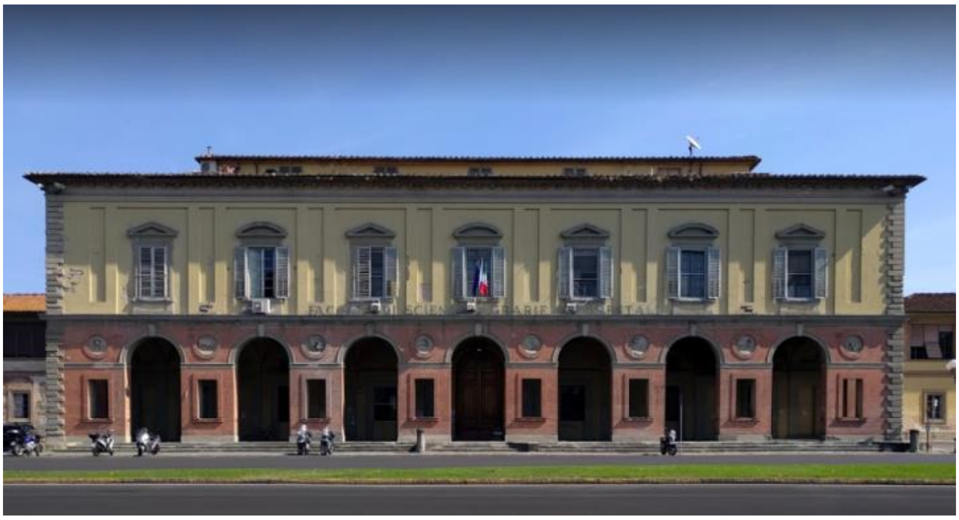

European PhD Network "Insect Science" - IX Annual Meeting

14-16 November 2018

SCIENTIFIC PROGRAMME & BOOK OF ABSTRACTS

Università degli Studi di Firenze
Scuola di Agraria
Piazzale delle Cascine, 18
50144 Florence

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European PhD Network “Insect Science”

PROGRAM

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ABSTRACTS

Real-time loop-mediated isothermal amplification: an early-detection tool for monitoring *Ceratocystis platani*

C. Aglietti[1,2], A. Santini[2], P. Bartolini[2], P. Capretti[1] and N. Luchi[2]

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An effective framework for early warning and rapid response is a crucial element to mitigate or prevent the impacts of biological invasions of plant pathogens, especially in an open door phytosanitary system as the European Union (EU) one in which inspections are concentrated on well-known pests and generally limited to visual examination of aerial parts of plants. Molecular detection of pathogens by using PCR-based methods requires a well-equipped laboratory. For preventing the spread of some pathogens rapid detection tools that can be applied as point-of-care diagnostic are highly desirable, including for *C. platani* a wound parasite ascomycete reported as the causal agent of *Platanus* trees canker stain. A Loop mediated isothermal amplification assay was developed to detect *C. platani* from plant tissue by using the portable instrument Genie ® II. The assay was able to recognize target pathogen with high levels of specificity and sensitivity (0.02 pg μl^{-1} of DNA in dd-water) in only 30 min also in environmental symptomatic samples, results confirmed by qPCR compared assays. As an example of field application, the developed LAMP assay, together with a battery drill to collect samples and an extraction Kit for field use, was used for monitoring *C. platani* spread in Florence. The use of such portable and handled LAMP-based assays allows a fast analysis of the collected sample reducing diagnosis time and making possible to apply it also in the field. Great simplicity, sensitivity, specificity, and minimum required equipment make it ideal for target diseases management and control at ports of entry, but also in nurseries, cities and forests.

Study of Diptera Hippoboscidae of the genus *Lipoptena*, parasites of ungulates, and morphological and bioecological investigations on *L. fortisetosa*, a new species for Italy

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Ippoboscids are parasites of various species and the representatives of the genus *Lipoptena* are specialised in infecting ungulates, although can accidentally infest other species including humans. They damage their hosts and maintain the transmission of pathogens and zoonoses.

The presence of ungulates in Italy has increased causing management issues and a consequent spread of their parasites.

During a research in Tuscany on *Lipoptena cervi* (Linnaeus, 1758), the presence of *Lipoptena fortisetosa* (Maa, 1965) has been revealed in Italy for the first time. This species is native to Japan but has spread into several countries. Its presence seems to be related to *Cervus nippon*, its original host, which was recently discovered in Italy. It could have spread through Europe carrying its ectoparasites.

Knowledge about these hippoboscid species remains sparse, although they could play an important role in sanitary risks.

This study provides a description of *L. cervi* and *L. fortisetosa* and several differences have been highlighted to facilitate their identification. Morphological features of ectoparasites show an extreme level of adaptation to parasitic life and a strong adaptive convergence in some parts of their body.

During the PhD the research will focus on the distribution of *L. fortisetosa* in central Italy and evaluate some aspects of its bioecology. The investigation will study the host location related to visual and olfactory stimuli; the morphology of antenna, in particular sensory structures, will be studied. Besides, specific analyses based on molecular techniques will be conducted to verify their role as vectors of pathogens and zoonoses.

Benzophenone as a primary mediator in host preference behavior of *Bagrada hilaris*

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The painted bug, *Bagrada hilaris* Burmeister (Pentatomidae; Heteroptera), is a stink bug native of Asia, Africa and invasive in the United States, Mexico, and more recently, South America. This species can cause heavy damage to several vegetable crops in the genus *Brassica*, in particular to young plants at seedling stage with marked preference for some species as *Raphanus sativus* L. and *Eruca sativa* L. Objective of this study was to evaluate the role of Volatile Organic Compounds (VOCs) emitted by seedlings of four *Brassica* species on the host location process of *B. hilaris.* Behavioral experiment were carried out using the vertical Y-shaped olfactometer, and VOCs emitted by seedlings were collected in headspace using solid phase micro-extraction (SPME) and analyzed by GC-MS. Results of behavioural experiment evidenced that *B. hilaris* prefer *R. sativus, E. sativa* and *B. rapa* over *B. carinata*. However, *B. hilaris* individual didn't elicit any significant preference among *R. sativus, E. sativa* and *B. rapa.* Chemical analyses evidenced the VOCs of *R. sativus, E. sativa* and *B. rapa* have in common the same main compound identified as benzophenone, which was not detected in *B. carinata*. Moreover *B hilaris* individuals were attracted to the crude extract of *E. sativa* seedlings, and to a liquid chromatography polar fraction. It was speculated that benzophenone, the main constituent of the active polar fraction, might act as primary mediator in this insect-plant interaction.

RNAI-MEDIATED IMMUNE SUPPRESSION OF PEST INSECTS TO ENHANCE THE IMPACT OF THEIR NATURAL ANTAGONISTS

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**These authors contributed equally*

The identification of new bioinsecticides of natural origin and of their appropriate delivery strategies is one of the strategies 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 bioinspired tools of pest control, based on the use of beneficials that goes beyond the organism level. The use of RNAinterference (RNAi) to artificially down-regulate host genes negatively targeted by virulence factors appears to be particularly promising. In this context, we have previously demonstrated that RNAi-mediated silencing of an immune gene (*102 Sl*), targeted by a polydnavirus associated with a parasitic wasp, generates an immunosuppressed phenotype in *Spodoptera littoralis* larvae and enhances the virulence of the entomopathogen *Bacillus thuringiensis*. Here we explored a delivery strategy of dsRNA and the effects of gene silencing on pathogen virulence. *S. littoralis* larvae were fed on artificial diet on which was layered a suspension of sonicated heat-killed *Escherichia coli* expressing *102 Sl* dsRNA. Experimental larvae showed marked transcriptional down-regulation of the targeted gene and impairment of the cellular immune response, associated with an enhanced susceptibility to the entomopathogen *B. thuringiensis.* These results indicate that efficient delivery of dsRNA molecules, through the use of bacterial cells as delivery capsules, can offer the possibility to new "bio-inspired" strategies of pest suppression.

INNOVATIVE DELIVERY OF Cu(II) IONS BY A NANO-STRUCTURED HYDROXYAPATITE TO ENHANCE THE SUSTAINABLE CONTROL OF *PLASMOPARA VITICOLA*

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In organic viticulture, the protection against *P. viticola* is essentially ensured by the use of cupric fungicides, which is expected to be restricted by the European countries, because of its ecotoxicological profile. The research on innovative forms of copper as well as the optimization of its distribution and persistence appear the most promising approach to enhance the control of *P. viticola*. The present research investigates the delivery properties of biomimetic synthetic hydroxyapatite (HA) to enhance the biological activity of Cu(II) ions. To this aim, four Cu(II) compounds were formulated with the HA and applied in a preliminary *in vitro* antifungal assay against *Botrytis cinerea* and in consecutives *in-planta* efficacy assays against *P. viticola* under greenhouse conditions. The *in vitro* results highlighted a different grade of inhibition by each Cu(II) compound according to their applied dosage and indicating also the delivery role potentially played by the HA, especially on the insoluble copper salts. Under greenhouse conditions, further findings were gained especially on the efficacy of variable percentages of HA into the formulations, on the influence of dose variation of the formulation and the treatment efficiency and persistence under rain–washing effect. The study revealed promising findings on the formulation based on the HA particles and the most soluble copper salt, which resulted highly efficient in reducing both the disease severity and incidence in all the experimental conditions, suggesting in the meantime a deeper investigation of functional models and the co-formulation process on the insoluble Cu(II) compounds.

A *Varroa destructor* salivary chitinase is involved in the regulation of infested honeybee pupae

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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, including proteins and viruses, through 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* candidate salivary proteins, along with qPCR and *in situ* hybridization detection to assess their expression in salivary glands. This process 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 then assessed by qRT-PCR and associated with the observed level of mortality on targeted *Varroa* mites. We finally scored the impact of this salivary effector on honeybee immune response by determining the expression profile of a set of immune genes in parasitized hosts.

Managing *Bactrocera oleae* endosymbiont *Ca*. E. dacicola in lab rearing: practical aspects for S.I.T. improvements.

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Abstract: *Bactrocera oleae* (Rossi) is a key pest of the olive crops in the Mediterranean basin. An important issue regarding *B. oleae* is its symbiosis with *Candidatus* Erwinia dacicola. This endosymbiosis is considered essential for the olive fly in field, whereas it had been found rarely in lab colonies, probably because of preservatives in artificial diets. So that we exposed eggs laid by wild females to two different treatments: a propionic acid solution and a mixture of sodium hypochlorite plus Triton X. The symbiont load on eggs was evaluated by real-time PCR and SEM. In the same time, we also tried a horizontal transfer among adults, exposing lab flies to ripe olives and gelled water contaminated by wild flies, wax domes containing eggs laid by wild females, cages dirtied by faeces dropped by wild flies in cohabitation with wild adults. In the first experiment, real-time PCR of eggs showed a significant reduction in *Ca*. E. dacicola abundance on eggs treated with propionic acid solution or the mixture of sodium hypochlorite plus Triton X compared to the control. Secondly, PCR-DGGE highlighted that the horizontal transfer of the endosymbiont occurred in the case of cohabitation. Thus, our results indicate that the use of preservatives in artificial rearing hinders the obtaining of a symbiotic strain and that cohabitation between wild flies and lab ones permits the horizontal transfer of the bacterium, opening further possibilities to establish a permanent symbiotic colony, a strategic tool for future SIT applications.

Characterization of *Hermetia illucens* (Diptera: Stratiomydae) larval midgut

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**These authors contributed equally to the work*

Hermetia illucens (Diptera: Stratiomyidae) larvae are among the most promising agents for the bioconversion of organic waste into nutritionally valuable proteins for production of environmentally and economically sustainable insect-based animal feed. Despite the great interest toward this species, there is a lack of knowledge about its biology: in particular, a deep understanding of the physiology of the midgut, the main site of nutrient digestion and absorption, is essential to better comprehend the extraordinary dietary plasticity of the larvae and to better exploit their bioconversion ability. To overcome this gap, we performed an in-depth morphofunctional characterization of *H. illucens* larval midgut. Our results demonstrate that this organ is composed of three distinct anatomical regions with different features and midgut epithelium is formed by different cell types that accomplish different functions. The characterization of the activity of enzymes involved in nutrient digestion and their mRNA expression levels show that digestion is associated to specific regions of the midgut. Moreover, we investigated the impact of different feeding substrates on the morphology and physiology of larval midgut. Our data show a diet-dependent adaptation process of the midgut. This plasticity may be responsible for the ability of *H. illucens* larvae to grow and develop on very different substrates. Finally, we analyzed the larval midgut microbiota. Our data demonstrate that both diet and midgut regionalization influence the microbial load and diversity. This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).

Ecology and phenology of spittlebug vectors of *Xylella fastidiosa*

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The plant pathogenic bacterium *Xylella fastidiosa* has been first identified in Europe in olive trees in Apulia in 2013; now is known to be present, with different genetic variants, in large areas of Southern France and Spain. The spittlebug *Philaenus spumarius* has been identified as a vector, and studies were undertaken to describe ecology and phenology of *P. spumarius* and other spittlebug potential vectors. Field surveys were conducted over the 2015-2018 period in olive groves of the Apulia and Liguria Regions of Italy. Structured population dynamics, host-plant selection, and seasonal movement between crop and wild species, were investigated. Nymph population in the herbaceous cover was estimated by direct counting, adults were collected by sweeping three different vegetational components: herbaceous cover, olive canopy, and alternative woody hosts. All samplings were conservative to avoid disturbance to spittlebug population. Phenology and reproductive biology were also investigated by rearing *P. spumarius* inside a mesocosm (insect-proof screenhouse) and several microcosms (cages hosting a single couple of *P. spumarius*). The main achievements on the ecology/phenology/biology of the spittlebugs will be presented. In conclusion, I will discuss how this information is implemented in designing control measures aimed at slowing disease progression and assessing the risk in non-infected areas.

Identification of volatiles released by fruit-associated yeasts for the biocontrol of *Drosophila suzukii*

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Chemical control of *Drosophila suzukii* (Matsumura) (Diptera: *Drosophilidae*) using synthetic insecticides is particularly challenging as it is difficult to respect pre-harvest intervals. In this research, a different approach to tackle the problems caused by *D. suzukii* has been proposed, taking in consideration the chemical ecology of *D. suzukii*. In particular, we aim to develop a new control strategy that will reduce or eliminate the residues of chemical insecticides on fruits, targeting fruit-associated yeasts to attract specifically the oviposing females of *D. suzukii* with a mass trapping approach. For this reason, we cultured eight yeast strains, previously found to be fruit-associated, to determine which volatile compounds (VOCs) they emit. The volatiles profile emitted by each living yeast culture was characterised using direct headspace and gas chromatography-mass spectrometry (DHS-GC-MS). Results showed that some yeasts are more prone to release alcohols and ethyl esters, while other strains release much more acetates. The strain *Saccharomycopsis vini* 1.23 was the only one emitting a specific monoterpene. In a parallel experiment, we determined the yeast strains that might function as phagostimulant components. To test if the yeast cultures could be perceived in different ways by *D. suzukii*, we are currently performing electroantennography (EAG) and gas chromatography-electroantennography detection (GC-EAD) puffing yeast volatile blends on *D. suzukii* antennae. Experiments are still in progress, but the preliminary results are promising.

Our research was kindly supported by the European EFRE project “Dromytal”.

Transmission mechanism of *Xylella fastidiosa*: known, unknown, ongoing

D. Cornara

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From a general point of view, researchers working on vector-borne plant pathogens attempt to eliminate confounding factors such as vector behaviour from their transmission experimental schemes, so that the role of targeted variables on pathogen transmission can be determined. However, vector behaviour has profound ecological and evolutionary implications for the pathogens they transmit, as the latter rely nearly entirely on their vectors for passage to new hosts. This is especially true for the bacterium *Xylella fastidiosa*, whose natural short-range spread depends exclusively on xylem-sap feeder insects. Therefore, our understanding of bacterial epidemiology pass through a deep comprehension of the several aspects of vector behaviour such as host range, within-host preference, dispersal, aggregation, and, last but not the least, feeding behaviour and patterns related to transmission. Despite decades of efforts, the feeding behavior/behaviors leading to *X. fastidiosa* transmission mechanism (acquisition and inoculation) remains unknown. Furthermore, all the background on bacterium transmission come from studies performed with sharpshooters (Hemiptera: Cicadellidae), the main American main vectors. On the other side, the spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae), to date considered the most important European vector of the bacterium, has shown elements of novelty compared to sharpshooters in respect to its relationship with the bacterium. Therefore, part of the outline valid for sharshooters should be questioned and further validated for those vector families widespread in Europe, and disregarded in previously studied outbreaks, such as spittlebugs and cicadas. Here, I briefly review evidences and hypothesis about the mechanism related to *X. fastidiosa* transmission, with a special focus on *P. spumarius*.

Evaluation of the biological activity of some essential oils against the meadow spittlebug *Philaenus spumarius*

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The “meadow spittlebug” *Philaenus spumarius* (L.) (Hemiptera, Aphrophoridae) is a highly polyphagous species and the main vector of the *Xylella fastidiosa* subspecies *pauca* strain involved in the “Olive Quick Decline Syndrome” in the Salento Peninsula. The identification of behaviorally-active compounds interfering in the host selection process could be of practical interest for developing control strategies towards this pest to prevent sap sucking from olive plants. Essential oils (EOs) extracted from *Lavandula officinalis, Citrus aurantium* var. *bergamia, Cymbopogon nardus, Pelargonium graveolens, Salvia officinalis, Helichrysum italicum, Rosmarinus officinalis, Thymus vulgaris, Origanum vulgare* and *Santolina chamaecyparissus,* reported as repellents to many harmful insects, were tested in electroantennographic (EAG) analyses. In addition, four terpenes, myrcene, methyl salicylate, R-(+)-limonene and S-(-)-limonene were used. Behavioral responses of *P. spumarius* to *L. officinalis*, *P. graveolens*, *C. nardus* and *C. a.* var. *bergamia* EOs were also evaluated using Y-tube bioassays. EAG demonstrated the capability of the peripheral olfactory system of female and male *P. spumarius* adults to perceive the EOs, and elicited dose-dependent EAG responses, in spite of the low number of antennal sensory structures described in *P. spumarius*. At certain concentrations, olfactory responses showed that males were attracted by *L. officinalis* and repelled by *C. a.* var. *bergamia* EOs. Females were attracted by *C. a.* var. *bergamia* at lower concentration and repelled at higher ones. *P. spumarius* responses to EOs provides interesting indications in the perspective of EOs practical applications like push and pull control strategies.

Research supported by Apulia Region, CISOL Project, DD n. 494, 2016.

Assessing Lepidopteran Biodiversity along various land use types in Mounts Choke, Ethiopia

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Ethiopia is endowed with a widely diverse genetic resource and recognized as one of the twenty Mega diverse country in the world; however, its biodiversity is regarded as poorly investigated. In recent decades, the country has experienced a huge impact of anthropogenic disturbances leading to a strong loss of biodiversity. Insects provide a wide range of ecosystem processes and services to sustain human life. Lepidoptera is the second largest and most diverse order of the class Insecta, with approximately 160,000 living species described so far. Lepidopteran assemblages were sampled across five different land use types with different anthropic impact (natural forest, remain of natural forest, shrub and grazing land, crop fields, human settlement mosaic) in Choke Mountain, with the aim to assess the impact of human activities on the structure and richness of Lepidoptera community. Fieldwork was carried out from August 2017 to July 2018 using light traps operating during nighttime simultaneously at four sites for each land use type, collected approximately 6,000 specimens currently under identification. Shannon and Margalef diversity indices were calculated. Here, results from the first month of field activity were presented. A total of 465 specimens of Lepidoptera, belonging to 130 morphospecies, were collected. Biodiversity indexes showed that natural forest and remain of natural forest maintained higher and comparable diversity level, whereas in the other three land use types index values were lower, with human settlement mosaic the lowest. Our preliminary results suggested that the level of habitat transformation had a direct impact on biodiversity levels.

Preliminary studies on the effects of high temperatures on some aphid life history traits and on aphid-parasitoid interactions

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Climate changes will increase the direct exposure of insects to stressful thermic conditions. Among insects, aphids are worldwide crop pests. They are ectothermic organisms and take part to complex trophic interactions. Extreme thermal variations could affect some aphid traits and, in an unpredictable way, its interactions with natural enemies such as endoparasitic wasps. In a laboratory study, we exposed the pea aphid *Acyrthosiphon pisum* to a high temperature (39.1°C for 30 min) and subsequently we recorded the effects on vitality, longevity and fecundity. Twenty-four hours after the exposure, survival rate was about 20%. Survived aphids were singularly kept on broad bean plants and the number of dead aphids and progeny were daily recorded in the following days. We found that, compared with the control, heat exposure decreases both longevity and fecundity. At the same experimental conditions, we investigated how the parasitoid *Aphidius ervi* copes with the exposure to a heat shock of its host. Five experimental treatments of parasitized and unparasitized aphids were established according to which day after parasitization the heat shock treatment was applied, that is, aphids heat shocked 1, 2, 3 4 o 5 days after parasitization. Aphid survival after heat shock was significantly higher for the parasitized individuals than for the unparasitized ones. Our studies show that high temperatures induce many relevant changes in aphid life history traits and that parasitism can affect host thermal sensitivity.

mTOR and honey bee health: a lab study

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Pollen is the main source of proteins, amino acids, and lipids for honey bees and plays a fundamental role in development and health. Recent studies showed that pollen is beneficial for honey bees infested by the parasitic mite *Varroa destructor* and the associated Deformed Wing Virus (DWV); in particular, results indicate that pollen can mitigate the negative effects of mite infestation, increasing the lifespan of bees. However, the mechanisms underlying this positive effect remains unclear.

We hypothesized that pollen could help bees to cope with parasites and associated pathogens by supporting the energetic balance disrupted by those biotic stressors. Therefore, to test our hypothesis we treated pollen fed bees with Rapamycin and assessed if the beneficial effects of pollen were lost; in fact, according to the literature, Rapamycin inhibits the Target of Rapamycin (mTOR) pathway, which is a major nutrient-sensing pathway that regulates growth and metabolism.

First, we show that Rapamycin does not reduce the beneficial effects of pollen; in contrast, this chemical seems to corroborate that effect.

Second, we underline a decrease in DWV load in drug treated honey bees, suggesting a possible involvement of mTOR pathway in honey bee immune response towards viruses.

Molecular mechanisms underlying intricate plant-insect-microbe interaction in plant-manipulating Gracillaridae

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Gall-inducing insects are iconic examples in the manipulation and reprogramming of plant development, inducing spectacular morphological and physiological changes of host-plant tissues within which the insect feeds and grows. Despite decades of research, basic mechanisms of gall formation remain unknown. Understanding the evolution and adaptive significance of plant manipulation needs to contrast feeding strategies of different species in an evolutionary framework. It also needs to understand how insects manipulate the physiology and the anatomy of their host-plant.

The leaf-mining moth *Phyllonorycter blancardella* (Lepidoptera: Gracillaridae) modifies the Cytokinin (CK) profile of mined leaf-tissues and the insect symbiotic bacteria *Wolbachia* is involved in the plant manipulation to the benefit of the insect host. To gain a deeper understanding into the possible origin and dynamics of CKs, we conducted an extensive characterization of CKs in larvae and in attacked apple leaves. Our results show that mines are enriched in CKs both on green and yellow leaves, allowing insects to control their nutritional supply under fluctuating environmental conditions. The spatial distribution of CKs within attacked leaves show that plant manipulation is strictly limited to the mine suggesting the absence of CK translocation from distant leaf areas towards the insect feeding site. They further show that CKs are detected in the highest levels in larvae reinforcing our hypothesis that CKs accumulating in the mines originate from the insect itself. Presence of bacteria-specific methylthio-CKs is consistent with previous results suggesting that insect bacterial symbionts contribute to the observed phenotype. Our study provides key findings towards the understanding of molecular mechanisms underlying this intricate plant-insect-microbe interaction. The ability of leaf-mining insects to manipulate their host plant physiology ask whether leaf-miners can also be considered to be plant reprogrammers.

Recent research suggests that some aspects of the plant manipulation shown by gall-inducers may be shared with other insect herbivorous life histories. Our current research focus on salivary effectors that are produced and delivered to the plant by the insects. We focus on leaf-miners, gall-inducers but also species that have evolved a unique mixed-feeding strategy, first larval instars being leaf-miners while last larval instars are gall-inducers. These species represent an intermediate state along the continuum between leaf-miners and gall-inducers and an unique opportunity to 1) identify genes that are responsible for the induction of galls; 2) understand the mechanisms of gall morphogenesis; and 3) shed light on the origin of gall induction by insects.

The *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) problem: the solution seems to be in its own mid-gut

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Bactrocera oleae (Olive fruit fly) is one of the major pest of olives in most commercial olive-growing regions worldwide. The main gut bacterium *Candidatus Erwinia* dacicola is essential for the fly's development. The aim of the present work was to inhibit the fitness of *B. oleae* altering the fly's microbiome through the use of microbial metabolites. Bioassays were performed using copper oxychloride and two selected metabolites of *Trichoderma* spp. (harzianic acid and 6-pentyl-α-pyrone). The repellent efficacy of smoke-water, obtained from pyrolysis of various plant materials was also evaluated. Genetic analysis was used to detect variation in the composition of the intestinal microbiota of flies. Harzianic acid and 6-pentyl-α-pyrone were isolated from culture filtrates of *T. harzianum* and *T. atroviride*, respectively, using different extraction protocols and chromatographic methods. Whereas, copper oxychloride solutions were obtained using a commercial product, and the antibiotic (Piperacillin) was used as positive control. Flies separated by sex and allowed to feed on the same compound were mixed for mating after 14 days and fresh olives were exposed to females for egg laying. The number of fly stings, eggs and larvae was calculated. Preliminary results from olfactometric tests using smoke-waters showed a significant repulsive effect in a concentration-dependent manner. Males were more sensitive than females. These results can be pursued in the development of new formulations useful in the Integrated Pest Management strategies for olive pests.

Vespa velutina in Italy: a modelling approach to predict the new annual colonized area and improve management practices

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The Asian yellow-legged hornet *Vespa velutina* (Lepeletier 1836) is an invasive alien species that is colonizing Italy since 2012. *V. velutina* preys honeybees and other native insect species, generating impacts on beekeeping, biodiversity and ecosystem services associated with pollinators, besides to concern among citizens and management economic costs. For these reasons, Europe considers *V. velutina* as an invasive alien species of Union Concern (IAS Regulation - EU 1143/2014), and member states should act to prevent, contain and limit its spread. In Italy, the European LIFE STOPVESPA project is acting since 2015 to contain the spread of *V. velutina*.

Control activities aimed to limit the spread of an invasive species requires the assessment of the colonized range besides to the prediction of the areas more feasible to be colonized. Therefore, the distribution of *V. velutina* records (colonial nests) in Italy were analysed in order to understand the factors that influence species distribution and develop predictive models of expansion; such models are useful to improve management practices.

The variables that contribute more in determining the distribution of hornet nests at the local scale are the elevation above sea level (95% of nests located within 521 m a.s.l.) and the distance from source sites (95% of nests within 1.4-6.2 km from nests of the previous year). The probabilities to found *V. velutina* nests over the limits of its colonization range decreases rapidly with the increasing of distances from source sites. These data were used to build predictive models of expansion for the species.

Is DNA barcoding a promising tool for Chrysomelidae identification? Developing data and testing its efficiency on Euro-Mediterranean species

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The advent of molecular taxonomy has allowed to identify and delimit species through a specific DNA sequence. Molecular tools have been postulated as the most promising approach to accelerate species identification and discovery, especially for the hyperdiverse group of insects. Chrysomelidae family, one of the largest within Coleoptera, includes species of interest for conservation aims and agricultural pests. The aims of this project are to 1) develop an accurate DNA-barcoding dataset for Chrysomelidae identification; 2) test its efficiency; 3) use integrated taxonomy approaches (morphology, DNA sequences and ecology) for delimiting species when disagreement between morphology and DNA barcode is observed; 4) test the efficiency of species delimitation methods. In the last years, collecting campaigns organized across the Euro-Mediterranean area allowed to collect more than 3000 Chrysomelidae individuals assigned to ~500 species. So far, barcode sequences were obtained for 889 specimens (250 species). These sequences plus others mined from online repositories (7237 sequences, 542 species) were used to test the efficiency of DNA-barcoding for leaf beetles identification, which resulted in 94%. Failure cases were mainly related to introgression and incomplete lineage sorting. In the case of *Chaetocnema* and *Phyllotreta* species, responsible for high damage to crops, DNA-barcoding resulted in 99% efficiency, confirming its interest in crop pest monitoring. Cases of inconsistencies between morphological and molecular identification are currently under investigation adopting integrated taxonomy. The 7237 Chrysomelidae barcodes dataset is used as model for testing the efficiency of distance and phylogeny-based species delimitation methods on different taxonomic levels (family, subfamily and genus).

Innov'api: innovative practices for a sustainable beekeeping

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The Interreg-Alcotra project "Innov'api" involves research institutions (INRA-Avignon and the University of Turin in cooperation with the CNR-IPSP) and beekeeping associations (ADA France, UNAAPI) from Provence-Alpes-Côte d'Azur, Occitanie and Piemonte regions. This collaboration aims at improving the defense strategy against the main parasite of honeybees, the mite *Varroa destructor*. In particular, we are comparing two beekeeping practices: the conventional treatment with a synthetic acaricide (Apivar$^{©}$) and a newly introduced practice based on brood removal in combination with oxalic acid applications. For this purpose, we are monitoring simultaneously in Italy and in France several colonies from selected apiaries, where both methods are used, by collecting data on multiple parameters all over the year. Quantitative data on adult and brood population, honey production and mite population are collected in the field. Since *V. destructor* has a major role in honeybee virus transmission, a special emphasis is given to the assessment of health parameters of the colonies by detecting and quantifying five viruses (DWV, ABPV, CBPV, BQCV, SBV) at eight different sampling periods. At the same dates, the gene expression of three markers of the physiological status of the colonies (vitellogenin, insulin-receptor 1, adipokinetic hormone receptor) is recorded as well. With this experimental plan, we aim at describing how different *V. destructor* control practices impact virus replication in the bees. The apiaries are being monitored for three years; here we present the results of the first year of the project.

Thousand canker disease moves south of the northern Apennines

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Thousand canker disease (TCD) is a serious disease of walnut native to North America, caused by the fungus *Geosmithia morbida* M. Kolařik, E. Freeland, C. Utley & N. Tisserat (Ascomycota, Hypocreales) and its insect vector *Pithyophthorus juglandis* Blackman (Coleoptera, Curculionidae). Italy is the only country where this insect/fungal disease complex has been found outside the North American continent, having been reported starting from 2013 in the Veneto region, and later in neighbouring areas. Given the economic importance of walnut cultivation in central and southern Italy, a field survey was carried out to ascertain the possible occurrence of the disease also in Tuscany. This survey led to the discovery of a disease outbreak in the province of Florence in 2018. More than 7000 *P. juglandis* individuals caught in two traps during the growing season indicated a massive occurrence of the vector. The fungus was isolated and identified from insect adults and larvae, as well as from cankers around entry holes and galleries. The finding is of prime importance because it is the first report of TCD south of the natural mountain barrier represented by the northern Apennines, into areas where the cultivation of walnut is both ecologically and economically important. The thermo-hygrometric requirements of both causal organisms suggest that climate is not a limiting factor for the establishment of the disease in the southern EPPO region. This insect/fungal interaction, if left unmanaged, has the potential to cause serious damage to both the important native *Juglans regia* and the introduced *Juglans nigra.*

One plus one does not make two. The interactive effect of multiple factors on bee health

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Bee health is threatened by a number of stressors, including parasites, pathogens, pesticides and adverse environmental factors. On the other hand, other factors, such as, for example, access to proper nutrition, can sustain challenged bee health. However, understanding the overall impact of this intricate network of factors is very difficult and progress in this direction is slow.
Using recently published work, I will illustrate some features of this complex system and present a few tools that could facilitate an effective approach to the study of the crucial problem of bee health as affected by multiple stressors.

Antisense oligonucleotides for the ABC-transporter in *Anopheles stephensi*: from gene silencing to vector control

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Nowadays strategies for the control of vector-borne diseases rely on chemical insecticide treatments against the vector populations. In general, the lack of vaccines for these vector-borne diseases, such as malaria, have made the insecticide applications in treated nets and indoor spraying (IRS), the best measures for the protection of humans. However, this required use has led to serious consequences over the years: the increase of environmental pollution and the spread of insecticide resistance in vector populations. Our proposal was to develop an eco-friendly control method, based on the inhibition of ABC-transporters, efflux pumps, mostly involved in mosquito resistance insurgence, and belonging to the "Defensome-machinery". The aim is to drop the cellular defenses of the asian malaria vector, *Anopheles stephensi*, to increase the efficacy of lower doses of insecticide. In particular, treatment with permethrin insecticide was combined with two gene- and specie-specific postrascritional silencing methods, siRNA (RNAi method) and antisense Morpholinos (MOs). The gene target for these silencing methods was the ABC-G4 gene, an ABC-transporter strongly involved in permethrin detoxification processes in *An. stephensi l*arvae. Firstly, larvae of *An. stephensi* were treated with specific siRNA against ABC-G4, and then exposed to LD_{50} of permethrin. Morpholino against ABC-G4 was used in another bioassay, following the same experimental procedure. Mortality of larvae and the relative expression of ABC-G4 gene were analyzed at different time points. Both siRNA and MOs treatments increase the mortality of treated larvae and confirmed the role of ABC-G4 in permethrin detoxification.

Citrus peel essential oil nano-formulations: bioactivity against *Tribolium confusum* (Coleoptera: Tenebrionidae)

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The whole grain chain is affected by the attack of many insect pests, which cause relevant product losses, with an estimated production loss around the 40%.

The withdrawal of authorisation for many pesticides has led researchers to look for new methods and approaches to control stored product pests.

Among these products the essential oils have been extensively studied due to their acknowledged antimicrobial, insecticidal, antioxidant and antifungal activity. Furthermore, essential oils are products with an elevate commercial value, mainly as insecticides.

In this framework new citrus peel essential oils (sweet orange, lemon and bergamot) nano-formulations were developed, physically characterized and tested against *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) adults. Bioassays aimed to evaluate both the repellent activity and the toxicity of the nano-formulations. Essential oils were chemically characterised (GC-MS) and the developed formulations were subjected to Dynamic Light Scattering analyses to evaluate the stability (Zeta potential), the particle dimension distribution and the polidispersion index.

The developed formulations had nanometric scales (<200 nm) and a good stability over time (ξ >-15mV). Sweet orange nano-formulation highlighted the best repellent activity as well as the highest toxicity (applied either as fumigant or as cold aerosol).

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The use of habitat management and elicitors to improve biological control in vineyard

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Nowadays, the reduction of intensive chemical product use and increasing agroecosystem complexity represent crucial strategies to boost ecosystem services and sustainability in agriculture. Among several biological control approaches, the use of elicitors so as to enhance plant induced resistance and the habitat management have showed positive results in attracting and improving efficacy of natural enemies. Though, only few field works have been carried out testing the impact of these two tools on functional biodiversity.

The goal of this work is to improve biological control against main key pests in Italian vineyard (*Lobesia botrana* Denis & Schiffermüller, *Planococcus ficus* Signoret), focusing especially on resistance inductors and habitat management strategy. Firstly, inter-row ground cover management, selected sown flower strips and the most promising elicitors are separately tested in different vineyards in the Province of Bologna and Reggio Emilia. Pest density, natural enemy abundance, key pest parasitization and predation are assessed.

Finally, the use of resistance inductors and habitat management will be combined in order to evaluate the potential of the “attract and reward” strategy in increasing functional biodiversity.

Preliminary results are showed and discussed.

You must know your enemy to cope with it: the newly introduced fig weevil *Aclees* sp. cf. *foveatus*

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Italian fig orchards are facing severe damages due to an exotic pest belonging to *Aclees* genus (Coleoptera Curculionidae). Plant damages are related to adults feeding on leaves and early stage fruits and, primarily, on xylophagous larvae, which feed on the woody tissues of the plant, causing complete destruction of xylem and phloem. This pest, initially identified as *A. cribratus*, has closer similarity in morphology with *A. foveatus* instead. However, since species attribution remains unclear, genetic analyses are in progress to characterize its phylogeny in relation to museum specimens belonging to *Aclees* genus, through comparison of Internal Transcribed Spacer regions from rRNA genes of samples collected in various Italian regions. Recently, adults of *A.* sp. cf. *foveatus* were collected in Tuscan fig orchards (Carmignano, Italy) and reared under laboratory conditions, using fig branches and fruits. At 25°C the embryonic development covers a period of 6 ± 1 days. Newly emerged first instar larvae, reared inside fig branches, were observed every two days in order to establish growth rate and biological cycle. *A.* sp. cf. *foveatus* exhibits five larval instars, lasting about 10 ± 2 days each. Larvae grow from 0.3 ± 0.1 cm to 2.0 ± 0.1 cm length and after 45 ± 3 days molt in pupae. Due to the lack of effective control strategies, search for pheromone glands throughout SEM as well as analyses of volatiles complexes emitted by males and females were conducted, in the perspective of developing a mass trapping system.

Cydia pomonella (Lepidoptera: Tortricidae) monitoring with lures implemented by acetic acid still needs an improvement in Italy

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Insect pest monitoring with sex pheromones can be implemented by the use of volatile organic compounds acting as kairomones. One of the best result was achieved with *Cydia pomonella* (Lepidoptera: Tortricidae) almost 20 years ago by adding the pear ester (*E,Z*-2,4-ethyl decadienoate) to the sex pheromone to obtain a bisexual lure, commercialized by Trécé as CM DA Combo®. This combo lure attracts also female moths and increases male catches compared to the sex pheromone alone. Despite the good results worldwide with such combo lure, in Italy the use of CM DA Combo® has been mainly limited to mating disrupted orchards, to better monitor the species in plots treated with the sex pheromone for mating disruption. CM DA Combo® lure was recently improved by Trécé with the addition of an acetic acid membrane cup dispenser. The aim of this study was to screen the attractiveness of this three-component lure in commercial pome fruit orchards without mating disruption and compare the catches with those of the commercial sex pheromone alone or with CM DA Combo®. During the first *C. pomonella* flight, a preliminary screening was performed in six commercial orchards, then during the second flight an experiment was realized with five replicates in two pear plots. The results highlights the effectiveness of all the tested baits in monitoring the target pest, but no statistical improvement was provided by either CM DA Combo® with or without acetic acid compared to the standard sex pheromone.

Damage of *Halyomorpha halys* (Stål), *Thyanta pallidovirens* (Stål), and *Leptoglossus zonatus* (Dallas) on pistachio nuts in different period of the season in California

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Stink bugs (Hemiptera: Pentatomidae) and leaffooted bugs (Hemiptera: Coreidae) are pests of numerous crops including pistachio, *Pistacia vera* L. (Sapindales: Anacardiaceae). Common pest species are the red-shouldered stink bug, *Thyanta pallidovirens* (Stål), and the leaffooted bug *Leptoglossus zonatus* (Dallas). Recently, damages on pistachio nuts have been described also for brown marmorated stink bug, *Halyomorpha halys* (Stål), an invasive pest native from Asia. In California, a cage experiment in field was conducted to investigate the impact on nuts caused by the three 'bug' species in different period of the vegetative season of pistachio. Each cage was put on a single branch, containing at least one nut cluster; one specimen of *T. pallidovirens*, *L. zonatus* (adult), or *H. halys* (adult or nymph) was added for a 5-days feeding period. The experiment was conducted at different times from the beginning of May to the end of August, and nut damages and nut drop evaluated and counted immediately after the feeding period. A general decrease of epicarp lesions and dropped nuts occurrence was observed for the three species, in particular for adults of *H. halys* and *L. zonatus*. The highest percentage of dropped nuts was observed with early periods of feeding activity (i.e., in May), likely related to the phenological stage of the plant. Damage level was different according to the species/instar used. The damage caused by the three species is discussed in the context of pistachio Integrated Pest Management, in order to plan a proper timing for pest control.

DNA metabarcoding to quantify the response of insect diversity to mountain forest die-offs in the French Pyrenees

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Mountain forests suffer from an increase in frequency and severity of summer droughts and infestations of pathogens and insects. Those factors are causing high mortality of some keystone tree species (forest die-offs). Yet, how tree diebacks and associated changes in forest composition will affect local diversity and ecosystem functions remains unknown. Here, we aim at quantifying the impact of climate-induced forest decline on biodiversity by measuring changes in taxonomic structure of invertebrate communities along gradients of silver fir (Abies alba) dieback in the French Pyrenees.

We use DNA metabarcoding to analyse 224 samples of Malaise traps placed on 56 silver fir dominated plots in the French Pyrenees from May to September 2017. Samples were sequenced using Illumina MiSeq and analysed using the DAMe twin-tagging pipeline approach. We conducted taxonomic assignment against reference DNA barcode libraries to streamline identification and recover biological information for ecological analysis. We discuss the results of our metabarcoding analysis and the utility of our approach to conduct biomonitoring across a large geographical scale.

Physiologically-Based Demographic Models for assessing the impact of invasive alien species in the light of climate change: a case study on *Ceratitis capitata*

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Future climatic scenarios are expected to influence the geographic distribution, abundance and impacts of Invasive Alien Species (IAS) affecting plant, animal and human health. Most of the modelling approaches projecting climate change effects on IAS are based on correlative approaches that simplify the analysis of the responses of IAS traits to the change in the environmental driving variables. There is a growing need to develop process-based modelling approaches able to capture the complexity of population system's responses to environmental changes. In this work, we explore the use of a Physiologically-Based Demographic Model (PBDM) to mechanistically represent the influence of environmental drivers on pest's distribution and impacts under climate change scenarios. As a case study, we applied the PBDM approach for the assessment of the area of potential establishment, abundance and impacts of the Mediterranean fruit fly (*Ceratitis capitata*) in Europe. Two climatic scenarios based on gas emission projections (Representative Concentration Pathways, RCP) are considered: RCP 4.5 corresponding to a peak of greenhouse gas emission by 2040 and RCP 8.5 corresponding to a steady increase of greenhouse gas emission throughout the 21st century. The model was calibrated against population dynamics datasets from field surveys collected in different European locations. The model allows predicting the current and projected distribution, abundance and impacts of *C. capitata* in the light of climate change and might be suitable for performing quantitative pest risk analysis.

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Susceptibility of grapevine cultivars to *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) infestation in field and in drying loft.

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The invasive pest *Drosophila suzukii* represents an important challenge for grapevine production. This species lays eggs in healthy ripening fruit. In addition to larval damage, *D. suzukii* can vehicle acetic acid bacteria. Grapevine represents a host fruit for *D. suzukii*; despite a lower susceptibility in respect to other fruits, infestation during ripening process has been described in varieties characterized by soft skin and low penetrating resistance. These two aspects, together with a complex of anatomical and physical proprieties determine the berry texture. Thus, an accurate evaluation of grape texture is required to determine the varietal susceptibility to infestation. Understanding which cultivar and ripening stage are the most susceptible to the oviposition would improve IPM practices and viticultural zoning.

The aim of this study was to identify the susceptibility to the infestation and development of *D. suzukii* in twelve highly valuable grape varieties during the ripening process, by measuring the ability of oviposition and the emergence rate, physical proprieties related to berry texture and chemical parameters. In addition, grapes devoted to drying process may represent a further substrate for *D. suzukii* oviposition and development. For this reason, three varieties were analyzed also during this postharvest period.

Differences for pest incidence were found. In PCA analysis, strong correlations among texture parameters, infestation rate and egg development were found. Infestation resulted related to skin toughness, skin elasticity and pulp consistency, whereas emerging rate was linked to skin elasticity and pulp consistency. During the drying process, increase in skin elasticity drives reduction in susceptibility.

POSTER PRESENTATIONS

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ABSTRACTS

Xylosandrus compactus (Eichhoff): a serious threat to Carobs in Sicily

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Xylosandrus compactus (Eichhoff) invaded the Italian peninsula in 2012. Thanks to the wide range of host plants, the beetle was able to colonize several regions in a short time arriving in Sicily 4 years later. The pest caused high levels of damage on carob (*Ceratonia siliqua* L.), that proved it to be highly susceptible to *X. compactus.*

The ability of the beetle to spread from the area of first invasion (summer 2016) was studied in the following two years. A bi-monthly monitoring program was performed, through the observation of 70 carob trees groups at a minimum distance of 500m from each other.

For each site trunk, branches and several tree twigs were carefully observed in order to check the occurrence of infestation symptoms. Potential infested material was examined at the laboratory to confirm the presence of the pest in the wood.

Data collected showed that *X. compactus* has a good dispersion capacity, being able to migrate more than 8 km/year from the first site of infestation. Moreover, results suggest that the pest moved from the initial site of invasion relatively slowly but continuously in both directions along the coastline. Among the monitored carobs, the 34% showed the presence of the beetle; all the infested trees showed the presence of the entry holes on twigs and branches, but only the 58% also on the trunk.

Further studies are ongoing to investigate the pest behaviour in the newly colonized environment, in order to establish a correct integrated pest management.

Wolbachia endosymbionts in velvet ants: a preliminary analysis of occurrence and variability

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Velvet ants (Hymenoptera: Mutillidae) are ectoparasitoids of mature larvae or pre-pupae of insects, especially aculeate Hymenoptera. Generally, they share an extreme sexual dimorphism with males winged and females invariably wingless, with few exceptions of apterous males. When they are on the alert for danger, they start running rapidly and evasively, they also show aposematic warning coloration patterns, and they produce chemical secretions and stridulation to deter attacks from enemies. The velvet ants enter into the host nests and seem to not be detected when they are inside, suggesting the presence of chemical mimicry. Mutillids parasite both solitary and social Hymenoptera, which sometimes live in symbiosis with the bacterium of the genus *Wolbachia*. The bacterium can be horizontally transmitted to a parasite that depend on the host for the development of the offspring, exactly as the velvet ants do. Nevertheless, no works have previously investigated the occurrence of the endosymbiont *Wolbachia* in the Mutillidae. Our aim was to do a screening for the presence of this bacterium in different velvet ant species collected in Tuscany (Italy) at "Parco Naturale della Maremma". We successfully detected *Wolbachia* in three of the seven species screened. Therefore, the symbiont could have coevolved with velvet ants and further studies are necessary to investigate if *Wolbachia* manipulate the biology of this host. Finally, we are analyzing the cuticular profile of these mutillids species to test the presence of chemical mimicry and to investigate if species infected with *Wolbachia* show a different cuticular profile compared to uninfected ones.

Beekeeping and honey bees: a conceptual framework for the classification of beekeeping management practices implemented in Europe

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Beekeeping is an important sector of the European agriculture generating employment and increasing incomes in rural areas. The role of the beekeeper is considered fundamental for maintaining the health status of a managed honey bee colony and, if intended, ensure its productivity. Several monitoring projects highlighted the direct and/or indirect role of the beekeeper for ensuring healthy honey bee colonies. However, a clear overview on the main actions carried out by beekeepers and their role for the successful management of honey bee colonies is missing. In this study, we aim at providing a generalized framework of classification for the Beekeeping Management Practices (BMPs) carried out by the European beekeepers and their influence on honey bees. Six BMPs were selected for their relevance at European level and their influence on a honey bee colony. Based on an extensive literature review each BMP was characterized in relation to i) the elements guiding their application, ii) the potential impacts on a honey bee colony and iii) the scenario-based variables that might influence their application and/or effectiveness. Knowledge gaps were filled through an Expert Knowledge Elicitation procedure. This work represents the first attempt to condense and further elaborate the knowledge available on the actions carried out by European beekeepers and quantify their influence on a honey bee colony. This work might support the development of realistic scenarios of beekeeping in Europe and the implementation of knowledge-based risk management actions.
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