barriers, enzymes and volatile compounds constitute the biochemical and physical, constitutive and induced defense mechanisms of the plants. In this framework, 12 species/varieties of *Solanum* spp. were tested in order to evaluate the plant defence response to the infestation by the tomato key pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). In particular, induced secondary metabolites, such as polyphenols, enzymes involved in oxidation processes (i.e., PPO and POD), and volatile compounds, e.g. terpenes, were identified and characterized. In addition, bio-assays on larval survival, larval development time, pupal weight and eroded leaf area were assessed using cut leaves from healthy plants and from plants previously infested with *T. absoluta* larvae (induced plants). In most of the tested plants, and especially in the tested eggplant variety, had an effect on *T. absoluta* larval survival and feeding activity. Furthermore, many compounds were identified among induced secondary metabolites, especially in wild species, *Solanum pennelli* and *Solanum habrochaites*. Some of the cultivated varieties showed an array of induced volatiles very likely implied in plant communication mechanisms related to their interaction with pests and/or natural enemies. The results suggested that these species/varieties have potential for induced plant resistance against this pest, potential direct antibiosis and antifeedant activities of induced substances on *T. absoluta* behavior and development is evidenced.

The black soldier fly *Hermetia illucens*: an innovative and alternative source of chitin and chitosan

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Chitin is the most widespread biopolymer on Earth, after cellulose. It is the major structural component of the arthropods' exoskeleton and the cell wall of fungi and yeasts. Due to their properties, like biodegradability, bio-compatibility, non-toxicity, antioxidant and antimicrobial activity, chitin and its main derivative, chitosan, find many applications in the industrial and biomedical fields, such as drug delivery, cosmetic, wound healing, food preservation, tissue engineering and wastewater treatment.
The main source of commercial chitin is represented by fish industry waste, chiefly crustaceans' shells. The use of insects is a valid alternative to crustaceans as a source of chitin and chitosan and represents a solution to the current demand for the set up of sustainable processes.
The PhD research project is aimed at producing chitin nanofibrils (CNs) for applications in cosmetic and biomedical fields. The chitin is extracted from pupal exuviae and adults of insect *Hermetia illucens*, which represent the only waste products of its rearing. *H. illucens*, the black soldier fly, feeds on organic wastes from the agri-food industry. The process of agri-food waste bioconversion, mediated by its larvae, allows to obtain products of high biological and economic value, perfectly fitting into a zero-waste circular economy production process.
Chitin, in form of chitin nanofibrils, proves to be excellent drug and cosmetic carrier. CNs, as cosmeceutical active compounds, have the ability to promote skin health. They are used both as active ingredients and as carriers capable of accelerating the penetration of emulsions and the skin repair.

Contrasting response of native and non-native plants to disturbance and herbivory in mountain environments

R

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Under global change mountain biodiversity has been increasingly threatened by non-native plant invasions. Although plant invasions are expected to be modulated by biotic interactions, it is still unclear how invertebrate herbivores can affect non-native establishment and success. Using a large manipulative experiment along the core elevational range of plant invasion in the European Alps, we disentangled the effects of abiotic and biotic drivers of native and non-native plant establishment after soil disturbance. Native and non-native species showed contrasting responses to soil disturbance and elevation. Warm temperatures and disturbance promoted non-native success over natives, suggesting that global change will probably favour the further spread of non-native plants in mountains. Most of the observed non-natives were not present in the surrounding vegetation as mature plants but emerged from the seed-bank, indicating that propagules were able to reach even remote natural areas. Moreover, invertebrate herbivores reduced native establishment and cover, showing that natural herbivory pressure from invertebrates might play a role in shifting competition hierarchies between natives and non-natives. Here, we showed that herbivores can influence how species respond to soil disturbance, making it necessary to account for changing biotic interactions when predicting future invasion dynamics.

RNAi silencing of an immune gene disrupts the embryonic development of *Spodoptera littoralis*

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The study of the immunosuppression syndrome in *Heliothis virescens* (Lepidoptera, Noctuidae) larvae parasitized by *Toxoneuron nigriceps* (Hymenoptera, Braconidae) allowed the isolation and characterization of a host gene that is negatively modulated soon after oviposition. This gene, denoted as *102*, has been functionally characterized in *H. virescens* as well as in *Spodoptera littoralis* (Lepidoptera, Noctuidae) *(Sl102)*; in both species it encodes a protein essential for the cellular immune response (encapsulation and nodulation of non-self organisms). The silencing of this gene by RNA interference (RNAi) enhances the septicaemia induced by *Bacillus thuringiensis* and the resulting killing activity on noctuid moth larvae and provides new opportunities for pest control. To further explore the potential of this pest control strategy, here we assess the effect of RNAi mediated silencing of this immune gene in *S.littoralis* eggs. The experimental results demonstrate that the expression of *Sl102* gene is significantly down regulated in eggs soaked in a solution of dsRNA targeting this gene, compared to control eggs soaked in a solution of dsRNA targeting the Green Fluorescence Protein. The observed gene silencing was associated with a drastic reduction of egg hatching and larval survival. This result appears very promising for the development of new control strategies of pre-imaginal stages of noctuid moths. The observed lethal phenotype indicates that *Sl102* gene, besides its immune function, has an important role in the regulation of embryonic development which has been totally ignored so far. Therefore, our data pave the way towards novel pest control application and offer the opportunity to unravel new molecular details of insect development.