

Book of Abstracts



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Sessions Lists

Sessions

I - Morphology, systematics and phylogeny

II - Physiology, ethology and interactions

III - Ecology and conservation

IV - Faunistics, biogeography and insularity

V - Agricultural entomology

VI - Insects and microorganisms

VII - Forensic, medical and
veterinary entomology

VIII - Forestry entomology

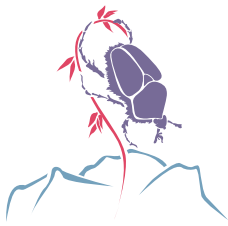
IX - Insects for bioconversion

X - Commodity treatment and urban entomology

XI - Genetics, genomics and biotechnology

XII - Social insects and apidology

XIII - Biological Control and
Integrated Pest Management



CNiE²⁰₂₃
P A L E R M O

XXVII Congresso Nazionale
Italiano di Entomologia

12-16 June 2023

Lectures

PLENARY LECTURE

History of an iconic Mediterranean insect, the pine processionary moth

Andrea Battisti

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The pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera Notodontidae), is an iconic insect in the Mediterranean culture for several reasons. First, the conspicuous silk tents built by the larvae in winter. Second, the devastating defoliations often called ‘fire without smoke’. Third, its use for criminal purposes. Despite this wide knowledge, its taxonomic status is unclear because the type material is not available and several species and subspecies have been described in the last centuries. A reconstruction of the origin of the material used for the first formal description has allowed to review the taxa, based on a molecular phylogeny of individuals originating from the whole Mediterranean range. The results revealed a complex history, largely explained by the biogeography of the region and by human activities. Interestingly, molecular information is reliable to separate species in most cases whereas morphology is not. In addition, hybridization among taxa makes it difficult to delimit species in contact zones when mating barriers are not present. Species delimitation based on both mitochondrial and nuclear markers allowed to retain three species of the complex (*T. cretensis*, *T. pityocampa*, and *T. wilkinsoni*) while more data are needed to conclude about the status of two recently described species, *T. hellenica* and *T. mediterranea*.

KEY WORDS: forest, insect, Lepidoptera.

PLENARY LECTURE

The Evolution of Coleoptera in the Permian and Mesozoic

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Beetles (Coleoptera) comprise ca. 380.000 described species and are therefore one of the most successful groups of organisms on this planet. I will address major transformations and morphological adaptations in the megadiverse order in the Late Palaeozoic and Mesozoic. The presence of arborescent plants with bark likely played a crucial role in the earliest evolution, providing microhabitats suitable for a strongly sclerotized body with hardened forewings in the Late Carboniferous. A first stage in the evolution of beetles was reached by the Lower Permian †Tshecardocoleidae, with incompletely sclerotized elytra without epipleura, loosely covering the dorsal side of the body in a tent-like manner, and a flexible cylindrical abdomen. During the Permian, infolded elytral epipleura evolved and a close fit of the elytra with the posterior thorax and abdomen, resulting in a largely secluded subelytral space. This optimized the morphology of adult beetles for moving in narrow spaces, but was also a predisposition for later invasions of aquatic habitats, by providing a chamber for breathing air storage. Moreover, this type of elytra could assume a multitude of different functions, for instance in the context of thermoregulation, mimicry, hind wing folding, or mating. Major changes of climate and land vegetation during and after the Permian-Triassic crisis were accompanied by major changes in the composition of beetle faunas. By that time, the split into the four extant suborders was already accomplished. Archostematan groups with wood-associated species with elytra with window punctures and tuberculate cuticle declined, whereas smaller forms with smooth elytra increased in abundance. Terrestrial Early Mesozoic groups such as Trachypachidae and Carabidae were likely primarily living in forests with litter of conifer needles. However, a distinct trend to invade aquatic environments is characteristic for the Triassic and Jurassic. This was enabled by the subelytral chamber and accompanied by smooth surfaces including the elytra and a more or less streamlined body. Independent invasions of aquatic habitats were achieved by different groups of Adephaga in the Triassic, with distinctly different adaptations in adults and larvae. Triassic species of the small suborder Myxophaga were also very likely aquatic. Whereas Adephaga are mainly characterized by predacious habits as larva and adults, major features of myxophagans are small body size and feeding on algae. A strong wave of radiation of aquatic beetles took place in the Jurassic, with a further diversification of the adephegan Dytiscoidea, and polyphagan aquatic lineages appearing on the stage, especially the Hydrophiloidea. The greatest trigger of megadiversification in the evolutionary history of beetles was the rise of angiosperms in the early Cretaceous. The ability to digest plant cell walls was likely crucial in the radiation of the megadiverse polyphagan Buprestoidea and Phytophaga. Specific tarsal attachment structures enabled phytophagous species to move efficiently on plant surfaces, and cryptonephric Malpighian tubules of cucujiform beetles reduced water loss, enabling them to stay for longer time on exposed plant surfaces. Today, Phytophaga, comprising weevils, leaf beetles and longhorn beetles, comprise about one third of all beetle species.

KEY WORDS: Beetles, evolutionary transformations, late Palaeozoic, Mesozoic.

PLENARY LECTURE

Insect attachment systems used in locomotion: bioinspirations for surface science and robotics

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Our research includes approaches of several disciplines: zoology, botany, structural biology, biomechanics, physics, and materials science. Using a wide variety of modern imaging techniques and experimental methods, we study mechanical systems and materials of insects. The research is mainly focused on the surfaces specialised for enhancement or reduction of frictional or/and adhesive forces. Such surfaces are composed of highly-specialised materials and bear surface structures optimised for a particular function. Some of these systems employ secretory substances, modulating forces in the contact area. Attachment systems of flies, beetles and bugs demonstrate interesting adhesion and friction properties and high reliability of contact. Experimental studies show that the effective elastic moduli of fiber arrays and spatula-like terminal elements in these systems are low, and this is of fundamental importance for enhancement of contact forces on rough substrata and for an increased tolerance to defects at the level of individual contacts. Based on the broad structural and experimental studies of insect attachment devices, the bioinspired reversible attachment devices were developed and their adhesive and frictional properties were characterised using variety of measurement techniques and compared with the flat surface made of the same polymer.

KEY WORDS: biomechanics, surfaces, adhesion, locomotion, biomimetics, legs.

PLENARY LECTURE

Speciation mechanisms of African Orthoptera with a focus on East Africa

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The East African region is known for its rich biodiversity, particularly in the mountainous areas of the Eastern Arcs that stretch through Tanzania and southern Kenya. Recent surveys of the Orthoptera fauna in these mountains have shown that they also harbor a diverse array of orthopteran insects with a high degree of endemism.

The geologically young volcanoes adjacent to the northern branch of the Eastern Arc chain, such as Mount Kilimanjaro and Mount Meru, were thrown up during the uplift of the rift valley system about 1–2 million years ago. The patterns of geographical species distribution found on these mountains can be explained by the expansion and retraction of forests during humid and dry periods, respectively. The vegetation corridors along rivers facilitated the dispersal of coastal taxa into the hinterland along the east African coast. During dry and warm periods, taxa that became trapped in montane habitats adapted to the montane climate or went extinct. The unique arrangement of mountain chains in the region likely facilitated speciation, and climate fluctuations and orogenesis, rather than a long-lasting and stable climate, are the most likely reasons for the high diversity found on the Eastern Arc Mountains and inland volcanoes.

On a larger scale diversification of East African taxa was triggered by large-scale climatic changes of the forest cover in Africa. African Tettigoniidae are thought to have been forest dwellers since humid Africa was more or less continuously covered by forests (Eocene pan-African rain forest). About 33 ma ago during the Oligocene a period of drastic global cooling occurred resulting in a drier climate at equatorial levels fragmenting the Eocene pan-African rain forest probably leading to a diversification in west and east African taxa. A trend from fully alate species to species with reduced wings is seen in e.g. Hexacentrinae.

The insights gained from studying Orthoptera can shed light on the factors that promote speciation and contribute to the rich biodiversity of African forests. Overall, understanding the mechanisms of speciation is crucial for conserving unique and irreplaceable ecosystems in Africa.

KEY WORDS: East Africa, Orthoptera, distribution, speciation.

PLENARY LECTURE

**Nectar-inhabiting microorganisms: hidden player in plant-insect interactions,
important impact on pollinators and parasitoids**

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Pollinators and adult parasitoids are frequent visitors of flowers and are well known to feed on nectar to cover their energetic needs. An increasing body of evidence has shown that nectar is ubiquitously colonized by microorganisms, particularly yeasts and bacteria, that have been dispersed into the nectar via air or flower-visiting animals. Microbial activity in nectar can alter sugar chemistry, often decreasing sugar concentration and alter sugar composition. Besides, the metabolic activity of nectar-inhabiting microorganisms affects others important nectar traits such as temperature, amino acid content, content of secondary metabolites and organic acids, and scent. Previous research has shown that microbe-induced changes in nectar chemical properties may impact pollinator fitness and behavior, and as a result plant reproductive success, but effects depend largely on the microbial species. More recently, evidence is accumulating that parasitoid attraction and performance are also strongly impacted by nectar-inhabiting microorganisms. Therefore, nectar-inhabiting microbes can no longer be denied when studying interactions between flower nectar and parasitoids. Moreover, they could have important implications in a conservation biological control perspective. Recent results obtained in this regard will be discussed.

PLENARY LECTURE

Taxonomic and functional biodiversity of agroecosystems: characterization and monitoring by using molecular methods

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The growing human population exerts increasing pressure on the biosphere, with severe negative impacts on natural ecosystems, society, and economy. Land-use intensification represents a major threat to biodiversity, with agriculture playing a prominent role in this process. To face this problem, it is necessary to better understand the impact of agriculture on the biodiversity underlying stable and resilient agroecosystems, including the entomological diversity, for maintaining the provision of ecosystem services to human beings. To pursue this ambitious objective, it is essential to develop innovative and expeditious approaches for better understanding, monitoring, and measuring biodiversity changes. In recent years, new molecular techniques including DNA barcoding and metabarcoding, as well as the more complex approaches based on DNA baits and shotgun metagenomics, have allowed characterizing the taxonomic and functional biodiversity of agroecosystems and natural/semi-natural environments. These new methods offer a unique opportunity to improve the knowledge of insect biodiversity responses to stresses and ideally, they can replace traditional biomonitoring approaches since they are expeditious, more accurate and sensitive, less invasive, and easy to standardize. However, some critical aspects should be considered. Indeed, besides some intrinsic technological issues that likely will be overcome in the near future, they strongly rely on reference databases of DNA sequences that have to be as complete as possible to allow the proper identification of the insect taxa. This last crucial aspect requires the implementation of national initiatives, involving taxonomists with different backgrounds, aimed at filling this knowledge gap. In conclusion, by leveraging the potential of molecular methods, we can design and manage agroecosystems that support sustainable agricultural production while preserving biodiversity and ecosystem functioning.

KEY WORDS: DNA-based taxa identification, environmental DNA, insect biodiversity characterization, biomonitoring.

PLENARY LECTURE

Sicilian Entomologists. Men and stories over three centuries

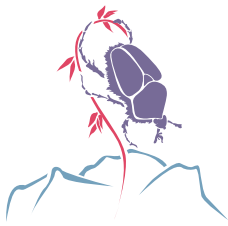
Marcello Romano

Naturalist

The author broadly traces the most important stages of entomological research in Sicily, following a chronological path from the mid-17th century to the end of the last century. The research focuses mainly on researchers on systematic entomology. The most well-known and recognized Sicilian protagonists are quickly reviewed, briefly illustrating their human and scientific figure and the events that most influenced their lives and consequently their entomological activity.

Particular attention is also paid to the many enthusiasts or simple connoisseurs who, despite not leaving written contributions, tackled this path with great commitment and dedication and contributed to the maintenance of entomological activity on the island, thus enabling the baton to be passed on to subsequent generations. Their memory must not be dispersed, as has unfortunately often been the case with their collections.

Finally, the presence in Sicily of numerous entomological collections that have come down to us more or less intact is emphasized, representing a historical, cultural and scientific heritage that can no longer be overlooked or ignored.



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Session I



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Dancers from the Cretaceous, phylogeny and reproductive ethology in Mesozoic empidooid flies (Diptera: Empidoidea)

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The Empidoidea are a highly diverse group (about 12,000 known species) of predatory and pollinating Diptera, present in almost all terrestrial ecosystems, despite they are often unnoticed due to their small size. Some representatives of the clade, such as the Empididae and the Dolichopodidae, are noteworthy for their complex courtship rituals characterized by dances, complex rhythmic movements, stationary flights, or the exchange of gifts in the form of prey or silk cocoons. The fossil record of the Empidoidea is quite extensive and dates to the Jurassic, though fragmentary and sometimes ambiguous. The study of ambers from Kachin (Myanmar) dating back to the Late Cretaceous (99 Mya) brought to light some unusual fossil empidooids, which have been assigned to a new genus, *Electrochoreutes*, represented by seven species. *Electrochoreutes* is characterized by a unique mosaic of plesiomorphic and apomorphic characters among all the known Diptera, complicating the reconstruction of the phylogenetic affinities of this taxon and throwing into crisis the entire phylogeny of the Empidoidea. The study of the fine anatomy of the fossils by means of high-resolution X-ray phase contrast micro-tomography allowed us to study in detail key morphological characters to reconstruct the relationships of these fossils within the empidooid clade through a cladistic analysis. Phylogenetic analyses, encompassing a wide selection of representatives of all clades of Empidoidea and all extinct Mesozoic genera and applying a wide range of analytical methods, reconstructed *Electrochoreutes* as the stem-group of the Dolichopodidae. One of the most remarkable features of *Electrochoreutes* is represented by the high sexual dimorphism and the presence of species-specific sexual characteristics on the forelimbs of the males of each species, suggesting that the latter played an important role in mating. The modern Dolichopodidae are often distinguished by the presence of secondary, sometimes exaggerated, sexual characters, which are displayed by the male during elaborate dances preceding the copulation and probably played an important role in intraspecific recognition. The diversity in form and structure of these characters in *Electrochoreutes* species suggests that complex mating rituals evolved in this lineage during the Cretaceous and shaped the evolution of the group since the beginning.

KEY WORDS: cladistics, Dolichopodidae, Eremoneura, fossils, X-ray phase contrast microtomography.

ORAL PRESENTATION



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Morphological description of the mouthparts of *Halyomorpha halys* (Hemiptera: Pentatomidae): focus on sensory receptors

Maria Chiara Battistelli, Paola Riolo, Nunzio Isidoro, Sara Ruschioni

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Halyomorpha halys (Stal) is a phytophagous Asian pentatomid hemiptera with biting-sucking mouthparts. The feeding mode of *H. halys*, associated with its high polyphagia and the gregarious behaviour, have made it an economically important pest in agriculture, especially in tree crops, where the feed bites are responsible for fruit deformation and decolouration and, consequently, the product unmarketability. The high specialization of the different structures of the mouthparts and the continuous interaction that these have with the food source suggest their important role not only in food intake but also in its evaluation and choice. In this context, the presence of the sensory receptors is essential. Therefore, knowledge of their characteristics, functions and organization is mandatory. The aim of the present study was to provide, for the first time, a detailed description of the mouthparts gnathites (upper lip, lower lip, mandibular and maxillary stylets) and sensilla of *H. halys* by using a Scanning Electron Microscope (SEM). On the basis of their morphology and size, the sensilla were linked to seven categories and fourteen types: ampullacea (two types), basiconica (two types), campaniformie (one type), coeloconica (two types), chaetica (four types), styloconica (two types) and trichodea (one type). The attribution of the functions performed by each typology of sensilla (chemoreceptive, thermoreceptive, hygroreceptive, mechanoreceptive) was based on their shape, number, distribution and position. Moreover, both male and female adults were observed to detect a possible sexual dimorphism. The information acquired with this study can be potentially used in the future for the design and implementation of new sustainable control strategies.

KEY WORDS: *Halyomorpha halys* (Stal), phytophagous, sensilla, biting-sucking mouthparts, scanning electron microscope.

ORAL PRESENTATION



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

German diversity of *Megaselia* (Diptera, Phoridae). A starting point for investigation of "dark taxa"

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Species extinction is increasing at a global scale, a rapid inventorization of our planet's biodiversity is becoming more and more important. It is a pressing need investigating insect biodiversity and accelerating species discovery and description, especially for species belonging to megadiverse yet understudied insect groups, also known as "dark taxa". Phoridae (Diptera) and are a great example of a "dark taxon", in particular the genus *Megaselia* Rondani, which contains more than 1,700 described species. The use of an integrative methodology, combining morphology with molecular methods, is probably the best approach to face hyper-diverse and "dark taxa". Species delimitation for these taxa should be based on multiple data sources (integrative taxonomy). Here, we use the large-scale integrative taxonomy (LIT) approach to sort 10,000 *Megaselia* into 277 preliminary species hypotheses based on next-generation sequencing (with MinION, ONT) barcode (658 bp) clusters, using Objective Clustering with a 3% threshold. These clusters were passed through the evaluation of the predictors for incongruence indexes between barcode clusters and morphology (maximum p-distance, stability index), and then a subset of specimens have been morphologically examined, finding many new species. Furthermore, we provided species estimates with Chao1, and results suggest that 21–80 more *Megaselia* species await discovery in the areas in Germany we sampled. As this estimate is based on collections from southern regions of Germany, the species count will likely increase with expanded geographic sampling. This work is part of the German Barcode Of Life III: dark taxa (GBOL) project, that is surveying the genetic diversity of animals, fungi and plants in Germany, with a focus on four "dark taxa" of insects, and lays the foundation for a more thorough investigation of highly diverse species globally.

KEY WORDS: Barcoding, dark taxa, diversity, Diptera, *Megaselia*, MinION, Phoridae, species delimitation.

ORAL PRESENTATION



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Rediscovery of *Psectrosema tamaricis* (De Stefani, 1902) (Diptera, Cecidomyiidae) in Italy and redescription of the species

Giuliano Cerasa, Gabriella Lo Verde

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The gall midge *Psectrosema tamaricis* (De Stefani, 1902) described under the genus *Rhopalomyia* Rübsaamen, 1892 was found in Palermo, Sicily, near the type locality more than 100 years after the original description; this species induces galls on *Tamarix gallica* and *T. tetrandra* (Tamaricaceae).

Gagné (1996) in the revision of the genus *Psectrosema* recorded 26 valid species all associated with *Tamarix* (Tamaricaceae) from the Mediterranean region to Central Asia and as far as China. Observations on biology and information on the species distribution, illustrations and diagnosis for immature stages, adults and galls, useful for redescription, are provided. Adults of *P. tamaricis* with one segmented palpus, clublike, coalesced last antennal flagellomeres, long pulvilli, empodia narrow, barely wider than pulvilli. Pupae with a short pupal prothoracic spiracle and lack of spines on the abdomen. *Psectrosema tamaricis* is similar to *P. album* and *P. nigrum*; however, *P. album* has a small lobe on the lower pupal frons and tarsal claws each with small tooth while *P. tamaricis* does not have a small lobe on the lower pupal frons and the tarsal claws are simple; *P. nigrum* has a pigmented pupal abdomen, tarsal claws each with small tooth and spatula broadens below the anterior teeth while *P. tamaricis* does not have a pigmented pupal abdomen the tarsal claws are simple and the spatula does not broaden below the front teeth. The galls consist of a swelling of the terminal parts of the branches, initially green then purplish-brown; frequently the apical portions of the scaly leaves incorporated in the galls protrude on its surface. The larval chamber is large and elongated, and over time the galls take on a woody consistency, leading to desiccation of the terminal part of the twig concerned. The adults emerge from December until mid-April often leaving the exuvia protruding from the emerging hole and the galls remain on the plant for several years after the gall inducer emergence.

KEY WORDS: Gall midge, *Tamarix*, taxonomy, Sicily.

POSTER



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

The sperm conjugation is a mechanism shared by several groups of Adephaga

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Sperm conjugation was described in Gyrinidae, Carabidae and Dytiscidae. In the process of sperm aggregation only the sperm heads are involved, while flagella are free. Extracellular material (spermatostyle), when present, can be associated to the sperm heads according to different modalities:

1. sperm pair joined in antiparallel manner, apparently without spermatostyle (some Dytiscidae);
2. sperm bundles attached with their heads to an elongated structure of extracellular material (spermatostyle) (Gyrinidae and some Carabidae);
3. sperm bundles with their heads protected by a cup of extracellular material (spermatostyle) (some Carabidae);
4. sperm rouleaux, with the tip of one sperm head adapted into the hollow, hooded portion of another sperm's head to form orderly stacks (some Dytiscidae). These sperm head stacks can be surrounded by extracellular material (spermatostyle) (Dytiscidae- Hydroporinae)

It was suggested that sperm conjugation, that is retained the ancestral condition of sperm transfer, at least in Dytiscidae, could be realized by convergent evolution in the whole Adephaga.

As to the functional significance of the sperm conjugation, several hypotheses, have been proposed other than that of a more rapid filling of the spermatheca. Among these, it has been suggested a physical protection from spermicidal environments, and a molecular exchange between sperm enhancing sperm motility and egg fertilization

KEY WORDS: Insect sperm, Adephaga sperm conjugation, sperm ultrastructure.



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Giant mating plug in a small diving beetle

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University of Siena, Italy

Males of insects experimented different reproductive strategies to avoid sperm competition and female remating with a second male. It is well known the case described by Parker (1970) on *Scatophaga stercoraria* with males remaining with the females up to oviposition.

Other male behaviours, however, have been described to reach the same purpose.

In the small dytiscid *Stictonectes optatus* (Seidlitz) (about 2.5 mm long), the male, at mating, produces with its accessory glands a secretion to form a large plug (0.5 mm in diameter). This plug will be introduced, together with the sperm, within the bursa copulatrix of the female, thus avoiding a second mating with a different male. The plug becomes stable into the female genital organs up to oviposition.

It is still under discussion whether the female can contribute to the formation of the plug.

KEY WORDS: Insect reproduction, sexual selection, female ultrastructure.

POSTER

SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

WID: World Ichneumonidae Database – A comprehensive database for the world fauna of Ichneumonidae (Hymenoptera, Ichneumonoidea)

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Today, taxonomists must face the daunting task of preserving biodiversity while much of it remains mostly unknown. This ambitious challenge requires the development of user-friendly tools to revive the interest in alpha-taxonomy and direct efforts towards the study of neglected taxa. Indeed, a lack of easily accessible resources is a major deterrent to new taxonomists taking up the study of poorly known groups. One of these is severely understudied taxa is Ichneumonidae, a hyper-diverse family of parasitoid Hymenoptera, that counts about 26.000 described species worldwide and approximately 60.000-100.000 estimated. Despite their importance as potential biological control agents in applied entomology, their diversity, distribution, and host associations remain largely unexplored. One of the main issues in dealing with such a hyper-diverse lineage is to manage a huge amount of data regarding nomenclature (valid/invalid names), geographical distribution, and biological aspects. Here, we present a comprehensive database for the world fauna of Ichneumonidae (WID: World Ichneumonidae Database), that facilitates the gathering of information on described species, nomenclature, distribution, and host association of this family, while serving as a repository for worldwide literature. WID is set up using a modular approach, beginning with the insertion of described taxa (valid/invalid names), followed by the inclusion of later citations, asserted geographical distribution, information on type series, biological associations, and images. WID is built in TaxonWorks, an integrated web-based workbench designed to organize and archive observations, images and notes for taxonomists. Differently from classical databases, TaxonWorks offers a pre-built scaffold that serves as a collaborative research environment for taxonomists working on the same taxonomic group. WID aims to be a ready-to-use, not individual-based tool, that allows to collect verifiable and cross-validated information. We believe that WID would be a valuable resource for the scholars working on Ichneumonidae, serving as a shared repository for world species knowledge and a stimulating research environment for the development of catalogues, interactive keys, matrices, and checklists.

KEY WORDS: biodiversity, catalogue, digitalization, checklists, nomenclature, parasitoids, taxonomy.

ORAL PRESENTATION

SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

**Integrative taxonomy of species of the genus *Anthophora* Latreille, 1803
(Hymenoptera, Apoidea) in Rome**

Diana Lucente, Lorenzo Fortini, Lucrezia Spagoni, Andrea Di Giulio, Marco A. Bologna, Alessandra Riccieri

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Anthophora is a genus of Hymenoptera Apoidea that counts about 400 species all over the world. In the region of interest for this study, Latium, have been recorded 25 of the 42 species registered in Italy. Most species emerge between March and April, but they are active also during summer, and in some cases until October. These bees are very common even in urban centers. Dichotomous keys for morphological identification of European *Anthophora* bees are currently not available and they can be easily mistaken with other species, like those of the genus *Amegilla*. Also, genetic and molecular knowledge about European *Anthophora* species in online databases is scarce. The aim of this work is to analyze the *Anthophora* species present in Rome and surroundings, by using both morphological and genetic traits. The sampling took place in 2022 and 2023, in urban parks in Rome and natural reserves in the roman countryside. The samples were dry-preserved, and a leg was collected in alcohol for molecular analysis. Morphological identifications were conducted mostly through the comparison with determined samples of museum's collections. The identified morphospecies were analyzed through DNA's extraction and COI (Cytochrome Oxidase I) sequencing and the results were compared with GenBank and BOLD databases to obtain a genetic identification. Then, a Neighbor-Joining phylogenetic tree was built to assess the monophyly of morphospecies and to confirm their identifications. This study has been conducted on a small area to date, but it should be extended in order to include more species and to acquire major knowledge about these important but taxonomically neglected pollinators. Also, this study could provide a solid basis to enlarge the research into the ecology and the conservation of the *Anthophora* bees in urban areas.

KEY WORDS: urban pollinators, morphology, Barcoding, COI.

POSTER

SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Antennal chemosensilla in the european ecto-parasitoid *Sclerodermus brevicornis* (Kieffer) (Hymenoptera: Bethylidae)

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Sclerodermus brevicornis (Kieffer) (Hymenoptera: Bethylidae) is a European flat wasp, ectoparasitoid of some longhorn beetle species (Coleoptera: Cerambycidae). Many studies have suggested that these bethylid wasps can be a candidate for the biological control of stored product insect pests. Additionally, the bethylid wasps can also sting humans causing dermatitis characterized by itch and erythematous papular lesions. To better understand the host location process and the interactions with humans the ultrastructure of antennal sensilla has been studied using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Adults of *S. brevicornis* used in this study were supplied by a stock laboratory culture from University of Milan and reared under laboratory conditions [25°C, 60% RH, and a photoperiod 16:8 (L:D) h]. The wasps were reared on larvae of *Corcyra cephalonica* (Lepidoptera: Pyralidae), following the protocol published by Abdi, Mohamed Khadar, Lupi et al. (2021).¹ The specimens were treated using the usual protocols for SEM and TEM investigations.

This study is the first that describes the antennal chemosensilla of the bethylid flat wasp *S. brevicornis*. For the insect nomenclature, we referred to a previous study on the other bethylid wasp species *Sclerodermus guani* Xiao & Wu, 1983.² The geniculate antenna of *S. brevicornis*, as all members of the genus *Sclerodermus* Latreille, 1809, consists of a proximal scape, a median pedicel and a distal flagellum. The latter is divided into 11 flagellomeres or annuli.

Sclerodermus brevicornis wasps exhibit a well evident sexual dimorphism in relation to type, number, length, and distribution of olfactory and gustatory sensilla. In addition, the antennae in males are longer than those of females. The chemosensory sensilla are represented by: sensilla placodea, double walled porous sensilla type I and type II, multiporous long sensilla basiconica, multiporous sensilla trichodea type I and type II, uniporous sensilla chetica. The chemosensilla LSB (Long Sensilla Basiconica), are present only in females, whereas other type of chemosensilla are located on the antennae of both sexes.

Numerous mechanosensilla are scattered on the antennal surface. The annuli that have a greater amount of sensilla are the last nine annuli.

The deep knowledge of the kind and distribution of antennal sensilla of the species belonging to the genus *Sclerodermus* can be important as taxonomic character to help to recognize the different species of flat wasps present in Italy and in the world. Moreover, this study represents a starting point in order to perform electrophysiological investigations to better understand the host research behaviours of *S. brevicornis* and the possible attraction mechanisms of bethylid wasps toward humans.

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KEY WORDS: *Sclerodermus brevicornis*, chemosensilla, sensilla, SEM, TEM

ORAL PRESENTATION



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Morphofunctional analysis of *Tribolium castaneum* during metamorphosis

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The red flour beetle, *Tribolium castaneum* Herbst 1797, is the most significant pest among Tenebrionidae (Coleoptera), causing huge losses to a wide range of stored agricultural products, such as flours and cereals. The control of this insect is mainly obtained through chemical compounds, whose use raised serious concerns due to environmental pollution, food contamination, and development of insecticide resistance. It is thus necessary to find new, environmentally-friendly alternatives that do not pose a risk to human health.

Although a wide literature on the molecular mechanisms that regulate the development of *T. castaneum* is available, little is known about the morphology of this coleopteron. To fill this gap of knowledge, we performed a preliminary, morphofunctional characterization of this insect during metamorphosis, with particular attention on the alimentary canal and the fat body, by using optical and electron microscopy, and synchrotron X-ray phase-contrast microtomography (SR-PhC micro-CT), which allowed an integrated analysis at different structural levels, from organs to cell organelles.

The midgut showed to be highly remodelled during larva-to-adult transition. In particular, the morphology and ultrastructure of this organ significantly changed during prepupal and pupal phase. SR-PhC micro-CT virtual slice and volumetric rendering provided high-resolution images of the gut structure, showing the replacement of the old epithelium by the newly formed one. On the contrary, only minor modifications were observed in the fat body. Since in other holometabolous insects the organization of both organs is profoundly affected during metamorphosis, the pattern observed in *T. castaneum* deserves further investigation to, possibly, confirm that it represents a peculiarity of this species.

Given that *T. castaneum* proved to be highly responsive to systemic RNAi treatment, these morphological studies, combined with knowledge on the genetic control of the development of this coleopteron, could hopefully represent the starting point to develop innovative and sustainable protocols based on this tool.

POSTER



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Spermiogenesis of *Halyomorpha halys* (Hemiptera: Pentatomidae) and Sterile Insect Technique: an ultrastructural study

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The brown marmorated stink bug (BMSB) *Halyomorpha halys* (Heteroptera: Pentatomidae) is an insect pest species native to the Far East that since 1990s invaded mid-Atlantic regions and, more recently, Europe, causing severe damages to several fruits and vegetal crops. Although various approaches have been adopted all over the world to contain the damage caused by this insect, so far the control of *H. halys* has proved to be difficult. Among the possible strategies to manage invasive pest species, the sterile insect technique (SIT) is considered one of the most sustainable and effective.

The SIT strategy consists in the sterilization of a large number of individuals of a target species by means of ionizing radiations so that the matings of wild individuals with irradiated ones are infertile.

As for the ultrastructure of the sperm of Heteroptera it is worth mentioning the presence of bridges that link the mitochondrial derivatives to the axonemal microtubules, the absence of accessory bodies, and the presence of two or three crystalline inclusions within the mitochondrial derivatives. Furthermore, in the centriolar region of Heteroptera there is an atypical microtubule organizing center (MTOC), unusual for that region in insects, whose formation and function need to be elucidated.

With the present contribution we illustrate, from the ultrastructural point of view, the spermiogenesis of *H. halys*, paying a particular attention to the development of the structures related to the centriolar region and investigating the potential effects of irradiation on the maturation of sperm cells.

KEY WORDS: Brown Marmorated Stink Bug, MTOC, SIT, spermatid, sperm.

SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Development of the antennal pathway in hemimetabolous insects: from sensilla to the brain

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Despite insect fauna dominating inland water ecosystems, we know little about its sensory biology and neuroanatomy. Among winged insects, Odonata is probably the oldest order, and together with Ephemeroptera they constitute the clade Paleoptera. Differently, Plecoptera is typically considered an order of basal Neoptera, closely related to terrestrial ones such as Orthoptera and Blattodea. The study of the sensory biology of aquatic insects undergoing incomplete metamorphosis, gradually passing from nymphal life in fresh water to adult aerial life, provides great opportunities to understand how Arthropod nervous systems can adapt to ecological challenges. In particular, considering that all aquatic insects derive from terrestrial ones, investigations on hemimetabolous Paleoptera and Neoptera can allow us to reconstruct their successful invasion of lentic and lotic ecosystems, through physiological adaptations such as new ways to breathe, move, acquire food, and sense the environment. In this context, we investigated the antennal sensilla, and the related sensory pathways in the brain of nymphs and adults of the dragonfly *Libellula depressa* (Paleoptera), and the stonefly *Dinocras cephalotes* (Neoptera). Antennal sensilla are dramatically different between nymphs and adults, to perceive different cues in water and air. For example, nymphal antennae of dragonflies are particularly rich in mechanoreceptors, which completely lack in adults. In contrast, mechanoreceptors are predominant in adult stoneflies, which use vibrational communication to meet and mate, while chemoreceptors are more numerous in nymphs. Still, the general morphology of the brain and the sensory circuitry do not show big changes during development, with dragonflies characterized by an aglomerular bi-partite antennal lobe and stoneflies showing a glomerular neuropil. Scanning electron microscopy describes the antennal sensilla, and the brain structures are visualized with an anti-synapsin antibody. The antennal receptor neurons and antennal lobe output neurons are mass-traced with tetramethylrhodamine. Our data suggest that the same brain centers can be able to process highly diverging information, provided through different sensory structures adapted to different environments. This agrees with developmental plasticity, which serves as a mechanism to maintain functionality throughout ontogenesis when the lack of a pupal stage does not allow metamorphic changes in the nervous system. The present data also advance the basal knowledge on the biology of Odonata and Plecoptera, threatened insects in fragile ecosystems, and thus present relevant perspectives in insect conservation.

KEY WORDS: aquatic insects, stonefly, dragonfly, insect brain, antennal sensilla, olfaction.

ORAL PRESENTATION

SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Morphology of the male reproductive apparatus and sperm of *Thaumascoris peregrinus* (Thaumastocoridae)

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The bronze bug, *Thaumascoris peregrinus* Carpintero & Dellape (Thaumastocoridae), is a tiny Heteroptera that causes severe damage in *Eucalyptus* plantations in Brazil, Australia and other parts of the world. The economic importance is pretty much known for this species and Thaumastocoridae, aspects such as their inner morphology, specifically of the male reproductive apparatus and sperm, are mostly unknown. One paper was published on *T. peregrinus* male reproductive system, but after some analysis, we felt the need to correct and update some information. Thus, we describe here the overall morphology of the male reproductive apparatus of *T. peregrinus*, as well as their sperm using light microscopy. This species shares the basic reproductive morphology features of most insects. They show paired testes, each presenting three follicles connected to short and dilated efferent ducts that merge and open to the deferent ducts. Two sets of paired tubular accessory glands were also connected to an ejaculatory duct, and no ejaculatory bulb was observed. Different from what was previously published, in the testes, we could observe what seemed to be mainly mature sperm forming bundles derived from individual cysts with no sequential zones of differentiation. Also, after being released from the follicles, the sperm was mainly stored in the efferent ducts dilated portion, with no clear, seminal vesicles derived from the deferent ducts, as is most usual. The sperm of *T. peregrinus* were filiform, being $463.6 \pm 6.9 \mu\text{m}$ long, and nuclei were very short and fusiform, measuring $5.3 \pm 0.2 \mu\text{m}$. We could not observe any sign of an acrosome. A paper by Kumar (1964) covers the male apparatus of *Thaumastocoris australicus*, which is similar to *T. peregrinus*, although they probably mistook the dilated efferent duct as a seminal vesicle by the provided illustration. In other Miroidea (sensu Schuh and Stys: (Thaumastocoridae + (Miridae + Tingidae)), there is continuous sperm production in male adult insects and the presence of typical seminal vesicles. Furthermore, in these two families, the sperm present longer nuclei in relation to their total length; their acrosomes are long and easily recognizable. All these features together may reflect a recent change in the systematics of the group. Thaumastocoridae, previously a member of the Miroidea (Cimicomorpha), was placed as a sister to Pentatomorpha when including morphological and molecular data in a single analysis. However, the monophyly and placement of the Thaumastocoridae are still ambiguous. Further studies describing alternative data sets, such as the male reproductive system for more species and the sperm ultrastructure, might help clarify this discussion.

KEY WORDS: Hemiptera, Internal morphology, Reproduction, Spermatozoa.

POSTER



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Ultrastructural characterization of maxillary palps in Coccinellidae

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Insect antennae are defined as the primary sensory organ, therefore mostly involved in intra- and interspecific communication. This trait is quite common within the whole class and throughout the different orders. As regards Coleoptera, this insect order represents the most diverse in terms of described species. Coccinellidae (ladybirds) is one of the families that attracted the interest of numerous researchers because of their impact, as natural enemies, of economically important pests. Ladybirds can be divided, according to their feeding habit, into phytophagous, entomophagous (mainly aphidophagous and coccidiphagous), mycophagous and polliniphagous. In this work, we investigated the maxillary palps ultrastructural organization of some economically important species characterized by a different feeding habit: entomophagous (*Harmonia axyridis*, *Scymnus interruptus*, *Delphastus catalinae*, *Cryptolaemus montrouzieri*) and phytophagous (*Subcoccinella vigintiquatuorpunctata*). The ultrastructural organization of maxillary palps of all species was investigated by Scanning Electron Microscopy and Transmission Electron Microscopy. In all species, the maxillary palps were segmented, with the main sensory area located at the apical part of the enlarged distal segment. No sexual dimorphism was observed. On maxillary palps, we found four sensilla types: multiporous gustatory sensilla I (MPG I) and II (MPG II), sensilla campaniformia and sensilla trichoidea. The sensilla shape was similar throughout all investigated species. The MPG I and MPG II occupied the major part of the apical sensory area, while sensilla campaniformia were positioned along the border of the area. As regards *H. axyridis*, the overall sensilla number located on the maxillary palps was higher compared to the number of antennal sensilla, and this reflects also on the number of sensory neurons innervating the structures. Moreover, the low number of putative gustatory sensilla on the antennae is counterbalanced by the high concentration of gustatory sensilla on the maxillary palps. We hypothesized that in Coccinellidae there are two primary sensory areas, the antennal sensory area and the maxillary palps sensory area. The data presented here represent a solid base for further detailed investigation of maxillary palps role in coccinellids chemical ecology.

KEY WORDS: Morphology, Sensilla, Mouthparts, *Harmonia axyridis*, *Cryptolaemus montrouzieri*, *Subcoccinella vigintiquatuorpunctata*.

ORAL PRESENTATION

SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

DNA barcoding of *Toumeyella parvicornis* (Hemiptera: Coccidae)

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The pine tortoise scale, *Toumeyella parvicornis* (Hemiptera: Coccidae), is a sap-sucking pest native to the Nearctic region that affects several *Pinus* species, whose heavy infestations may contribute to host tree declines. Recently, the scale insect was accidentally introduced to Europe where it was first discovered in Italy in 2014 and later in southern France in 2021. Since its introduction, *T. parvicornis* has rapidly spread across several regions in Italy and is contributing to pine tree losses, particularly in the areas surrounding Naples and Rome. The infestation caused by the pest is of great concern as it has the potential to significantly impact the local pine forests and cause direct and indirect damage to urban areas. The lack of a reliable system to trace the origins and spread of *T. parvicornis* infestation limits our capacity to identify the main entry routes of this alien species and to develop effective control measures. In this study, we used the mitochondrial COI and ribosomal 28S-D2 markers to assess the genetic diversity of pine tortoise scale populations and to study its invasive process in Europe. Samples were collected from Italy, France and USA. Alignment of the 28S-D2 sequences revealed a unique haplotype, while COI showed a single haplotype in Europe which differs by five nucleotide substitutions from USA population of *T. parvicornis*. Moreover, our dataset differs by approximately 10% identity from the only two sequences of *T. parvicornis* available in GenBank at the moment, suggesting a likely erroneous taxonomic annotation of these samples collected in Canada. These results highlight the importance of morphological identification and haplotype analysis in tracing the origins and spread of invasive pests. Further studies and additional samples from different countries are needed to fully reconstruct the invasive process of *T. parvicornis* and to implement effective control measures.

KEY WORDS: invasive species, mitochondrial gene, soft scale insect.

POSTER

SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

Cuticular structures of Buprestidae (Coleoptera: Buprestoidea): do we really know everything?

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Buprestidae are a beetle family with approximately 15,000 described species, present in all continents except Antarctica. The group, also commonly known as Jewel Beetles, is characterized by the variety of patterns and richness of colors, both physical and chemical, of their integuments. It is not clear yet the eco-ethological meaning of these colors, even if it is hypothesized that they may contribute to cryptic mimicry, constitute an aposematic signal in some groups or have role in the interaction with conspecifics. In addition, if we exclude the studies conducted on the infrared receptors of the Melanophilini and the antennal sensilla of some species of phytosanitary interest, very little is known about the structures present on the integument of Buprestidae and the role they may have in their biology. In this study the main cuticular structures of the Buprestidae are examined, with particular emphasis on those little known or not described yet. In particular, through the use of scanning electron microscopy (SEM), also combined with ion beam microscopy (FIB/SEM), these structures are investigated and characterized and, when possible, new structures are identified in the main subfamilies and tribes. The preliminary results show that within the family there are peculiar traits (i.e. the morphology of the bristles, often in association with pores for secretion), potentially useful in determining phylogenetic relationships. Furthermore, the discovery of putative sensory pores on the thorax of the genus *Agrilus* associated with the presence of subgular stridulatory organs suggests the existence of a complex set of interactions with conspecifics based on chemical-vibrational stimuli. Understanding the functionality of these structures could, beyond the eco-ethological aspects, constitute an important element in the development of more effective methods of detection and monitoring of invasive species.

KEY WORDS: Buprestoidea, beetles, fine morphology, integuments, ultrastructure.

ORAL PRESENTATION



SESSION I

MORPHOLOGY, SYSTEMATICS AND PHYLOGENY

An integrated approach solves the alpha taxonomy of cryptic species in Scelionidae (Hymenoptera), egg parasitoids of bugs (Hemiptera: Pentatomoidea)

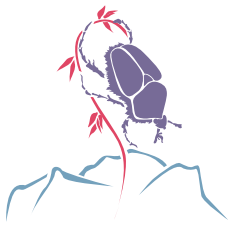
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The subfamily Telenominae (Hymenoptera: Scelionidae), and especially the genera *Telenomus* Haliday and *Trissolcus* Ashmead are widely studied because of their potential as biological control agents of pests. Species of the genus *Trissolcus* are egg parasitoids of Pentatomoidea (Hemiptera) and particularly of the families Pentatomidae and Scutelleridae. *Telenomus* species share the same hosts with *Trissolcus* species, but they are also able to attack eggs of other Heteroptera, Auchenorrhyncha, Lepidoptera, Diptera and Neuroptera. The taxonomy of *Telenomus* and *Trissolcus* has received increased attention in recent years following the invasion of brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Great progress has been made in the taxonomic study of the Palearctic species of the genus *Trissolcus*, and some of these cryptic species have been well characterized as a result. Contrarily, *Telenomus*, the largest genus of the family, still includes a considerable number of species in the Palearctic area with insufficient information for the species-level identification. Therefore, many authors have identified species of the group *Telenomus podisi* Ashmead, known as parasitoids of pentatomids and scutellerids, as *Telenomus* sp. or *Te. chloropus* (Thomson). From analyses of parasitoids emerged from field-collected eggs, a multidisciplinary approach — biological (reproductive isolation tests), molecular and morphological — applied to the alpha taxonomic study of cryptic species in the two scelionid genera showed the presence of four valid species within the species *Tr. semistriatus* (Nees von Esenbeck). This approach also reinforced the statement of morphological characters previously proposed to distinguish *Tr. japonicus* (Ashmead) from the species *Tr. kozlovi* Ryachovsky. Molecular and morphological analysis have also made it possible to identify the suite of morphological characters to distinguish *Te. heydeni* Mayr and *Te. turesis* Walker. The results obtained in recent years in the integrative taxonomy of cryptic species in *Trissolcus* and of *Te. podisi*-group from the Palearctic region are here reported.

KEY WORDS: *Trissolcus*; *Telenomus*; reproductive isolation; egg parasitoid; molecular analysis.



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P A L E R M O

XXVII Congresso Nazionale
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12-16 June 2023

Session II



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Plastivorous activity of *Hermetia illucens* larvae (Diptera Stratiomyidae)

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The world's production of plastics has increased dramatically in recent years, going from 234 million tons in 2000 to 460 million tons today. The same phenomenon is evident in the production of plastic waste which has currently reached 353 million tons. Globally, the mismanagement of plastic waste has led to its accumulation in freshwaters, oceans and land in any form, including microplastics. Reducing plastic pollution has become one of the most pressing environmental challenges that require effective and integrated strategies. Recently scientific research is focusing on the identification of insect species which, thanks to their intestinal microbiota, have the ability to effectively degrade synthetic polymers. Although the larvae of some coleopterans and lepidopterans are the most studied organisms for the biodegradation of plastics, other species could offer similar capabilities. Therefore, the present research has had the aim of evaluating the "plastivorous" activity of *Hermetia illucens* (Diptera Stratiomyidae), reared on a diet contaminated by fluorescent polystyrene (PS) microparticles (latex beads, carboxylate-modified polystyrene, fluorescent yellow-green, 2 µm, aqueous suspension 2.5%). Evaluation of larval performance did not reveal significant differences in development and mortality between the larval population reared on the PS-contaminated diet compared to that reared on the control diet. The examination of the larval intestinal tissues through the laser scanning confocal microscope, in a bright field and with fluorescence, allowed us to observe the abundant presence of the fluorescent particles of PS in the foregut and midgut and minimally in the hindgut, with translocation of the fluorescent particles of PS within the cells of the midgut epithelium. Moreover, the formation of low molecular weight products from the biodegradation of PS was investigated by GC-MS. This research, which should be completed with the analysis of the intestinal microbiota and proteomic analysis to identify the microorganisms and enzymes involved in the degradation of PS, provides interesting results on the potential "plastivorous" capacity of *H. illucens*.

KEY WORDS: plastic, biodegradation, black soldier fly, plastivorous capacity, microbiota, polystyrene

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Trichoderma harzianum* enhances direct and indirect tomato plant defenses against the stink bug *Halyomorpha halys

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Beneficial soil-borne fungi belonging to the genus *Trichoderma* provide several benefits to plants by promoting their growth, enhancing nutrient uptake or suppressing plant pathogens. In recent years, there is increasing evidence showing that these beneficial soil microbes also mediate plant defenses against insect herbivores and increase the attraction of their associated natural enemies to plants under insect herbivore attack. However, limited knowledge is up to now available regarding the impact of *Trichoderma* species on trophic systems consisting of plants, stink bugs and their egg parasitoids. Thus, we here investigated how *Trichoderma harzianum* strain T22 modulates tomato plant defenses in response to the brown marmorated stink bug (BMSB), *Halyomorpha halys*. We first determined the effect of *T. harzianum* on direct tomato plant defenses against *H. halys* feeding activity. The results showed that *T. harzianum* reduces the relative growth rate of BMSB nymphs that develop on inoculated plants. At the molecular level, Trichoderma-mediated effects were observed with higher transcript levels of the JA-dependent gene PIN2 and SA-marker gene ToPR1 in inoculated and BMSB-infested plants compared to infested and non-inoculated ones. Furthermore, we examined the role of *T. harzianum* in modulating indirect tomato plant defenses by investigating the behavior of *Trissolcus japonicus*, one of the main *H. halys* associated egg parasitoid, in response to volatile released from BMSB induced plants in Y-tube olfactometer. We found that *T. harzianum* T22 root colonization enhances the attraction of *T. japonicus* females to oviposition-induced plant volatiles. The importance of these findings will be discussed within molecular and chemical perspectives.

KEY WORDS: plant growth-promoting fungi, multitrophic interaction, defense signaling pathways, *Trissolcus japonicus*.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

**Courtship, mating, and behavioural asymmetries in two aphid parasitoids,
Aphidius ervi and *Aphidius matricariae***

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Biological control agents (BCAs), such as parasitoids, are widely employed to control agricultural pests. Baseline knowledge on their biology and ecology can contribute to the success of biocontrol programs. Knowing parasitoids' reproductive habits is crucial for assessing their potential as BCAs, as well as for mass-rearing development and implementation. The study of sexually selected displays, such as male wing fanning, may aid in the selection of strains with high reproductive success, through the promotion of successful male phenotypes. This data can be utilized for improving mass-rearing processes. Indeed, prolonged mass-rearing might result in mating failures, which can impact the overall success of insect control programs. Herein, we analysed the courtship and mating behaviour of two braconid parasitoids belonging to the genus *Aphidius*, i.e., *Aphidius ervi* Haliday and *Aphidius matricariae* Haliday, which are successfully used for the biocontrol of various aphid species. Besides quantifying key displays leading to copulation, we investigated the presence of population-level lateralized traits. Results showed that antennal tapping, wing fanning, as well as the overall precopula and copula phases were longer in *A. matricariae* than in *A. ervi*. Only *A. matricariae* exhibited post-copulatory behaviour. At variance with other braconid species, male mating success of *A. ervi* and *A. matricariae* was not affected by the duration of wing fanning, chasing, and antennal tapping. Female kicking behaviour during the precopula was right-biased at population level in both species. Overall, our study adds useful insights on the basic biology of braconid parasitoids of commercial importance for aphid biocontrol, and reports the presence of a population-level lateralized mating display in braconid parasitoids.

KEY WORDS: biological control, Braconidae, lateralization, mass-rearing, parasitic wasps, reproductive behaviour.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

The role of trophobiosis and ant behaviour in the integrated pest management of the vine mealybug *Planococcus ficus* (Hemiptera: Pseudococcidae)

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The vine mealybug *Planococcus ficus* is one of the most problematic agricultural pest worldwide. Due to the huge economic importance of this type of plantations, favoring the development of effective methods of integrated pest management (IPM) is of main importance. Many ant species establish mutualistic relationships with mealybugs mainly based on trophobiosis where the honeydew produced by the mealybugs is a valuable food source for the ants, which provide protection from predators in return. Therefore, IPM programs often suggest the control of ants to enhance the effect of mealybug natural predators.

This research is based on the idea that the trophobiosis can play an important role in the pest-monitoring phase and that the analysis of ant behaviour can be used to locate the presence of *P. ficus* and to measure the infestation levels. A group of vineyards in Trentino region (Italy) was used as study model. The collected data were firstly used to compile an ethogram of the useful behaviours, and then used to build a pest monitoring protocol based on ant behaviour. The new protocol was compared with a standard one based on the count of mealybugs on the vine leaves.

Results suggest that the new protocol is more efficient in the early detection of the pest, in the detection of under-bark clusters that will not eventually reach the leaves (false-negatives) and of pest latent presence after the use of chemical control strategies. The ant behaviour protocol represents a more efficient and precise tool both for the pest population monitoring, in relation with the evaluation of opportunities and timing of intervention, and for the evaluation of the efficacy of the strategies used for pest control. In conclusion, this new protocol is proposed as a valid complementary tool for IPM of the vine mealybug and its successful achievement encourage searching for further agricultural contexts in which ants may be reevaluated as a monitoring tool.

KEY WORDS: IPM, animal behaviour, vine mealybugs, ants, Trentino, vineyards.

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

A multimodal trap enhances the capture of the invasive pest *Halyomorpha halys*

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The brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is native to Asia and now widespread in all continents of the Northern Hemisphere. In the areas where it has become established, this invasive pest is causing considerable agricultural losses to several crops. Evaluating the population density of *H. halys* in the field is crucial for farmers to better determine the most effective control strategies. Pheromone-based traps are effective, but have also some limitations, as the insects often tend to remain in the proximity of traps and are not captured. Therefore, multimodal traps, i.e. traps combining different stimuli, have been designed. In this study, we developed and evaluated multimodal traps in which visual and olfactory stimuli were present. Visual stimuli consisted of LEDs emitting UV-A and visible light. Olfactory stimuli consisted of the synthetic aggregation pheromone and odours from already trapped *H. halys* individuals. In laboratory two-choice bioassays, we evaluated the attraction of the stink bug to different wavelengths. Moreover, in natural, agricultural, and urban environments we tested different prototypes of the trap. Our results showed that traps presenting a combination of olfactory stimuli with UV-A and blue or green visible wavelengths exerted a greater attraction on *H. halys* (up to ~8-fold) compared to traditional sticky traps or small semi-pyramidal traps with only aggregation pheromone. Thus, the simultaneous presence of synthetic pheromone and LED had a synergistic effect on *H. halys* positive phototaxis. This multimodal trap could be used not only for *H. halys* monitoring but also in other IPM applications, such as mass trapping.

KEY WORDS: stink bug, monitoring, mass trapping.

POSTER



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Investigation on seasonal movements of the woolly aphid in the aerial part of the plant and its parasitoid *Aphelinus mali*

Serena Giorgia Chiesa, Cristina Tomasi, Mario Baldessari, Gino Angeli

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Eriosoma lanigerum represents one of species of aphid complex threatening the apple cultivation and it is considered one of the apple key pests. As a result of aphid trophic action the tree suffers from altered growing of the tissues, desiccation of the branches and roots, damages on wood and the honeydew drips onto fruit. The woolly aphid management is based on pre and post-bloom insecticide treatments and resistant rootstock available on the market, however it is the contribution of the biological control by its specific parasitoid *Aphelinus mali* that is generally decisive for solve summer infestations.

The woolly aphid overwinters as nymph on the roots under the soil and, also due to the higher frequency of mild winter events, in the trunk cracks and branches in the aerial part of plants.

Its parasitoid mainly overwinters in the stage of diapausing mature larva, it has a high mortality induced by low winter temperatures; from the mummies present in the field at the end of summer, only a small percentage can generate live individuals that will emerge in early spring and can thus be able to begin the parasitism phase.

Populations of *E. lanigerum* vary over the years depending on biotic and abiotic factors but in recent years there has been a significant increase in populations in all apple orchards in Northern Italy.

In order to understanding how climatic factors can influence populations of *E. lanigerum* and its movements on the aerial part of plant, a monitoring was carried out in apple orchards in the province of Trento. This work shows a study on movements dynamics in the aerial part of plant of *E. lanigerum* and the differences from what was recorded in the past at the same latitudes.

The movements of the aphid were monitored from spring to winter with adhesive trap bands in several points of the trunk and on branches identified as overwintering points. It was observed that in the dynamics highlighted, as reported in the bibliography, are prevailing upward movements in spring and downward movements in autumn. Also due to the mild climate, the stopping of movements in the upper part of the canopy was noted late in the season (11/23/2022) while the migratory activity detected in the area closest to rootstocks continued until late winter.

Moreover, the monitoring of *A. mali* was supported by means of yellow chromotropic traps and adhesive bands placed on trunks and branches.

KEY WORDS: *Eriosoma lanigerum*, migration.

POSTER



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Effects of environmental stressors on associative learning of *Apis mellifera*

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Environmental stressors, such as exposure to insecticides in agricultural areas, can affect insect learning and memory. Several techniques and experimental procedures have been developed to study learning. Among these, the proboscis extension reflex (PER) is widely used, for example, in the honey bee *Apis mellifera*. PER protocols require careful handling of the insect and precise timing of the application of the desired stimuli needed to train honey bees and assess their learning response. Here we summarise some of our ongoing research activities using PER to assess the cognitive abilities of honey bees.

In a first study, honey bees were collected from hives located in agricultural areas (industrial crops) or in natural areas. The studies took place from 2020 to 2022. In the laboratory, bees were trained to associate a specific odour with a sucrose reward using a specially developed PER protocol and apparatus. The results were mixed. For the most part, the PER response did not differ between hives in agricultural and natural sites. However, some variability was observed between hives, suggesting that it is important to keep hives in as homogeneous conditions as possible.

In a second study, honey bees were artificially exposed to low doses (LD20) of a commercial formulation of the natural insecticide Spinosad. This insecticide is known to be highly toxic to honey bees. However, no assessment of associative learning deficits has been carried out to date. Again, bees were trained to associate a specific odour with a sugar reward using the PER protocol. Bees exposed to DL20 showed impaired associative learning compared to the control. Our results add relevant information to the knowledge on the neurotoxic effects of natural insecticides and may have implications for the management and conservation of honey bees in agricultural ecosystems.

KEY WORDS: olfaction, spinosad, sublethal effects.

POSTER



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

The rearing host species and adult conditioning differently influence female parasitoid olfactory responses

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Female egg parasitoids face challenges when foraging for their hosts, which are often inconspicuous and hard to detect. To optimize their searching behaviour, egg parasitoids rely on direct and indirect host-related cues, such as host-induced plant volatiles and volatiles from adults, respectively. However, parasitoid ability to exploit host-related cues can be influenced by learning as well as by the species used for rearing. The East Asian egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is a classical biological control agent of *Halyomorpha halys* Stål (Hemiptera: Pentatomidae). This invasive stink bug is a serious pest of several agricultural crops in Europe and North America. Adventive populations of *T. japonicus* were detected in several countries, including Italy. Furthermore, in Italy, field releases of this parasitoid have been authorized as part of a classical biological control program. Laboratory no-choice and choice tests showed parasitoid potential to develop in native non-target stink bugs. In this study, we evaluated the effect of the rearing host on the parasitoid olfactory response to host related cues. Furthermore, we assessed whether different combinations of the rearing host and adult conditioning with host-related volatiles can induce changes in the host-location behaviour of the egg parasitoid. Results demonstrated that, when reared on *H. halys*, *T. japonicus* positively responded only to plant volatiles induced by this target host. Conversely, when reared on native non-target pentatomid species, *T. japonicus* did not respond to any pentatomid-related cues. Notably, positive olfactory responses were partially recovered after adult conditioning with host related cues, but only towards volatiles related with *H. halys*. Our results are discussed in relation to parasitoid host range, parasitoid conditioning, biocontrol efficacy, and drawbacks when using alternative hosts for parasitoid mass production as biocontrol agents.

KEY WORDS: *Trissolcus japonicus*, *Halyomorpha halys*, tri-trophic interactions.

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

**Evaluation of proboscis extension response to sucrose containing imidacloprid in
Musca domestica L.**

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The housefly *Musca domestica* L. (Diptera, Muscidae) is a synanthropic species that is a major pest of livestock farms. Its management is mostly performed with chemical treatments and many cases of resistance to different insecticides have been recorded worldwide. Studying the mechanisms of development of insecticide resistance is crucial to improve the management of this pest.

The aim of the present work was to understand the behavioral resistance of *M. domestica* strain that was previously selected for behavioral resistance to imidacloprid, a widely used neonicotinoid insecticide. Specifically, the objectives were to: i) characterize the detection and behavior of susceptible and resistant fly strains by analyzing the proboscis extension response (PER) to different concentrations of imidacloprid (high: 4000 µg/ml or low: 10 µg/ml); ii) discern whether imidacloprid detection occurs in the sensory system of the tarsi or proboscis.

Adult house flies (3–6 d old) were placed into plastic holding cages without food (only water) for a starvation period of 1 d for the susceptible strain and 2-3 d for the resistant strain. Following the starvation period, flies were chilled briefly in a –20°C freezer and then sorted by sex on a chill table. Female flies from each strain were glued to wooden toothpicks using clear nail polish applied to the dorsal thorax of each female, allowing for easy handling of the flies. Two assays were performed using separate groups of flies: tarsal contact and proboscis contact test. In the tarsal contact test, 150 female flies of each fly strain that responded correctly to a negative (water) and positive (30% sucrose) test solution (control) were subsequently exposed to a 30% sucrose solution containing imidacloprid at one of the two concentrations indicated above with only the tarsi allowed to contact the sucrose solution. PER was recorded for each fly after 0, 2 and 10 s of continuous tarsal contact. In the proboscis contact test, 150 females were tested similarly to the previous test, except that the flies were allowed to feed on a 30% sucrose solution (positive control) for 2 seconds to confirm a positive response before they were exposed to the 30% sucrose solution containing imidacloprid at one of the two concentrations indicated above. PER was recorded as in the previous test.

The results show that in the tarsal contact test there was no statistical difference in PER for flies of both strains or for flies exposed to either imidacloprid concentration, suggesting that imidacloprid was not detected by the tarsal sensilla. In the proboscis contact test, PER was statistically different between fly strains and varied between imidacloprid concentrations only for the resistant fly strain, suggesting that imidacloprid detection occurred at level of the proboscis sensory system.

KEY WORDS: housefly, behavioral response, diptera, insecticide, imidacloprid, resistance.

ORAL PRESENTATION

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Increased aggressive response towards adults and larvae exposed to a fungal biopesticide in *Polistes dominula* wasps

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Nestmate recognition plays a key role in colony integrity in many social insects. This ability enables the discrimination of nestmates from foreign individuals and relies primarily on the identification of a blend of chemical compounds, particularly cuticular hydrocarbons (CHCs). Many factors can alter this chemical signature, especially in environments where anthropogenic impact is most pronounced. In recent years, much attention has been focused on the effects that some substances, e.g. biopesticides, can have on the integrity of colonies of common pollinators of agro-economic interest, such as honeybees and bumblebees. Other social Hymenoptera, such as paper wasps, have sadly received less attention despite their ecological relevance as predator of pest arthropods. In this study, we investigate the potential adverse effects of a common biopesticide, the entomopathogenic fungus *Beauveria bassiana*, on the nestmate recognition ability of the paper wasp *Polistes dominula* at different stages of the colony lifecycle. To do so, we subjected larvae and workers to topical exposure using a fungal solution at the field-recommended concentration (109 spores/ml). Firstly, we investigated the effects of exposure to *B. bassiana* on adult-brood recognition by testing the wasps' ability to discriminate between exposed and unexposed larvae. Then, we focused our attention on the adult-adult interaction to test whether exposure affects nestmate recognition. Our results show a selective removal of exposed larvae by adults. On the other hand, in workers, fungus increases aggressive behaviours toward exposed nestmate. This is likely due to a change in the CHCs profile of treated individuals. Chemical extracts from exposed and unexposed larvae and adults have been collected for GC-MS analyses for qualitative and quantitative examination of cuticular compounds. CHCs profile alteration induced by biopesticides could affect the nestmate recognition ability, undermining both behaviour and social interactions. In *Polistes* wasps, which provide key ecosystem services, selective removal of exposed larvae and nestmates can have destructive effects, delaying nest development and reducing the number of colony members needed to maintain colony integrity.

KEY WORDS: Chemical profile, social paper wasp, nestmate recognition.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Fruit odour coding in the brain of the agricultural pest *Drosophila suzukii*: Towards olfactory-guided pest management

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Many species of insects severely damage our food production. The best protection so far is heavy pesticide application which has proven to also affect beneficial species leading to the alarming decline of pollinating insects. One example is the fly *Drosophila suzukii*, a close relative of *D. melanogaster* which severely damages the production of berry and stone fruits including strawberries, raspberries, blueberries and cherries. Since its first captures in Trentino in 2008, its population has increased and established in Italy and the rest of Europe.

Flies locate fruit hosts by their attractive odours, detected and encoded by a highly specialized olfactory system before being translated into behaviour. The corresponding information processing pathway is not yet fully understood, especially the step that evaluates the attractiveness of odours. An alternative pest management method would be to use of odours to which *D. suzukii* adults are particularly susceptible. They are thus lured into traps or repelled from fruits, without affecting other species. The damaging effect of *D. suzukii* is the female's preference to lay eggs in ripening fruits on plants, and the larva feeding from the fruits resulting in damages of up to 80% of harvests. They are occupying a diverse ecological niche from *D. melanogaster* thriving on overripe and fermenting fruits on the ground.

With evidence of genetic changes in the repertoire of olfactory genes, the host shift appears to be associated with changes in the detection of ripe fruit odours and fermentation odours in *D. suzukii*. However, it is unclear what distinguishes the brains of *D. suzukii* and *D. melanogaster* to cause the crucial difference in host selection, also because one of the tools that have proved extremely useful in studying the brain of *D. melanogaster* was not available for *D. suzukii*, namely transgenic insect lines that allow brain activity to be mapped.

We are studying the olfactory pathway leading to attraction to ripe fruits in *D. suzukii* using a transgenic fly line expressing the genetically encoded calcium sensor GCaMP7. We use minimally invasive 3D in vivo two-photon microscopy to image neural activities to characterize the neuronal responses to environmental odours and to follow the processing pathway of these stimuli in a comparative study with *D. melanogaster*. We present the first differences between the two species in the olfactory code with 3D mapping of the responses to selected odours on the antennal lobe. We expect the results to improve the general understanding of olfactory coding and its role in the host shift.

KEY WORDS: *Drosophila suzukii*, olfactory system, berry fruits, microscopy, transgenic flies.

ORAL PRESENTATION

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Influence of vibrational cues on *G1 Ganaspis cf. brasiliensis* host searching behaviour

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The larval parasitoid *G1 Ganaspis cf. brasiliensis* (Hymenoptera: Figitidae) has been selected as the best candidate to release for classical biocontrol programs of spotted-wing drosophila (SWD). The parasitoid shows high specificity and prefers to attack 1st and 2nd instar host larvae within freshly infested fruits. While long- and medium-range host location heavily rely on chemical stimuli, the detection of the host in its microhabitat is likely mediated by the vibrations produced by the larvae inside the fruit pulp. We identified and described a variety of vibrational cues emitted by SWD larvae of different age and at different infestation densities, and create an artificial signal that could be reproduced into uninfested fruit. We performed bioassays to investigate whether *G. brasiliensis* uses mechanical cues to discriminate between suitable and unsuitable host patches. The wasp reactions were analyzed considering the time spent foraging on the fruit and the number of ovipositor insertions. We discuss our findings in relation to the parasitoid's behavioural ecology.

KEY WORDS: *Drosophila suzukii*, *Ganaspis brasiliensis*, Classical Biological Control, Biotremology, Insect Behaviour.



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Short and long-term effects of a heat shock on the longevity and fecundity of the pea aphid, *Acyrtosiphon pisum* Harris, and on the progeny body size

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Rapid temperature increments may affect the life history traits of insects as well as their populations dynamics. The effects of heat stress on the fecundity and longevity of the pea aphid, *Acyrtosiphon pisum* Harris, were studied by exposing groups of aphids to a temperature of 39.0°C for 30 minutes in a thermostatic bath. Mortality and fecundity were recorded 24 hours after heat treatment and then daily in the following weeks. The survival rate at 24 h was 20% with higher residual mortality also on subsequent days. Significant differences were found between treatments ($\chi^2(1) = 8.5$, $P = 0.0036$) using Cox's proportional hazards model. Aphids that survived heat stress showed reduced fecundity compared with those in the control group. The ANOVA performed on fecundity values showed significant differences among days ($F_{21,947} = 25.9$, $P < 0.001$), between treatments ($F_{1,947} = 21.6$, $P < 0.001$) and a significant "day by treatment" interaction ($F_{21,947} = 4.5$, $P < 0.001$). During the first nine days after the heat exposure, significant differences in daily fecundity between heat shocked and control aphids were found (Tukey's HSD post-hoc test, $P < 0.001$). Thereafter, in the following days, the fecundity of the treated aphids was similar to that of the control, not so much due to recovery in the stressed aphids but rather due to reduced fecundity in the control group as a consequence of aphid senescence. This result matched by the reduction of the average number of mature embryos in the stressed aphids, assessed by dissecting a portion of the aphids at different days after heat exposure (days 1, 4, 7, and 11). In addition, in heat-stressed aphids, embryo size was smaller. This reduction in body size was maintained during post-embryonic development. Reduced longevity and fecundity, and smaller offspring size may be the consequence, at least in part, of the significant decrease in the population of the bacterium *Buchnera aphidicola* measured in aphids exposed to heat stress.

KEY WORDS: heat stress, aphids.



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

The three-dimensional structure of the forest influences the spatial distribution of moths

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The spatial distribution of species within the ecosystem is primarily driven by the presence of trophic resources. In a given forest type, insects trophically related to the dominant tree species are expected to be evenly distributed due to the abundant presence of their food plant. However, the distribution of species and individuals within a forest ecosystem is driven not only by the presence of trophic resources, but also by more complex relationships with biotic and abiotic parameters such as microclimate, predatory pressure and morphometric traits. We investigated how the three-dimensional structure of space below the canopy may affect the composition of nocturnal lepidoptera communities on a micro-spatial scale. In order to synthesise the complexity of dispersal behaviour of this group of insects, we evaluated easily measurable traits such as wingspan and the presence of tympanic organs, both connected to their mobility and thus potentially influenced by the structure of the available flight space. The study area is entirely included in Sila National Park (Catanzaro, Calabria), where 12 sampling sites were selected in pine forests (*Pinus nigra* subsp. *laricio*) and 12 in beech forests (*Fagus sylvatica*). Forest spatial structure was investigated using a portable laser scanner GeoSLAM ZEB-REVO. Nocturnal lepidoptera monitoring was carried out using UV LED light traps placed in the middle of each of the 24 sampling sites, at a height of 1,30 metres from the ground. Traps were activated one night per month from May to October 2019 in pine forests and from May to October 2020 in beech forests during nights with favourable conditions for moths sampling. We observed that the portion of space above three metres from the ground is the only one that influences community composition. Particularly, larger species with tympanic organs prefer environments with less space below tree canopies. Our findings could be the results of a defence strategy against their main nocturnal predators. In fact, the tympanate and larger species not only actively avoid chiropter predation, but also seem to actively choose denser forests where bat predation is known to be lower. The micro-spatial distribution of species and their abundance thus appear to be significantly influenced by the spatial structure of forests.

KEY WORDS: Lepidoptera, Community, Sila.

POSTER

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Attracting bees: a preference expressed by *Anthidium* spp. to *Salvia yangii*

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Anthidium is a genus of bees in the family Megachilidae, cosmopolitan in its distribution, with mostly solitary species that fly all summer. Females forage on numerous plant species and males, peculiar since they are larger than females, patrol and defend flower patches looking for a mate. During the project API-GIS, we monitored wild pollinators in urban parks in the city of Milan and this genus resulted especially frequent. Its frequency resulted associated with an ornamental species, *Salvia yangii*, previously known as *Perovskia atriplicifolia* and commonly called Russian sage. This plant shows an extended flowering season (June - October), with blue to violet blossoms arranged into branched and showy panicles. It grows over a wide range of climate and soil conditions and it is recently used in gardens and landscaping. We, therefore, decided to investigate with more detail this relationship by a) carrying out comparative monitoring in urban and rural areas, through patch records on the ornamental; b) describing the characteristics of the plant, as for its morphology, histochemical analyses and trichomes description.

We experimentally placed *S. yangii* plants in a rural environment, to check for the attractiveness of this species towards *Anthidium* in conditions different from that of the city, and recorded the frequency of visits. We carried out 61 patch records of 5-minuts of interval, 45 in urban parks and 16 at one site in the rural area. Simultaneously with the observations, we recorded data on the number of flowers and flower stems (visual display). In the same season, we obtained morphometric data from 60 flowers, selected among 3 plants and collected twice along the flowering season. Plants came from the same genetical pool, potted with soil of the same type, quantity, and relative disposition as the one placed in the rural area, to minimize variability (genetics of individuals, attraction linked to the abundance and relative distance of plants). We also proceeded with histochemical analyses on 30 flowers, selected in the same phenological phase, and described trichomes from leaves, calyx and floral parts.

Our results confirm a strong attractiveness of *S. yangii* on *Anthidium* species, expressed by the frequency of these bees compared to other species both in urban as well as in rural areas. A different bee community visited *S. yangii* in the two study areas, as expected. *Anthidium* were the most abundant visitors, even more frequent than the honeybee in urban contexts. In the rural area, *Anthidium*, visited the plants even if in that area it was not previously recorded. Both males and females were attracted by the flowering species; in the urban parks, males also adopted the plant as resting stems during the night. The flower characteristics reflected that of the genus *Salvia*, confirming the convenience of its employment to combine enhancement of resources for pollinators and visual amusement by people.

Even if not native to our country, *S. yangii* is a non-invasive species whose use could be boosted and recommended to attract and sustain wild pollinators, in urban and rural gardens.

KEY WORDS: Megachilidae, russian sage, foraging, *Apis mellifera*.

POSTER

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SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Insight into the male behavior of solitary and quasi-social Bethyloid wasps

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Nowadays, many Hymenoptera parasitoid species are used or under study as biological control agents. Among them there is the Bethyloidea family which includes several species with various degrees of sociality. For instance, *Sclerodermus brevicornis* is a quasi-social ectoparasitoid as females can cooperate to paralyze big *Psacotheta hilaris hilaris* larvae, oviposit on them and take care of the offspring. This species is an important pest of *Ficus carica*, a major worldwide cultivated tree and especially throughout the Mediterranean basin (Turkey, Italy and Spain). On the contrary, *Goniozus legneri* females behave as a solitary species seeing that a single female lays eggs on pest larvae, excluding other conspecific females. In this case, host larvae usually belong to the Lepidoptera order, such as the species *Corcyra cephalonica*, a foodstuffs pest. *Sclerodermus brevicornis* and *G. legneri* males are usually winged and have a quite short lifetime, compared to females. They usually emerge before females but detailed information on the mating behaviour is missing. This study therefore aims to compare biological and behavioural traits of *S. brevicornis* males to those of *G. legneri*, through choice and no-choice tests, more specifically on survival length and mating behaviour.

KEY WORDS: *Sclerodermus brevicornis*, *Goniozus legneri*, parasitoid, survival length, mating.



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Innovation in Biotremology applied to pest control: vibrotraps and vibroplates

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In recent years, biotremology has been rapidly developing, especially in the field of agricultural entomology. This discipline studies the vibrational communication of animals and the use of vibrational signals to manipulate the behavior of harmful species. These signals belong to the category of semiophysicals that, like semiochemicals, mediate the intra- and interspecific communication of many insect species. It follows that the emission into the environment of vibrational signals, specifically developed to interact with target species, can interfere with their natural behavior. This principle inspired the development of new techniques for plant protection from which, recently, two new tools for crop protection have been tested. The first one is a bimodal trap (vibrations + pheromones) to capture the brown marmorated stinkbug, *Halyomorpha halys*. This is the first example of a crop protection device based on the combination of vibrational signals with pheromones. In fact, the stinkbug aggregation pheromone was associated with a highly specific vibration, a sexual signal, which was selected in the laboratory through multiple choice tests conducted on individuals in special arenas. This signal has led to a significant increase in captures in field tests, especially when the addition of a battery to the device solar panel allowed the vibration to be maintained overnight. The second tool is the "vibro-plate" for the control of greenhouse whiteflies. Vibro-plates were developed to support a platform on which potted greenhouse crops can be placed and to which is transmitted a disturbance vibration specifically designed to cover the vibrational communication of the greenhouse whitefly, *Trialeurodes vaporariorum*. Tests conducted in greenhouses on tomatoes, showed that the synergistic use of plant derived products (orange oil and extracts of *Clitoria ternatea*) and disturbing vibrations is effective in containing the whitefly infestation. Biotremology techniques therefore seem to open new scenarios, hitherto little explored, in the field of sustainable crop protection. Thanks also to the rapid technical and technological progress, these instruments are becoming of great interest to companies in the crop protection sector because they are perfectly in line with the principles of eco-compatible (the vibrations leave no residues nor generate electromagnetic radiation) and the digital agriculture (the devices are associated with electricity supply systems and therefore capable of hosting electronic platforms and related sensors). In conclusion, we expect further development of biotremology techniques in the near future with a significant extension of such methods to other target crops and insects.

KEY WORDS: biotremology, semiophysicals, sustainable crop protection, new technologies.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Personality and behavioural syndromes in dung beetles: evidence from multiple behaviours in *Copris umbilicatus* Abeille de Perrin, 1901 (Coleoptera, Coprini)

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Although personality studies have primarily focused on vertebrates, the evidence showing invertebrates to be capable of displaying personalities (expressed as the repetition of certain behaviours over time) and behavioural syndromes (a set of correlated behaviours) is steadily growing. Dung beetles have never been considered in this framework, despite having been extensively studied in many fields of ethology, ecology and evolution. In this study, to investigate the possibility of personality and behavioural syndromes in *Copris umbilicatus* – an optimal species model thanks to its subsocial complex behaviour – we analysed three multiple behaviours (activity, thanatosis and distress call emission) which may be differentially expressed as distinct behavioural traits.

We found moderate to excellent levels of repeatability in all behavioural traits considered, indicating individuals to have distinct personalities. Repeatability estimates were noticeably higher in the behavioural traits related to sound emission, modest in traits related to locomotory activity and lower in the trait related to thanatosis. Furthermore, we showed that the duration of thanatosis was negatively correlated with two activity traits, namely ‘distance moved’ and ‘locomotory speed’. This suggests the existence of a behavioural syndrome involving thanatosis and activity, with bolder individuals exhibiting shorter thanatosis and higher locomotor activity, in contrast with fearful individuals which display longer thanatosis and poor locomotor activity. No relationships were found between the behavioural traits and body size or sex. Principal component analysis (PCA), performed on each separate behaviour as well as on all behaviours together, showed a well scattered distribution of individuals across the PCA bi-dimensional space, suggesting that each displays a behavioural mix that distinguishes it from the others. This noticeable difference between individuals, which was not due to differences in sex or body size, could be attributable to differences in personality among individuals.

The study of personality in invertebrates could contribute towards a better understanding of the insect dynamics present in other fields, such as population and community ecology. Personality affects individual fitness, which in turn influences the population growth rate, population stability and resource-seeking dynamics. By consequence, personality may also have different implications in terms of invasion biology, conservation and biological control. Dung beetles provide an impressive variety of ecosystem services. Through the manipulation of livestock faeces for their feeding and nesting processes, dung beetles contribute, first and foremost, to dung removal, but also to bioturbation, nutrient cycling, mineralization processes, plant nutrient uptake and plant growth enhancement, all of which may benefit agricultural and pastoral ecosystems. Since the provision of these services may depend on the personalities represented in local populations and communities, future research might reveal bolder or more active individuals to be those able to remove more dung.

KEY WORDS: distress call, locomotor activity, thanatosis, repeatability, behavioural traits, locomotory arena, sex, body size.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Study on the effects of oral exposure to brake system wear particulate matter (PM) on springtail *Orthonychiurus folsomi* and *Folsomia candida*

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Soil is one of the most species-rich habitats among terrestrial ecosystems. Collembola are soil-dwelling animals that play a key role in plant litter decomposition processes by forming soil microstructure, regulating soil fertility and energy flow through above- and below-ground food webs, and contributing to soil microbial community dispersion and biodiversity maintenance. Soil ecosystems are experiencing increasing pressure due to anthropocentric activities. Road traffic is an important contributor of pollution in soils due to both exhaust (fossil fuel combustion) and non-exhaust (abrasion of vehicle components, e.g., braking system, tires, and friction with road surfaces) emissions. Here, we investigated the effects of oral long-term exposure to metal-based particulate matter (PM) from brake system (brake pads and brake discs) on two collembola species: *Orthonychiurus folsomi* (Onychiuridae) and *Folsomia candida* (Isotomidae). The two species were treated with a high concentration (HC) and a low concentration (LC) dose of brake wear debris. Our results show that chronic exposure to this pollutant PM can have sub-lethal effects (i.e., reduced number of surviving adults and the number of eggs) both at low and high concentration for both species. Scanning electron microscope coupled with X-ray spectroscopy (SEM-EDX) analyses, revealed brake wear PM markers in the gut and faeces of collembola, and histological analyses detected alterations in the digestive and reproductive systems, and in the abdominal fat body at high concentration.

KEY WORDS: Collembola, non-exhaust emissions, ecotoxicology, histology.



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Giannutri, an open air laboratory to evaluate the competition between *Apis mellifera* and wild bees

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Despite the numerous benefits provided by beekeeping, the massive presence of the honey bees (*Apis mellifera*) has the potential to competitively exclude the local pollinator from access to resources. In functionally low-diversified Apoidea communities and environments with reduced diversity of plant species, such as small Mediterranean islands, these effects get exacerbated. Giannutri Island represents a perfect open-air laboratory to investigate and quantify these effects. Indeed, it is a small island (2.6 km²) and it is possible to experimentally exclude the honey bees by closing the hives.

This study aims to perform a behavioural monitoring of wild pollinators to quantify the effect of honey bees on them and to identify the maximum sustainable load of *A. mellifera* for the island. Giannutri island hosts a simplified and fragile ecosystem in which the wild Apoidea community is dominated by a few taxa, which are also the target species of this study, *Anthophora dispar* and *Bombus terrestris*. The main trophic resources for pollinators, and the most abundant flowering species, are *Rosmarinus officinalis* and *Teucrium fruticans*. Since the entire island is subjected to the same competitive pressure, we used an experimental design that involved the closure of the hives on certain days to temporarily exclude the honey bees from the island. We thus obtained the experimental condition of presence (hives regularly open) and absence (hives experimentally closed) of honey bees. The potential impact was measured thanks to a multidisciplinary approach. For these purposes, we measured the availability and consumption of nectar from pollinators. Moreover, we analysed the spatial distribution and the foraging behaviour of wild Apoidea in both experimental conditions.

After the first year of experiments, significant results emerged regarding the existence of competitive interactions on the island. First of all, there was a strong overlap between the trophic niche of the target species and *A. mellifera*, paired with a high consumption of nectar. The foraging behaviour of *A. dispar* appeared to be altered in hives-closed condition showing a significative reduction of foraging time on flowers. The same ethological response was observed on flowers closer to the hives, along with an increase in the frequency of visited flowers. The shorter foraging time, compensated by a higher visitation rate, can result in a lower trophic supply and higher energy consumption. These behavioural alterations have thus the potential to negatively impact the wild Apoidea fitness. The abundance and potential decline of pollinators is monitored through years with standardized transects. Further experimental data will allow us to identify the maximum load of honey bees for Giannutri, providing guidelines for the development of sustainable beekeeping in this context. Although the results of this study are related to the Giannutri context, the experimental approach here described could be replicated in similar environments to promote sustainable beekeeping in other fragile ecosystems.

KEY WORDS: competitive interactions, foraging behaviour, wild Apoidea, mediterranean island.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Maintenance of the female polymorphism in odonata: contribution from the study of a natural population of *Ischnura elegans* in central Italy

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Ischnura elegans (Odonata, Coenagrionidae) is a common damselfly in Europe, widespread in small shallow ponds rich in vegetation. Like many Coenagrionidae species, it is characterized by an evident polymorphism, with androchrome females resembling males and gynochrome females not. Male learning to recognize and preferentially harass the majority morph, thus selecting it negatively, appeared to be an appealing, explanatory hypothesis for polymorphism maintenance. Still, recent investigations under field conditions demonstrated that a male's experience with a given female morph does not affect his subsequent reactions to female morphs. Additionally, the female morphs of *I. elegans* do not differ in rates of harassment by males or in the eggs laid. Despite the extensive research on the reproductive behavior of polymorphic Coenagrionidae, the mechanism of the maintenance of polymorphism remains to be determined. Available data suggest a subtler proximal mechanism than male learning, at least in *I. elegans*. In detail, in our long-term study population, which breeds on ponds of a fish hatchery adjacent to Lago Trasimeno, we performed insect samplings along the pond shores in the morning, samplings of insects in copula in the late morning, and samplings of ovipositing females in the afternoon, every September from 2017 to 2023 (except in 2019 because of restrictions due to the COVID-19 pandemic conditions). The aim was to calculate the ratio between androchrome and gynochrome females in the different samplings over the years, to investigate variations in morph frequencies in the population, and search for evidence of sexual selection mechanisms. We found that gynochrome females dominate our population, but this dominance was exceptionally high in 2017 and 2021, decreased in 2022, and almost disappeared in 2018 and 2020. That oscillation suggests a proximal mechanism to maintain genetic polymorphisms in females by negative frequency-dependent selection. In addition, the percentage of androchrome females in the couples differed from that in the population samplings along the shoreline, thus clearly demonstrating a nonrandom pair formation. The percentage of androchrome among ovipositing females was similar to that in couples. Female morphs never differed in the rate of eggs laid. Morph-specific differences in the appearance and probability of being detected and recognized by males, coupled with flexible morph behavior, could give wild females considerable control over their apparency to males and hence, over the frequency of intersexual encounters. Our preliminary data, in particular, suggest that different mechanisms used by androchrome and gynochrome females to avoid male harassment, respectively, mimesis with males and with the vegetation background, could decrease their effectiveness as the frequency of the corresponding morph increases in the population. A more extended series of data, together with additional behavioral observation on males and females in the field, will allow us to test this hypothesis and help to clarify the main mechanisms of maintenance of female polymorphism in *I. elegans*.

KEY WORDS: Coenagrionidae, reproduction, polymorphism, sexual selection, investigation in field.

POSTER



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

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

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The asparagus moth, *Parahypopta caestrum* (Hübner) (Lepidoptera, Cossidae), is one of the most damaging pest of *Asparagus* spp. in many Mediterranean areas, including Foggia province, the widest producing area of green asparagus in Italy. The soil-borne larvae of this pest bore galleries into the asparagus roots and shoots causing destruction of the plantations after 2-3 years. Control of this pest is difficult due to the cryptic habitats of the larvae and the adult emergence during the harvest time that limit the use of chemical means. The identification of chemical cues involved in intraspecific interactions of *P. caestrum* could greatly contribute to the development of effective monitoring and control tools. To date, no studies have attempted to identify semiochemicals of this species. To contribute to the knowledge on the sex pheromone of this pest chemical, electrophysiological, and field tests were carried out. Virgin females and males were obtained from pupae collected in a highly infested plantation in Foggia (Italy) area 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 electroantennography (GC-MS-EAD) revealed the presence of four EAG-active peaks whose mass spectra matched those of three monounsaturated C14 aliphatic acetate and one monounsaturated C14 aliphatic alcohol, respectively. Isomerism and double bond position in the structure of these EAG-active compounds were elucidated by comparing their GC retention times and MS fragmentation patterns with those of authentic standards. Double-bond position and configuration of sex pheromone candidates were also confirmed by comparative EAG experiments with series of monounsaturated cis (Z) and trans (E) C14 aliphatic acetates and alcohols. To define the relative ratio of different sex pheromone components in female glands, quantitative analyses of different gland extracts were performed. The sensitivity of male antennae to each sex pheromone component was characterized by calculating the corresponding EAG dose-response curves. In field trapping trials, individual compounds were not attractive. Some binary and even more ternary mixtures of acetates were attractive to *P. caestrum* males. The addition of the monounsaturated C14 alcohol to the attractive blends did not improve male trap catches. Oil traps baited with rubber septa dispensers loaded with the identified sex attractant blend successfully detected the presence and flight activity of *P. caestrum* in the main areas of asparagus cultivation in Foggia province and Molise region. Monitoring activity showed that adult *P. caestrum* emerge from the second half of May to the first half of June with some differences among areas. Studies are in progress to develop further pheromone-based control methods (i.e., mass trapping) for a sustainable management of this pest.

KEY WORDS: Lepidoptera, semiochemicals, GC-MS-EAD, EAG, biological control, Integrated Pest Management (IPM).

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Interacting with biomimetic robots to shape behavioural processes in insects

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Robotics is increasingly applied in many contexts, generating a wave of new scientific and technological advancements with high impact on human society and the environment. Animal-robot interaction, a field at the interface between robotics and behavioural ecology, emerges from the deep integration of artificial agents into animal populations and communities. Here, robotic agents and animals behave collectively and share their abilities in a hybrid organism-robot augmented system. In animal-robot mixed societies, as in cyborgs, the technological component is used to control, repair, or enhance some functions of the biological system (e.g., an insect colony) with which it interacts. Conversely, animal sensing and intelligence can inform engineered systems, boosting their performances during real-world challenges. The application fields are many, ranging from biological investigation to bioinspired engineering design. A major aim of animal-robot interaction is biodiversity preservation and improvement of agriculture and environmental sustainability. Herein, we present two case studies related to Diptera species of economic interest. In the first case study, we used biomimetic agents to transfer social information in adult individuals of the green bottle fly *Lucilia sericata* (Diptera: Calliphoridae), an insect species of medical and veterinary importance, also commercialized as a pollinator for several crop species. Observational conditioning produced by bio-robotic demonstrators enabled us to reproduce different flower foraging choices, significantly influencing the following fly selection process. *Lucilia sericata* adults avoided coloured disks previously visited by a bio-robotic predator and preferred those previously visited by a bio-robotic conspecific indicating the complex risk-benefit trade-off that this fly undertakes. In the second case study, we focused on the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). The medfly is a polyphagous fruit pest, mass-reared for SIT purposes or as host of endoparasitoid species exploited for biocontrol purposes. Rearing conditions can affect insect performances in terms of competitiveness, resulting in individuals with less effective foraging, dispersal, and mating behaviours. Since the temporary territorial behaviour of medfly males is closely related to their sexual performance, we proposed an innovative bionic approach for influencing the behaviour of mass-reared *C. capitata*. We investigated if agonistic interactions with biomimetic conspecific agents lead to more competitive experienced individuals during subsequent mating interactions with real females. Our findings outlined a notable effect of aggressive interactions, occurring during territorial behaviour against the robotic fly, on the subsequent courtship and mating sequences. These improved reproductive performances could be neuromodulated by octopamine, which is surged in the haemolymph by specific octopaminergic neurones during physical exertion and fight. So, specific neuromodulators with a proven involvement in insect motivation and learning abilities may have been activated by the biohybrid aggressive interaction established in this study. These results provide a unique perspective on the behavioural ecology of social learning in solitary insects; they also highlight the high potential of the animal-robot interaction approach for unveiling the full spectrum of invertebrate social dynamics. Overall, these results pointed out that robotics can have a key role in future environmental and agricultural management methods aimed at increasing sustainability.

KEY WORDS: animal-robot interaction; bionics; ethorobotics; Diptera; social learning; reproductive behavior; agonistic behavior; Calliphoridae; Tephritidae.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Cuticular hydrocarbon profiles in velvet ants (Hymenoptera: Mutillidae) are highly complex and do not chemically mimic their hosts

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Although chemical recognition using cuticular chemistry is important for host-parasite interactions within aculeate Hymenoptera, cuticular hydrocarbon (CHC) profiles of only a few host-parasite pairs were characterized and compared in this order. One largely neglected family in this context is the Mutillidae (velvet ants), whose species are ectoparasitoids of bees and wasps. In our study, we characterized and compared the CHC profiles of 5 species of Mutillidae and 7 host species. The CHC profile of velvet ants differed among species and included large proportions of n-alkanes and methyl-branched alkanes. Alkenes were much less abundant in the CHC profiles of three species of velvet ants compared with their hosts, while the other two species possessed a much lower abundance of methyl-branched alkanes, than their hosts. Both the number of peaks and compound diversity were generally higher in velvet ants compared with their hosts. Thus, CHC profiles of parasitoids did not show signs of mimicry when compared with their hosts. In dyadic encounters, performed only in one species of velvet ant and its host, the ectoparasitoid mainly avoided to interact, while aggression by the host was rare. Our results suggest that velvet ants did not evolve chemical mimicry, perhaps in accordance with their wide host spectrum which would limit chemical specialization. However, the reduction of alkenes in social bee-attacking species and the reduction of methyl-branched alkanes in social wasp-attacking species may favour host nest invasion, since these two CHC classes are known to be important in nestmate recognition for social bees and wasps respectively. A larger, phylogeny-corrected comparison of Mutillidae and hosts may help clarifying the evolution the CHC profile of these parasitoids.

KEY WORDS: Cuticular hydrocarbon profile, Mutillidae, bee hosts, wasp hosts, chemical mimicry.

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Insect-plant interaction in the oligophagous ladybird *Chnootriba elaterii*.

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Host-plant selection, crucial to the success of insect species, is a complex phenomenon involving different insect senses and plant cues. Using as model insect the oligophagous melon ladybird *Chnootriba elaterii* (Coleoptera, Coccinellidae), feeding on different species of Cucurbitaceae, and eight plant species represented by *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita pepo*, *Cucurbita moschata*, *Lagenaria siceraria*, *Ecballium elaterium* (all host plants) and *Luffa cylindrica* (non-host plant), the present research aims to shed light on 1) the different cues involved in host location process, at long and at short distances; 2) the adult preference and larval performance on the different plant species; 3) the mechanical interaction of the insect when walking on the plant surface. For these purposes, we used different techniques represented by electroantennographic recordings (EAG) and scanning electron microscopy (SEM) to characterize antennal olfactory sensilla and their sensitivity to the VOCs produced by the seven cucurbits, open and closed Y olfactometer to evaluate the involvement of chemical and visual cues in the host plant selection, two-choice feeding tests to evaluate adult preference among cucurbits, and larval performance bioassays to evaluate the development time, mortality, and morphometric adult parameters grown on different plants. To investigate insect attachment ability, we performed traction force and centrifuge experiments measuring larval and adult friction force exerted on the different plant species and SEM observations to characterize insect attachment devices and plant surfaces. SEM investigations described for the first time the antennal olfactory sensilla of the melon ladybug, and EAG recordings demonstrated that insect antennae respond to both standard chemical compounds and plant VOCs from Cucurbitaceae (except *L. cylindrica*, the non-host species). Despite EAG responses, olfaction did not guide females in the Y-tube olfactometer between different host-plant species, nor could allow the ladybirds to distinguish between Cucurbitaceae and non-Cucurbitaceae. When *C. elaterii* were tested in an open arena olfactometer (where they could see the plant), they were able to move toward the cucurbit hosts only when olfactory were presented together with visual cues proving that olfaction is not the most important sense involved in the host location. Two-choice feeding tests using cucurbits showed that females preferred some of the host plants (watermelon, and melon) probably because gustatory stimuli are involved in addition to olfactory and visual cues. Larval experiments were conducted to determine the correspondence between adult preferences and offspring performance; indeed, larvae develop faster on watermelon than on other cucurbits. No difference in larval mortality was recorded except on *L. cylindrica* where larvae died and were never chosen by females. Among host species, no differences were encountered in the morphometric adult's parameters (length of elytra and width of pronotum). *L. siceraria*, whose leaves were firmly refused by adults, was eaten by larvae, and this divergence may be due to plant mechanical barriers such as trichomes which in this species are dense, short, and flexible and reduce attachment ability in all developmental stages. These results clarify the host-plant selection process of an oligophagous insect and can be potentially useful to improve biocontrol strategies.

KEY WORDS: Melon ladybird, host-plant selection, olfaction, mechanical defense, Cucurbitaceae.

ORAL PRESENTATION

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Insect attachment ability: basic research and implications in host selection

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The ability to firmly adhere to the surface along the whole life cycle is of pivotal importance for insect survival and reproductive success. For this reason, insects developed very efficient attachment devices located on their tarsi represented by claws and smooth or hairy pads, supplemented with fluids, which make them able to cope with different slopes and with substrates of varying roughness and wettability. In this context, the relationship between phytophagous insects and plants, or between parasitoids and egg surface, represent interesting examples of antagonistic coevolution between the insect need to firmly adhere to the host surface for feeding/oviposition behaviour and the host necessity to reduce such adhesion with specialised antiadhesive surfaces. Here we report the data obtained measuring the attachment ability of different pest insect species belonging to different orders represented by *Nezara viridula* (Hemiptera, Pentatomidae), *Bactocera oleae*, *Ceratitis capitata* (both Diptera, Tephritidae), *Chnootriba elaterii* (Coleoptera, Coccinellidae) and of some ooparasitoids such as *Anastatus bifasciatus* (Hymenoptera, Eupelmidae) to artificial and natural surfaces in order to shed light on the adaptations developed in the leg attachment devices of these insects to adhere to the plant leaves/fruits to the egg surface in relation to the different surface features and properties (roughness, wettability, presence of trichomes, waxes, etc.). Detailed knowledge regarding insect pest attachment ability can be potentially useful to develop environmental friendly pest control strategies. In this regard, some examples concerning the use of nanoparticle films in reducing insect attachment ability and therefore their reproductive success are shown. Nanoparticles can represent a good alternative to the use of insecticides for the control of pentatomid bugs and other pest insects.

KEY WORDS: Smooth pads, hairy pads, pulvilli, claws, friction, biomechanics.

SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Masters of deception: multimodal mimicry in clearwing moths [Lepidoptera: Sesiidae]

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The evolutionary arms race between predator and prey has driven the formation of incredible survival strategies. Many hymenopterans, for example, have developed active defence mechanisms which they demonstrate to predators through aposematic warning displays, such as yellow and black bands on the abdomen of numerous wasps and bees. Predators learn to recognise these warning signals in order to avoid unpleasant encounters. In a phenomenon known as *Batesian mimicry*, harmless species (i.e. those without active defence mechanisms) have evolved imitations of models who can sting, bite or otherwise defend themselves. Bates noticed visual aspects of the phenomenon, however further studies revealed the existence of complex, multimodal mimicry, in which morphological resemblances go in pair with imitations of behaviour, sounds and chemical signals.

Few other invertebrates have evolved more remarkable resemblances to their evolutionary models than those of the lepidopteran family Sesiidae (clearwing moths). Sesiids are diurnal and rarely crepuscular moths whose most striking feature are narrow wings, either entirely transparent or with hyaline patches. Apart from their morphological similarity to wasps and bees, it has been demonstrated that some clearwing moths mimic their models' behaviour, yet due to their rarity, difficulty in locating them in the field, as well as seasonal occurrence, these aspects of mimicry in sesiids are poorly studied. An entirely unexplored type of deception in sesiids is acoustic mimicry. While performing fieldwork in Malaysian rainforests, we heard a clearwing moth buzzing in flight – a sound typically produced by bees and wasps. We recorded the buzzing sounds of two species of Southeast Asian sesiids, *Malayomelittia pahangensis* and *Heterosphecia hyaloptera* and compared them to their visual model bee, *Tetragonilla collina*, and two control species of bees occurring in the same habitat. Recordings were performed on untethered, flying insects in nature. Based on eight acoustic parameters, as well as wingbeat frequencies calculated from slow-motion videos, we found that the buzzes produced by both clearwing moths highly resemble those of *T. collina* but differ from the two control species of bees. Acoustic similarities to hymenopterans they morphologically resemble, alongside behavioural imitations, indicate that clearwing moths display multimodal mimicry of their evolutionary models.

KEY WORDS: mimicry, bioacoustics, clearwing moths, Sesiidae.

ORAL PRESENTATION



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Performance of *Macrosiphum euphorbiae* on tomato plants irrigated by wastewater and colonised by *Trichoderma harzianum*

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A major challenge for smallholder farmers is to improve crop productivity while ensuring food security and quality. Among the numerous factors that make agriculture increasingly difficult, pest management and water scarcity are two of the most important. A solution may be to use biostimulants such as *Trichoderma* spp. fungi and reuse wastewater for irrigation. However, direct wastewater reuse may pose a risk of introducing organic micro-pollutants, excessive content of nutrients, and/or increase soil salinity and consequently, a tertiary low-cost wastewater treatment is needed. In addition, the activity of *Trichoderma* can be affected by environmental conditions (i.e., soils of different properties-structure, pH, and organic matter, availability of water and nutrients, climatic conditions), and wastewater irrigation could influence plant growth, and the insect community associated with crop species.

The aim of the present study is to investigate the effects of *Trichoderma harzianum* T-22 and wastewater irrigation on pest insect performance on tomato plants. A model system consisting of tomato plants (*Solanum lycopersicum* L. cvs. Bobcat), a sap-sucking insect (*Macrosiphum euphorbiae* - Hemiptera: Aphididae) and synthetic wastewater was selected for preliminary experiments. The first step was to investigate the fungus colonization and the possible effect on the tomato plant using a simulated wastewater effluent. The objectives are also to investigate the aphid behavior as follow: i) settlement preference under choice tests; ii) colony growth rate; iii) individual biological parameters.

The results of the present research address the effects of wastewater irrigation on *Trichoderma* activity in terms of aphid behaviour. They also introduce the possibilities of the interaction *Trichoderma*-wastewater irrigation for smallholder farmers. This research is part of the project S.A.F.E. (Sustainable water reuse practices improving safety in agriculture, food and environment) that is founded through the PRIMA programme, supported by Horizon 2020, the European Union's Framework Programme for Research and Innovation.

KEY WORDS: wastewater irrigation, adaptation, *Trichoderma harzianum*, *Macrosiphum euphorbiae*, *Solanum lycopersicum*.



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Phenotypic plasticity and maternal effects in aphids reared in a food-restricted environment

Vincenzo Trotta, Andrea Alfredo Buongusto, Pierluigi Forlano, Vittoria Caccavo, Donatella Battaglia

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The changing environmental conditions faced by insects can impact the phenotype and, in general, the fitness of individuals over multiple generations. Small changes in the environment can greatly affect species distributions, community composition, ecosystem function and the life-history traits of organisms. Phenotypic plasticity (the ability of a given genotype to vary its phenotype in response to the environment) is thought to be particularly important in overcoming environmental variations. Transgenerational plasticity occurs when the environment where the parents developed influences the phenotype of the offspring, that is, parents are able to change the phenotype of their offspring in response to local environmental conditions. Such responses can be adaptive when they generate a fitness advantage in the offspring.

In this study, the plastic responses of body size, productivity, and survival of the pea aphid *Acyrtosiphon pisum* reared in a resource-poor environment such as a cut leaf were investigated. In particular, effects related to transgenerational phenotypic plasticity were estimated, as well as the relationship between transgenerational plasticity and the cause that induced it. Maternal and phenotypic plasticity effects were observed on productivity, survival, body size, and, indirectly, on the developmental time. Specifically, aphids perform better when grown on plant than on cut leaf (phenotypic plasticity) and perform worse in both environments if they come from mothers grown on leaf (maternal effects). However, no transgenerational plasticity effects were observed for the traits considered: offspring do not appear to be advantaged in the environment that their mothers have already experienced. The results of this study support the hypothesis that adaptive transgenerational plasticity effects are masked in a particularly resource-deprived extreme environment, where developmental problems prevail instead.

KEY WORDS: *Acyrtosiphon pisum*, transgenerational phenotypic plasticity, productivity, survival, body size.



SESSION II

PHYSIOLOGY, ETHOLOGY AND INTERACTIONS

Unveiling electromagnetic fields' effects on ant brains: changes in biogenic amines levels

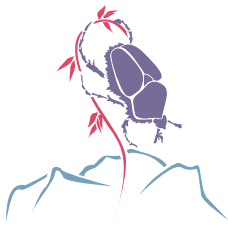
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Electromagnetic fields (EMFs) are ubiquitous in human-modified environments and have been shown to have a significant impact on living organisms. Recent research has highlighted that EMF radiation can affect physiology and behavior of arthropods. EMFs can act as stressors, triggering a complex array of neuronal, molecular, and neurochemical responses to change the physiology of the central nervous system, which, in turn, modulate animal behavior. Ants have been found very sensitive to EMFs. These insects play a critical role in maintaining ecosystem stability by regulating a variety of ecological processes. Their importance is reflected in their abundance, biomass, and diversity, which make them essential consumers and ecosystem engineers. Ants are highly effective at detecting hazardous electromagnetic radiation waves, as even low-strength EMFs can cause them to exhibit altered behavior. The present study aimed to investigate whether low frequency EMFs induce changes in the physiology of the ant nervous system by examining their impact on biogenic amines levels (BAs), and how it translates in changes in their aggressive behavior. BAs function as neurotransmitters, neuromodulators and neurohormones in the nervous system of vertebrates and invertebrates. They play a crucial role in the diversification and modulation of various behaviors, including aggression, communication, and cooperation. We exposed *Formica japonica* Motschoulsky (Hymenoptera: Formicidae) workers to low frequency EMFs for 5 min, 10 min, and 1 hour. Electromagnetic fields were generated with a custom-made Helmholtz coil that produced homogenous 60Hz sinusoidal AC electromagnetic fields with the strength of 100 μ T. The levels of BAs in the ant brain were measured by using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). We examined changes in dopamine (DA), serotonin (5-HT), octopamine (OA) and its precursor tyramine (TA) levels, as well as some of their N-acetylated metabolites, i.e., NacDA, Nac5HT and NacTA. Results showed that even a 10-minute exposure to EMFs led to changes in OA and Nac5HT levels in the brain, with ants exposed to EMFs having higher levels of these two amines compared to the control. These findings suggest that low frequency EMFs may impact the levels of BAs in the central nervous systems of the ants, potentially altering their behavior and physiological processes. Understanding how EMFs can affect BAs levels in ants' brains is important for gaining insight into the potential effects of EMFs on insects and ecosystems, as well as for understanding the fundamental mechanisms underlying the behavior and physiology of these important model insects.

KEY WORDS: *Formica japonica*; EMF; octopamine; neurophysiology; social insects.

ORAL PRESENTATION



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Session III

SESSION III

ECOLOGY AND CONSERVATION

**Arthropods used as bioindicators of microplastics in the terrestrial environment:
the case of carabid beetles (Coleoptera, Carabidae)**

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Microplastics (MPs) are defined as plastic particles with a range size varying from 1µm to 5mm and with regular or irregular shapes. The widespread of MPs in various ecosystems including oceans, soils, and freshwater, makes organisms exposed to these emerging contaminants. MPs' bioavailability and toxicity impact the environment negatively. Therefore, it is essential to monitor the presence of MPs in different environments using a sustainable method as the employment of insects as biological control agents (bioindicators). Bioindicators refer to living organisms with specific characteristics that make them suitable to monitor the contaminants in different environments. In this research, carabidae beetles (Coleoptera: Carabidae), a predominantly terrestrial insect group, have been used for the first time as bioindicators: their biological and morphological characteristics and their widespread make them ideal for studying the impact of MPs on terrestrial environment. Samplings have been carried out by placing pitfall traps along the coastal of the Conero area, Ancona, Italy. The traps have been checked every two weeks from July to October 2022, and insects were sampled on seasonal bases. The caught specimens have been identified at the family level, counted, and dissected in order to obtain digestive tracts for the subsequent MPs extraction and characterization. The results indicated that carabidae beetles ingest MPs with a frequency ranging between 40 to 90% depending on the site and seasonal periods, mainly shaped on fragments, and sized between 0.1-1mm, while polyester and silicon are the main represented polymers. Considering the few information present in the literature and the need to identify good biological control agents, the results of this study could be useful for the future and applied in other environmental contexts. Besides Carabidae, identification and analysis of other arthropods present in traps will be considered a plus to have further information about the MPs presence.

KEY WORDS: carabid beetles, bioindicators, microplastics.

SESSION III

ECOLOGY AND CONSERVATION

Monitoring of species of Community interest in the Special Areas of Conservation (SAC) IT 9220135 "Gravine di Matera": two cases of population estimation

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Monitoring species of Community interest included in Annexes II, IV and V of the Habitats Directive is an obligation for all member states of the European Union, due to the provisions of art. 11 of Directive 92/43 "Habitats". In fact, the article 11 says: "Member States shall undertake surveillance of the conservation status of the natural habitats and species referred to in Article 2 with particular regard to priority natural habitat types and priority species". The surveillance of species is aimed to set the conservation status at site and member State level, as well as allowing to estimate the conservation status at EU level. The estimation of the species population is aimed to defining their state of conservation in the studied site as required by the Standard Data Form (SDF) which is to be filled in, for each site of the European Natura 2000 Network. In this study we want to present two cases of population estimation of two species included in the annexes of the Directive: *Cerambyx cerdo* L. (Coleoptera, Cerambycidae) and the amphibian *Bombina pachypus* (Bonaparte) (Anura, Bombinatoridae), also to promote the research and scientific activities necessary to achieve the obligation set out in Article 11 of the Habitat Directive (Art. 18). All data comes from two projects implemented by the authors on behalf of the Murgia Materana Regional Park in Matera (Basilicata, Italy). *Bombina pachypus* has been monitored within the "LIFE ARUPA" project, while *Cerambyx cerdo* has been monitored within the project "Centre for studies and conservation of biodiversity" funded by PO-FESR Basilicata 2014-2020. In the second year of monitoring of *Cerambyx cerdo* (2021), we innovated the monitoring process, designing and developing a dedicated software system for monitoring the species. All the field data necessary for the population estimate and for the tracking and documentation of the survey activities about *Cerambyx cerdo* population, were collected through application software for android smartphones, called "Great cAPPricorn" used for the first time in the present study. The software allows to mark georeferenced data, accompanied by all textual and multimedia information needed, such as data about traps, surveys, photos or used baits. The software is also capable of processing population estimates using the Schnabel method. The android software guides the user to apply the methodology of the "Manual for monitoring species and habitats of Community interest (Directive 92/43/EEC) in Italy: animal species, published by ISPRA, Manuals and Guidelines Series 141/2016 (Stoch & Genovesi, 2016). The survey of data directly from the field, considerably facilitate the post-survey activities such as archiving and processing the data collected. In the specific case, the monitoring system archives all the data entered in dedicated databases and processes all the data entered during the surveys, calculating the population estimate of the species. The software also edits a detailed report of all the survey activities, in addition to the data processing performed.

KEY WORDS: Natura 2000 Network, Habitat Directive, Monitoring of species, state of conservation, *Cerambyx cerdo*, *Bombina pachypus*.

ORAL PRESENTATION

SESSION III

ECOLOGY AND CONSERVATION

Study on the diversity of wild bees (Apoidea: Anthophila) and butterflies (Lepidoptera: Rhopalocera) in the Culuccia peninsula, Sardinia

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In the last few years, several studies have been highlighting the global decline of insects, in particular pollinators. This is a raising concern for the long-term sustainability of wild vegetation and major agricultural crops. The Mediterranean Basin is considered one of the main biodiversity hotspot. Indeed, Sardinia hosts numerous endemic species of fauna and flora. The aim of this study is to characterize the diversity of Apoidea (Anthophila) and Lepidoptera (Rhopalocera) in the Culuccia peninsula, located in the north-east of Sardinia. The area of approximately 300 hectares is protected as a "Permanent Oasis of fauna protection and capture", where the Culuccia Island Nature Observatory is located. The territory is characterised by a great diversity of natural environments: Mediterranean scrub, thermophilic plant associations, rocky systems and rocky coastlines, grazed meadows, dune systems and sandy coastlines, garrigue and a permanent pond. Currently, the human activities are limited to a few areas which are dedicated to sustainable agri-food production (Biru Agricultural Society). The sampling was conducted from spring to autumn 2022, in all the different environments. Individuals were collected in seven fixed transects (400 m x 4 m), using a entomological net. Also, for Apoidea, the floral species on which the insect was captured was recorded. In addition, two Malaise traps were installed from May to October 2022, in the environments alternating between scrubland and pastures. The collected material was prepared and stored dry in entomological boxes. At the time of capture, a mesothoracic leg was taken from the Apoidea and stored in 96° alcohol in order to sequence the mitochondrial COI gene (Cytochrome Oxidase I), for a total of 204 individuals. The identification of Lepidoptera was conducted through the use of dichotomous keys. The same approach was used for Apoidea combined with DNA Barcoding. The results of α - and β -diversity of the Lepidoptera and Apoidea community are presented in relation to the different peninsula environment.

KEY WORDS: wild bees, butterflies, Sardinia, DNA Barcoding, key dichotomous.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

**Diversity and abundance of pollinating butterflies in the Sila National Park
(Lepidoptera: Hesperioidea, Papilionoidea)**

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The present monitoring work was carried out at several sites within the Sila National Park under the impetus of new directives aimed at the protection and conservation of pollinating insects, among which Lepidoptera are of particular importance. Sampling was carried out from May to September, biweekly consistent with optimal weather conditions. Eighteen sites having different ecological, structural and functional characteristics were selected, 500 meters long and divided into 10 sections each. Specifically, transects were identified in areas under conventional cultivation, areas under organic cultivation, meadows and pastures subject to more or less intensive grazing. By comparing sampled communities, we assessed the relationships of species diversity and abundance with environmental parameters of sites because of the rapid reaction of lepidopterans to environmental changes of natural and anthropogenic origin. Analysis of data was carried out using a species/station matrix with abundance values. In the 18 transects monitored, 71 species and 6,355 individuals belonging to 5 families were counted. We found that the areas with the highest diversity values were those that were not under cultivation showing no major differences between organic and conventional cultivations. In fact, communities were still very similar to each other as shown by the Correspondence Analysis. From the conservation point of view, of great importance was the finding of new populations of species included in the Habitats Directive such as *Zerynthia cassandra*, *Phengaris arion* and *Parnassius mnemosyne*. Due to the start of monitoring in late spring, *Z. cassandra* was detected only in two transects one of which was characterized by meadows and the other subject to light grazing, with the latter in need of stronger protection. *Phengaris arion* was found in two sites, one of which subject to a very intensive grazing that should be managed in order to reduce the threat to which the population is subjected. Among species of conservation concern, *Parnassius mnemosyne* is the most frequently found, confirming that the Sila Massif probably hosts the most conspicuous populations of the Italian peninsula. In addition, new occurrence sites of *Brenthis ino* were found. This species, linked to humid habitats, has been found in Italy outside the Alpine Arc only in this massif, where populations are likely threatened by overgrazing and the expected rainfall reduction.

KEY WORDS: Rhopalocera, conservation, biodiversity.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

QBS-ar index as tool to investigate different Hexapoda communities in apple orchards, strawberry and tomato fields across Europe

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QBS-ar (Soil Biological Quality focused on microarthropods) is considered a consistent method to investigate soil biodiversity, regarding the analysis of the soil mesofauna. The method is based on the use of the Berlese extractor, which provides a selection of those individuals thinner than 2 mm, and their consequent classification using the so-called Ecomorphological Index (EMI) as a measure of their adaptation to soil life. This method does not require a specific taxonomical classification but focuses on those morphological characteristics that permit to classify individuals in biological forms (BFs) well adapted to epigeic, emiedaphic or euedaphic soil conditions. Higher adaptation implies a higher EMI, and the sum of EMIs determines the community QBS-ar value. Thus, insects belonging to mesofauna are considered in this index and cover a relevant role in soil health, contributing to the nutrient cycling performing the assimilation of nutrients in the food web (organic matter decomposition and bacterial grazer), and to the water regulation helping in the maintenance of soil porosity.

In this work, the QBS-ar index was evaluated, as well as the presence and density per square meter of BFs belonging to the Hexapoda subphylum, as defined by the QBS-ar protocol, in 17 agricultural sites located in 5 European Member States within the H2020 Excalibur project. The structure of BFs community was evaluated according to different crops (apple, strawberry and tomato, representing perennial arboreal crops, herbaceous perennials and annual herbaceous crops, respectively).

In comparison with the QBS-ar index, the Hexapoda community was evaluated by alpha diversity indices: richness, Shannon index, and Pielou Evenness index, using Hexapoda BFs as information for the biodiversity level and identifying as outliers those values greater than 1.5 times the interquartile range. QBS-ar, richness, and Shannon index showed a decreasing trend of diversity from perennial crops to annual herbaceous crops. However, only the QBS-ar and Shannon indices showed significant differences depending on crops, suggesting a higher QBS-ar value in apple orchards and a lower Shannon index value in tomatoes. The QBS-ar index evidenced a greater capacity for statistical discrimination and a greater magnitude among the biodiversity indices investigated. The community in the three crops showed a similar number of BFs of different Hexapoda, with a uniform distribution of abundance, suggesting the absence of dominance phenomena. In conclusion, QBS-ar index allowed a greater discrimination among crops with different pluriennial cycle stability respect to focusing only on the Hexapoda community diversity, confirming the importance of considering the whole mesofauna to better highlight the differences between the different crops.

KEY WORDS: QBS-ar index, Hexapoda, soil biodiversity, microarthropods, mesofauna.

ORAL PRESENTATION

SESSION III

ECOLOGY AND CONSERVATION

Some like it cold: Mediterranean butterflies cope with extreme temperature by moving to woody areas

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In Mediterranean environments, climate change is expected to cause frequent periods of high drought and extreme temperatures. Most European butterflies live in grassy areas that are particularly influenced by these environmental conditions. Therefore, butterflies may be dramatically affected by climate change, especially during summer months. On the other hand, butterflies are known for their remarkable ability to move to find suitable climatic or microclimatic situations. However, few studies in Mediterranean ecosystems have evaluated the behaviors at a small-scale level exhibited by diurnal Lepidoptera to survive during hot and dry periods when resources are scarce. The aim of this study is to evaluate the possibility that several butterfly species can use different vegetation units at different times of the year and to identify the combination of natural elements that allow for their survival. The study was conducted in heterogeneous environments in the Mediterranean area inside the MAB UNESCO World Biosphere Reserve of Monte Peglia (maximum altitude 837m). To obtain a complete picture of environmental preference over time, 90 plots (50 x 50 m) were sampled every two weeks from April to October 2022, in 5 macro-areas within the Core and Buffer areas of the reserve. Plots were chosen by selecting a comparable number of grassland and forest habitats at different altitudes. In each plot, data on abundance of diurnal Lepidoptera species, as well as environmental and vegetation coverage characteristics were collected. The latter was estimated in the field and confirmed by photo interpretation and remote sensing data. Fourteen sampling sessions were carried out for a total of 1137 plots sampled. Overall, 81 butterfly species were identified, two of which were present in the Habitat Directive. Comparison with historical data showed that in the reserve, 12 species, previously reported and generally rare in the region or associated with low mountain environments, were not confirmed during our samplings. In addition, during the hottest months, different butterfly species tend to decrease their presence in grassy areas and become more common in wooded areas. In particular, areas that showed higher butterfly richness over time were characterized by specific environmental conditions: small clearings near streams surrounded by forests. This finding contrasts with results obtained in central European areas where open grassland areas host richer communities of butterflies and other pollinators. Our results extend the habitat of butterflies from grassy areas, where they find trophic resources for adults and larvae (flowers and host plants), to wooded areas where they find shelter from high summer temperatures. The ability to actively move from grassy to wooded areas may prove to be the key to the survival of Lepidoptera in Mediterranean environments. This evidence provides clear indications of the importance of environmental heterogeneity in conservation strategies for Mediterranean areas to mitigate the effects of climate change.

KEY WORDS: Lepidoptera, Monte Peglia Reserve, aestivation, climate change, environmental mosaic.

ORAL PRESENTATION

SESSION III
ECOLOGY AND CONSERVATION

Hymenoptera pollinators in natural ecosystems in Calabria

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Pollinators play a key ecological role in ecosystems: the majority of Angiosperms require insects for their reproduction, and pollinating insects are dependent on resources for their reproduction or energy needs. Therefore, plants and pollinators share a long mutualistic relationship. In the last years populations of pollinators have decreased throughout Europe; for this reason, conservation strategies and monitoring plans are urgently needed.

The cause of this decline is multifactorial: degradation and fragmentation of habitat, intensive agriculture and pesticide exposure, the reduction of quality and abundance of food resources, pathogens attack such as virus, bacteria and fungi, but also parasites such as other insects and mites. In addition, climate change exerts pressure and exacerbate these threats on pollinators. In addition to altering diversity and abundance of pollinators, climate change may impact the species' phenology and may lead to a mismatch between the timing of pollinator activity and flowering period of the plants, further reducing pollination efficiency.

Our research is conducted inside Sila National Park, Pollino National Park and Serre Regional Natural park and in rural areas of Calabria and the focus is the study of pollinator species, with particular emphasis on their abundance and diversity. Pollinators are collected by pan traps with different colour (yellow, blue and white) and with the use of hand net along selected transects. The collected material is sorted at the General and Applied Entomology laboratory of the University of Calabria and identified using available identification keys.

This sampling is focused to collect samples belong to different family such as Andrenidae with genus *Andrena* Fabricius, 1775 and *Panurgus* Panzer, 1806; Apidae with the genus *Anthophora* Latreille, 1803, *Bombus* Latreille, 1802, *Eucera* Scopoli, 1770, *Xylocopa* Latreille, 1802; Colletidae with genus *Colletes* Latreille, 1802 and *Hylaeus* Fabricius, 1793; Halictidae with the genus *Halictus* Latreille, 1804 and *Lasioglossum* Curtis, 1833, and Megachilidae with genus *Megachile* Latreille, 1802 and *Osmia* Panzer, 1806.

KEY WORDS: Hymenoptera, Bees, Pollinators, Pollination, Conservation.

SESSION III
ECOLOGY AND CONSERVATION

Pollinators of Orchids of genus *Anacamptis* Rich

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Because of climatic change and human activities, some orchid species and the associated pollinator fauna have seen a reduction in their populations in the last few decades. For this reason, it is fundamental to apply monitoring plans to avoid this drastic loss of biodiversity.

The purpose of this project is to study the strategies of reproduction of orchids, especially the genus *Anacamptis*, but also to study the ecology, biology and phenology of pollinators living in the same habitats as the Orchids.

This research lasts 2 years and is still ongoing. The sampling is performed in some areas with flowering of orchids in Sila National Park and in Pollino National Park (Calabria, Southern Italy) and it involves the use of some traps, such as pan traps with different colour (yellow, blue and white) and Malaise traps. In addition, hand net is used to collect insects along transects; finally, direct observations of visiting insects on the flowers of the orchids are also performed. In this last case we record with picture and video the pollination process to document the pollinator behavior during flower visits.

Every collected insect is inspected for the presence of pollinia and their position on the insect body. Subsequently the pollinia are analysed using genetical tools to identify their species.

All collected samples are identified in the General and Apply Entomology Lab at the University of Calabria using dichotomic keys and a reference entomological collection.

There are several pollinators which are been collected and recorded, mainly Hymenoptera (Apoidea), like the genera *Andrena* Fabricius, 1775, *Apis mellifera* Linnaeus, 1758, *Bombus* Latreille, 1802, *Eucera* Scopoli, 1770, *Halictus* Latreille, 1804 and *Lasioglossum* Curtis, 1833, but also Diptera: genera *Stevenia* Robineau-Desvoidy, 1830, *Tachina* Meigen, 1803 and *Villa* Lioy, 1864 ; Coleoptera: genera *Anthaxia* Eschscholtz, 1829, *Chlorophorus* Chevrolat, 1863, *Oedemera* Olivier, 1789, *Oxythyrea* Mulsant, 1842 and *Tropinota* Mulsant, 1842; and Lepidoptera: genera *Pieris* Schrank, 1801 and *Zygaena* Fabricius, 1775.

These first observations suggest that the main pollinators of the genus *Anacamptis* of the study areas belong to the genera *Apis*, *Bombus* and *Eucera*. This research is pioneer for southern Italy and it will allow us to evaluate the community structure of insects associated with orchids. This research aims to obtain new and interesting data on the entomological communities closely related to the native Italian orchids, with particular reference to the populations present in the southern regions of our peninsula and to the different pollination strategies by more or less specialized groups.

KEY WORDS: Pollination, Pollinator, Apoidea, Orchidaceae, *Anacamptis*.

POSTER

SESSION III
ECOLOGY AND CONSERVATION

Resampling nocturnal Macrolepidoptera in the Lucca Apennines

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The family Noctuidae is the third largest family of the order Lepidoptera, with more than 12,000 described species and thousands yet to be named. They are represented on every major continent (except in Antarctica), colonising a broad spectrum of habitats from hydricwet to ultra-xeric. Noctuid moths also play an important role in the ecosystems where they are one of the dominant groups of insects pollinating at night. Unfortunately, the climate change is affecting the distribution of many species and populations inhabiting several areas, especially mountain zones, appear particularly vulnerable to these threats because they are constrained in altitudinal shifts. To better evaluate the influence of these changes, on a local scale, we decided to return to sample a little-explored area of the Lucca Apennines to understand the quality of night moth populations, 40 years after the last monitoring. Our sampling confirmed the presence of species already captured in the area but also highlighted a change in the lepidoptera fauna, with the addition of previously unrecorded species whose presence could be related to the changes taking place.

KEY WORDS: moths, climate change, biodiversity.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Spiders (Arachnida, Araneae) from Città Studi's Botanical Garden (Milan, Northern Italy)

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Urbanisation, which causes habitat reduction and fragmentation, is one of the major causes of biodiversity loss. Green areas within urban contexts, including parks, gardens, flower beds and botanical gardens, can act as refugia for different taxa. The city of Milan is characterized by having green areas of historical, recreational and naturalistic value, such as the Città Studi Botanical Garden of the Università degli Studi di Milano. Because of that, we decided to investigate the Garden's spider fauna (Arachnida, Araneae) and make a faunal comparison with an urbanized area adjacent to it (Polo Universitario Città Studi). In this way we aimed to understand if the Garden could act as a refugium for non-urban spider species. Consequently, the Garden was divided into 7 macro-areas: uncultivated meadows subject to mowing, oak-hornbeam, wooded area under development (with alien species such as *Nonea lutea*, *Ailanthus altissima* and *Morus nigra*), mixed environments (including greenhouses of succulent plants and an ancient wall), pond, greenhouse of exotic plants and a transept of mixed habitats. In contrast, the Polo, was characterized by the presence of some flower beds surrounded by *Photinia x fraseri* and *Prunus laurocerasus* hedges, with some arboreal species, in a more anthropized context. Both areas were surveyed in the period between April and September 2021, using pitfall traps (9 sessions in the Garden and 8 in the Polo), sweeping net, entomological umbrella and sight collection (10 in the Garden and 9 in the Polo). A total of 1104 spiders belonging to 85 different species were collected. Of these, 68 are exclusive to the Garden (80%), 5 to the Polo (6%) and 12 are shared by both the areas (14%). The most represented families in the Garden are Linyphiidae, Thomisidae and Theridiidae (each 14%), while in the Polo Theridiidae (24%) are the most numerous, followed by Thomisidae and Salticidae (each 18%). Among the most significant species found in the Garden there are: *Tmarus stellio* (Simon, 1875) and *Theridion hemerobium* (Simon, 1914), respectively a Thomisidae and a Theridiidae new to the fauna of Lombardy, and *Cicurina japonica* (Simon, 1886), an alien troglophilic Hahniidae from Japan, found in Italy only in the province of Bergamo. *Porrhoclubiona leucaspis* (Simon, 1932), a new Clubionidae for Lombardy, and *Platnickina nigropunctata* (Lucas, 1846), a new Theridiidae for Northern Italy, were found in the Polo. Additional 21 new species for the province of Milan are also reported (15 in the Garden, 1 in the Polo and 5 in both the areas). The Garden's spider community appears very diversified, probably due to environmental heterogeneity. In the Garden 23 hygrophilous and/or litter-related species (only 1 of them is shared with the Polo) are recorded. Instead, the Polo hosts 11 species, 8 of which in common with the Garden, related to dryer, synanthropic and detritic environments. Therefore, we confirm the Garden as a refugium for not urban-contexts adapted spiders. However, the finding of *C. japonica* suggests that the apparent expansion of this species should be monitored, since in the future it could colonize hypogean environments in Italy.

KEY WORDS: alien species, new records, spiders, urban fauna, urbanisation.

POSTER

SESSION III
ECOLOGY AND CONSERVATION

Check list of orthoptera species of the sic “calanchi di rigoroso, sottovalle e carrosio” and of the neighboring areas in the piedmont apennine

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In the north-western Italy (Piedmont, Liguria, Val d'Aosta) 147 species of Orthoptera are reported, 70 of which are present in the Ligurian-Western Piedmont Apennines. However, there is little knowledge relating to the orthoptera communities inhabiting the xeric meadows of the Piedmont Apennines. Therefore, in this study we wanted to deepen the knowledge on the orthoptera communities living in these habitats, with a particular focus on the species present in the SCI "CALANCHI DI RIGOROSO, SOTTOVALLE E CARROSIO" and in the neighboring areas. The research was carried out within the arid meadows of a hilly area of the Alessandria Province, in particular in the municipalities of Arquata Scrivia and Gavi Ligure, one of the two areas also fell within the SCI IT1180030, Calanchi di Rigoroso, Sottovalle and Carrosio. These territories are characterized by a diversified landscape, dominated by broad-leaved forest interrupted by significant gully formations, fragrant broom shrublands and xeric grasslands. The latter include the investigated habitats, often attributable to habitats 6210 and 6210* (Important Orchid Site). Two study areas have been identified (Arquata and Sottovalle), about 3km apart, located respectively in the municipalities of Arquata Scrivia and Gavi Ligure (AL). In the Arquata station we implemented three sampling areas (A1, A2, A3) while in Sottovalle station consists of a single area (B1). Sampling was carried out from 12 May to 15 July for 11 sampling sessions (one session per week) along 16 standard 10x2m transects (4 transects per area) at least 2m apart, covered with a mowing net at about 10m/min. The orthoptera community investigated is made up of at least 42 species out of the 70 reported for the western Ligurian-Piedmont Apennines (corresponding to 60%). This result is absolutely relevant considering that in this study only one habitat type was investigated, that belonging to semi-natural dry grassy formations (H 6210). Among the species identified, we found a new species *Antaxius difformis* with respect to the above mentioned list. In total, 3500 specimens belonging to 16 subfamilies were collected. The two most abundant subfamilies were the Gomphocerinae and Tettigoniinae with respectively 1030 and 871 specimens collected. These two subfamilies were followed by the Phaneropterinae with 674 specimens and the Calliptaminae with 647 specimens. The subfamilies present in the study area well represent the Italian and in particular the Piedmont orthopterological diversity. The wide range of eco-ethological characteristics of the species of these subfamilies denotes the diversity of habitats which characterize the study area and which do not always reflect the typical conditions of these territories.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Ecology and abundance Of a *Saga pedo* population (Orthoptera Tettigoniidae) in the Piedmont Apennine

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The increasing of forest cover and the significant intensification of agriculture has contributed to the rarefaction of the large bush cricket *Saga pedo*, now considered Vulnerable at a global level by the IUCN and included in Annex IV of the Habitat Directive. Since the populations are not abundant and highly localized, it is not easy to acquire sufficient information on the ecology of the species and on the size of its populations. Furthermore, due to the strong elusiveness and cryptic nature of the specimens, the probability of observing their presence in the wild is extremely reduced and the abundance of the populations risks being greatly underestimated. This study aims to investigate the conservation status of the species in the Piedmont Apennines through 1) an estimate of the size of the population under study in relation to the environmental characteristics of the surrounding landscape and the availability of prey; 2) the elaboration of an habitat suitability model useful for the implementation of future monitoring in the territories close to the study area. The research was carried out in the dry meadows of a hilly area in the Alessandria Province, in the municipalities of Arquata Scrivia and Gavi Ligure. The samplings were carried out from May to July for 11 sessions along 16 10x2m transects crossed with a grass net. In addition to *Saga pedo*, all the other Orthoptera present were identified and classified at the subfamily level and, where possible, at the species level. Population size and detection probability of *Saga pedo* were obtained by applying the N-Mixture models. To verify the existence of an effect of prey (such as the abundance of specimens for each subfamily of Orthoptera; the total abundance of Orthoptera and the number of subfamilies observed simultaneously with *Saga pedo*) on the abundance of *Saga pedo* we performed a GLM regression. To spatially visualize the relationship between *Saga pedo* and the other Orthoptera subfamilies, we used the PCO metric sorting method.

The population of *Saga pedo* in the study area consists of 197 (± 115) specimens which, in natural conditions, have a probability of being detected not higher than 2.38%. This probability is further reduced on windy days. The investigated population of *Saga pedo* occupies sub-optimal areas. This is evident not only from the habitat suitability model produced but also from the evident spatial overlap between *Saga pedo* and other subfamilies that are ecologically very distant from it and mostly linked to mesophilic elements. The sub-optimal conditions in which the population under study lives are indirectly confirmed also by the small body dimensions of the specimens and by the short duration of the life cycle which did not exceed 60 days. Most of the specimens observed are concentrated in small clearings completely immersed in a wooded matrix and therefore isolated from each other. On the basis of the results obtained from this study, it is probable that the advancement of the forest represents the main threat to this population, transforming the clearings and xeric meadows to which it is linked into small fragments that are suboptimal and insufficient to host viable populations.

ORAL PRESENTATION

SESSION III
ECOLOGY AND CONSERVATION

Functional Biodiversity in Coastal Terraced Vineyards in National Park Cinque Terre.

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In the Italian viticulture context, the terraced vineyards of the Cinque Terre Natural Park (CTNP) are an integral part of the landscape declared UNESCO World Heritage Site. The well-functioning of vineyards of local white grape cultivar is important for high quality wine production and for the integrity of the territory affected by high hydro-geological risk. Management practices which support biodiversity can improve soil health, reduce the use of pesticides and fertilizers, allowing to preserve yields (2030 Soil Strategy). In this context, the microfauna of the CTNP vineyards and the soil biological functionality were investigated focusing on 1) analysis of the community of microarthropods that colonize the leaves of the Albarola, Bosco and Vermentino cultivar, 2) characterization of the ecological processes of the first 10 cm of the soil, near the vine plants.

In the Park area, 6 sites were selected at different altitudes and distances from the sea. From 2020 to 2022, for each terraced vineyard and vine variety, 25 leaves were sampled in spring and autumn to identify the phytophagous/predatory arthropods present. Soil samples were analyzed for biochemical properties and calculated the arthropod community by total abundance and biodiversity indices and soil biological quality (QBSar index).

Sampling site and cultivar influenced phytoseiid density on leaves (GLM, $p < 0.001$; HSD Tukey test). Considering conventional management, phytoseiids were abundant (2.4 ind./leaf) and identified species were *Kampimodromus aberrans*, *Typhlodromus pyri*, *T. exhilaratus*, *Phytoseius finitimus*, *Euseius stipulatus*. The phytophagous functional groups such as cochineal were sporadic, Tetranychid and Eriophyid mites absent. The organic carbon content varied from 53.6 to 72.2 g Corg/kg of soil and the microbial biomass from 60 to 123 mg dsDNA/g of soil. Concerning biogeochemical processes, enzymes linked to the P cycle were abundant in the highest sites (>200 m asl); the abundances of enzymes involved in the carbon cycle and the microarthropod community were highest in no-tillage vineyard site.

The total abundance of edaphic microarthropods was over 16,000 specimens: 86 % mites, 8 % springtails, 2 % ants and followed by other groups belonging to Myriapoda, Arachnida, Crustacea and Insecta. In Autumn, the abundance of edaphic arthropods is lower than in spring: maybe due to vertical migrations for optimal micro-environmental conditions. The vineyards located at higher altitudes showed low QBSar values and a simplification of the community structure denoting soil functionality loss.

The complexity of the communities of organisms and functional biodiversity can entail a high environmental resilience and mitigate land degradation. This multi-disciplinary approach may allow applications in smart agriculture and conservative productive policies in a protected area such as Cinque Terre.

KEY WORDS: soil biodiversity, phytoseiids, QBSar, enzymatic activity, microarthropods.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Wild bees in warming cities

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Urbanization is considered one of the major threats to biodiversity worldwide. However, recent research highlighted the potential role of cities as refuges for pollinators. Main drivers of cities' ability to sustain pollinators are the amount and the spatial organisation of green areas. In addition, temperature is one of the key determinants of insect pollinators' activities. The urban heat island effect makes cities warmer than surrounding natural areas, providing an ideal system to study warming effects. Here, we studied the effects of temperature, open habitat cover and distance from the city centre on wild bee communities in the city of Rome (Italy). We selected 36 sites along two independent gradients of open habitat and of surface temperatures, and we sampled bee communities using pan traps for four months. Then, we measured functional traits of wild bees, i.e., body size, social behaviour, nesting strategy, and diet breadth. Temperature drove the abundance and composition of wild bee communities irrespective of landscape metrics. At warmer temperatures, communities were richer in species and individuals. By contrast, colder sites were characterized by less species and less individuals. In addition, temperature changed the functional composition of bee communities. With increasing temperatures, bee communities showed a higher percentage of polylectic and small size species. In conclusion, warming seemed beneficial for bee abundance, but it might homogenise communities selecting for those traits that make species more easily adaptable. Further research is needed to understand the potential role of cities as pollinator refuge under global change, focusing not only on wild bees, but even on other fundamental pollinator taxa such as Coleoptera, Diptera, and Lepidoptera.

KEY WORDS: bees, biodiversity, climate change, functional traits, urban environment.

SESSION III

ECOLOGY AND CONSERVATION

Spiders take their place: Spider communities segregate into different strata in the wild

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The spiders (Araneae) are an abundant and diverse group of arthropods that play contrasting roles in ecosystems, both as (mainly) generalist predators and prey for vertebrate and invertebrates. In addition, they show an outstanding functional diversity due to a morphological variability that favours niche partitioning among species with different hunting strategies occupying different microhabitats. However, spider microhabitat segregation has been, paradoxically, rarely approached at a small spatial scale among spider communities located just metres apart. In this study we did so and analyzed spider inter-specific segregation among microhabitats at open woodlands in central Iberia.

We defined four microhabitats associated with different strata (tree canopy, bushes, leaf litter beneath the canopy and open grassland). At two open woodlands we selected 10 focal trees at which we collected spiders by shaking the branches of each one six times in each cardinal direction. We placed a white sheet below the branches and immediately collected all the spiders falling onto it. Bush spiders were sampled using a sweeping net along two 10-m long transects 10-15 metres apart from the trees. At this distance we also set up a pair pit-fall traps separated by 10 meters (open grassland spider sampling). Lastly, we placed another two pitfall traps beneath the canopy (leaf litter). Biological communities of spiders were analysed using species diversity indexes (Hill's numbers of true diversity), similarity of strata (SIMPROF and NDMS), segregation (Venn diagrams) and generation of functional guilds (UPGMA) from different standardized samplings.

We obtain a clear and precise segregation of the spider communities in the four different defined strata, both at the level of effective (q_1) and dominant (q_2) species richness. The main segregation was between species linked to tree and bush branches and those living on the ground (collected by pitfall traps). Interestingly, there were also some differences between the communities of ground spiders living beneath the canopies and in open grassland next to the trees. Also, the results of the guilds at species level support this segregation, with relationships existing between spider functions and occupied strata.

These results show us that microhabitat segregation among spider communities takes place at a very small spatial scale. They also warn about the loss of certain landscape elements (like isolated trees in open woodlands) that may occupy a small surface proportionally but are essential for the conservation of the spider communities linked to them. Differentiated and specialized spider communities will be very vulnerable to changes in the environment and land cover at a microhabitat scale.

KEY WORDS: Spiders, Chelicerata, Biological Communities, Microhabitat.

SESSION III

ECOLOGY AND CONSERVATION

Monitoring of protected insect species with citizen science: is it feasible and effective? Results and perspectives of an Italian long-term initiative

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Recent projects and initiatives aimed at monitoring species, habitats or environmental variables, have proven that citizen science (i.e., scientific research carried out by non-experts in collaboration with professionals and scientific institutions) is a valuable tool to collect data and gather information at large scales, covering long periods of time and a large scale of landscape. This approach can be of particular interest, especially if it meets the requirements of the Habitats Directive. In fact, according to this European directive, each country must undertake surveillance of the conservation status of protected species and habitats present in its national territory (art. 11) and provide a national report on their status every 6 years (art. 17). These objectives require a remarkable sampling effort, both in terms of time and space, especially if small, inconspicuous species, such as insects are concerned, as their distribution is in many cases still partially unknown.

In Italy, only few citizen science projects are dedicated to the collection of data on insect species, and these mainly focus on butterflies, pollinators, or invasive alien species. As a consequence, protected insects are rarely targeted by citizen science projects.

In this context, we present the results from a long-term citizen science initiative which started as the "LIFE MIPP" project (LIFE11NAT/IT/000252) and continued afterwards thanks to funding from the Italian Ministry of the Environment. This initiative focuses on protected insect species, including butterflies, beetles, dragonflies, and crickets, and so far more than 1,400 volunteers participated and collected more than 7,000 records. Analyses of these data show a clear increase in the number of volunteers and number of records as well as greater spatial coverage for the target species over the years.

As this initiative has been ongoing for a long time, we wanted to know if our project has already reached its goals and if continuing this initiative was still scientifically meaningful. Our analyses show that the project has not yet reached its maximum potential, revealing the importance of long-term projects in collecting data on the distribution of the targets. New information and data are still being recorded.

Furthermore, projects of this kind can also have an important social role in educating and empowering citizens. In the present case, a sociological survey has been carried out to assess the level of involvement of volunteers and whether this initiative also has educational and awareness-raising implications towards biodiversity and nature conservation. Preliminary results are encouraging.

In conclusion, we demonstrate that citizen science is a powerful tool for the study, the monitoring and the conservation of biodiversity, including neglected species, like insects. In fact, the data collected during this initiative not only have confirmed and strengthened the current knowledge, but also added new spatial information, resulting in a better definition of the species conservation status as required by the Habitats Directive. In addition, the direct involvement of volunteers in data collection also has an important social implication since people are trained, educated, empowered, and made aware about insects and the importance of their conservation.

KEY WORDS: Citizen Science, Conservation, Biodiversity, Protected species, Habitats Directives.

ORAL PRESENTATION

SESSION III

ECOLOGY AND CONSERVATION

Results of three-year surveys conducted on quarantine insects in the context of the new EU regulatory framework framework

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Globalized trade and climate change have exponentially increased the risk of introduction of alien species harmful to plants and Italy, due to its specific soil and climatic characteristics and richness in biodiversity, is particularly susceptible to it.

One of the most important actions to prevent the establishment and spread of these new pests is prevention implemented through surveillance of the territory.

To this end, the EU's new regulatory framework requires all member states to monitor their territories through specific surveys for quarantine pests and regulated by sector legislation.

Since 2020, EU countries are obliged to draw up and implement a National Survey Program for certain harmful organisms and which in the Italian legal system is implemented by art. 27 of Legislative Decree 19/2021.

National planning takes into account:

- The 20 priority pests identified by Regulation (EU) 2019/1702;
- Of all organisms regulated by Emergency Measures;
- The EU-relevant quarantine pests referred to Regulation (EU) 2019/2072.

In this paper we report the summary results of the entomological surveys carried out in the three-year period 2020-2022 by the Plant Protection Service of the Campania Region, jointly with its scientific partners, and descending from the implementation of the Regional Survey Plan and covering an average of 50 phytophagous insects/year with particular attention to "priority 1":

Agrilus anxius, *Agrilus planipennis*, *Anastrepha ludens*, *Anoplophora chinensis*, *Anoplophora glabripennis*, *Anthonomus eugenii*, *Aromia bungii*, *Bactericera cockerelli*, *Bactrocera dorsalis*, *Bactrocera zonata*, *Conotrachelus nenuphar*, *Dendrolimus sibiricus*, *Popillia japonica*, *Rhagoletis pomonella*, *Spodoptera frugiperda*, *Thacoumatota*.

From the survey findings, the cerambycicide *Aromia bungii*, which has been present in Campania since 2012, deserves attention. Following the monitoring activities of host plants in vacant areas, some infested plants were found in Castelvoturno (Ce) in the year 2021. Timely eradication measures have been taken and the infested area and the buffer zone have been demarcated. Further investigations in the delimited area have not led to any additional findings so there are grounds to hope for its eradication to be made official to the EU Commission according to the timelines and procedures set out in Implementing Decision (EU) 2018/1503. Also through the Survey Plan deployed by the Campania Region, in the year 2022, monitoring with traps made it possible to identify the presence of *Bactrocera dorsalis* in some areas of the Neapolitan area where single specimens had already been captured in 2018. The Campania Region, through its Plant Protection Service, it has adopted a specific Action Plan with the identification of a whole series of phytosanitary measures aimed at countering the spread of the oriental fruit fly. Furthermore, it was possible to detect in Campania the widespread presence of *Aleurochantus spiniferus* and *Toumeyella parvicornis* as well as other phytophages to a lesser extent such as *Halyomorpha halys*, *Drosophila suzukii*, *Cryptoblabes gnidiella*.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Predicting the effect of global warming on dung removal of three Geotrupidae species (Coleoptera, Geotrupidae)

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Global warming is affecting spatial and temporal distribution of insects that in turn affect their ability to provide ecosystem services to society. Dung beetles are an important component of Alpine areas because of their ability to provide key ecological processes. This is particularly true for dung removal that promotes multiple ecosystem services such as nutrient cycling, pest removal and seed dispersal. However, the effect of global warming on dung removal has been scarcely investigated. Here we quantified dung removal efficiency of three Geotrupidae species (*Anoplotrupes stercorosus* (Scriba, 1791), *Geotrupes stercorarius* (Linnaeus, 1758), *Trypocopris pyrenaeus* (Charpentier, 1825)) in experimentally warming treatments and used experimental results as the basis for spatio-temporal modelling of dung removal based on past (1981-2005) and future (2011-2100) temperature scenarios in the altitudinal belt 600-1400 m of the Piedmont region. For each species, dung removal efficiency of dung beetle pairs (i.e. one female + one male) was quantified at different temperatures (i.e. 5°C, 10°C, 15°C, 20°C, 28°C and 36°C). A biological model between dung removal and temperature was built from experimental data for each species separately and then used for spatio-temporal modelling. In this regard, high resolution (1x1km) temperature data were coupled with the biological model to obtain estimates of dung removal for the entire study area. For the past scenario changes in dung removal due to global warming were quantified by taking into account changes in pasture extent. Three scenarios of global warming (low, moderate and high carbon emissions) and in three time intervals (2011-2040, 2041-2070 and 2071-2100) were considered for modelling future trends. Our results show how increasing temperatures in the past (1981-2005) may have boosted rates of spring dung removal in the entire study area (expressed as amount of dung removed per month per pair), potentially compensating for the reduction of grassland extension in pasture-based livestock farming systems. Quite interestingly, models for future scenarios showed a weak increase in dung removal limited to the most extreme scenarios, suggesting a resilience of this ecosystem service. Our inferences on changes in dung removal rates for the past and our predictions for the future can inform land-use planning and decision making for adaptation strategies of livestock management in Alpine areas.

KEY WORDS: dung beetles, dung removal, ecosystem services, global warming.

ORAL PRESENTATION

SESSION III

ECOLOGY AND CONSERVATION

Handling and marking effects on survival, capture probability and mobility of non-territorial damselfly *Coenagrion castellani* (Odonata, Coenagrionidae)

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Studies that yield positive and innovative results tend to generate more attention than those with negative or null findings. This has led to a problem known as the “file drawer” issue, where some relevant research results are not published. However, negative results play an essential role in advancing research, and their publication is crucial for scientific progress. In some cases, negative results can indicate underlying assumptions that must be met to conduct investigations properly. For instance, in Capture Mark and Recapture (CMR) surveys, an essential assumption is that marking animals does not affect their recapture rates or life expectancy. Despite being negative, such assumptions are crucial to the validity of the research and must be considered carefully. To explore the effects of manipulation and marking in non-territorial dragonflies, a population of *Coenagrion castellani* Robert, 1948 was investigated by means of mark-recapture method in a small watercourse in Umbria, Central Italy. During the flight season, corresponding to the June-July period, 849 adult individuals were marked with a numerical code on their right hindwing in eight separate sessions. The individuals were marked with five distinct colours based on the stretch where they were initially collected. The study area was restricted to a 250-meter long transect, which was divided into five sections ranging from 40–70 m each. After marking, damselflies were immediately released, and the manipulation required less than 30 seconds. It is interesting to note that no evidence of deficit related to the handling and marking effect was found. No significant effect of any colour was detected on the survival of both males and females, and couples with a single or both marked partners were recorded on several occasions. Individuals marked in red unexpectedly showed a lower probability in recapture, whereas in the other stretches, no color effect was observed. In addition, a significant proportion of marked individuals exhibited a low mobility with a natural inclination towards philopatry. The individuals marked in black had notably high sedentary rates, with a median travel distance of 33 meters along the watercourse, in a stretch where the vegetation and overall habitat characteristics may have been more favourable than in other areas. The results of the mark-recapture exercise indicate that both careful manipulation and the use of wing marks with different colours have no discernible effects on the survival probability, mobility and behaviour of this non-territorial species. These results, despite being negative, are significant for entomological research since this species is included in Annex II of the Habitat Directive and therefore subject to periodic monitoring to assess its conservation status.

KEY WORDS: CMR, Odonata, philopatry, dispersal, RMark.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Evaluation of different anthropical pressure on diurnal lepidoptera biodiversity of Aspromonte National Park.

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The study of insect biodiversity can represent a valid tool to understand the conservation status of specific environments. The diurnal lepidoptera, because of their high relationship with the angiosperms and their susceptibility to sudden changes in environments, are used to assess the effect of environmental disturbance on specific areas.

In this study the results about a monitoring activity aimed at using diurnal lepidoptera as bioindicators to assess the conservation status of Calabrian populations were reported. Both qualitative and quantitative composition of these pollinators were studied in different scenarios and environments located into National Park of Aspromonte (Calabria, Italy).

Ten transects, 500 m long, located in different environmental contexts (intensive and extensive grazing, cultivated field, burned area, natural environments) were surveyed twice a week, from May to September, and the different species of flying adults were annotated.

The results obtained, although referring to the first period of observations, highlighted how the human activity negatively affects the structure and composition of the studied lepidoptera. During the monitoring activity 43 species, belonging a 5 family were surveyed. In the most degraded environments (grazed and burned areas), which presented a low abundance of flower plants, biodiversity of butterfly species was lower compared with that registered in environments in which the human pressure was limited or absent.

KEY WORDS: Pollinators, anthropic activity, biodiversity, butterfly.

SESSION III
ECOLOGY AND CONSERVATION

Monitoring and conservation of protected species of insects: the LIFE ESC360 approach

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When it comes to biodiversity-focused research, a few projects involve non-expert volunteers in data collection on protected species and habitats. Even fewer involve young volunteers (18 to 30-year old) and do focus on the monitoring of insects. In this context, Life ESC360 was the first project in Italy dealing with these characteristics.

The project lasted four years (2018-2022) and engaged almost 360 volunteers working in 22 Nature State Reserves (corresponding to 23 Natura 2000 sites) in Lombardy, Tuscany, Lazio, Abruzzi and Apulia. After a specific training, and thanks to the support and coordination of the project experts, volunteers applied specific national protocols and collected data on more than 60 targets of habitats, plants and animals (including insects, birds, reptiles, amphibians and mammals) listed in annexes of the EU Birds and Habitats Directives.

The present contribution aims at analysing and presenting the insect monitoring activities of LIFE ESC360. These focused on, 5 saproxylic beetles (*Lucanus cervus*, *Morimus asper*, *Rosalia alpina*, *Osmoderma eremita*, *Cerambyx cerdo*) and 4 butterflies (*Zerynthia cassandra*, *Z. polyxena*, *Euplagia quadripunctaria*, *Parnassius mnemosyne*) as well as the investigation on butterfly and cricket/grasshopper species communities. A total of 374 sampling sessions were performed in spring-summer of 2019, 2020 and 2021, and presence data of about 4260 individuals were collected.

The project provided different types of data, resulting in i. the contribution to already existing and well-established monitoring programmes, ii. the confirmation of the presence of protected species in some areas in which information concerning the population and its conservation status were lacking, iii. the first records of protected species presence in some Natura 2000 sites and Reserves, and iv. the consequent activation of new monitoring programmes.

Moreover, the investigation of the butterfly communities reveals valuable information concerning the habitat management, highlighting the importance of maintaining some areas closed to the public, thus preserving patches of biodiversity within a pre-existing anthropic matrix.

These data constitute remarkable information both in terms of scientific knowledge and management of Nature Reserves, Natura 2000 sites and protected species, revealing the potential and the effectiveness of citizen science initiatives.

Additionally, several implications resulted concerning the human dimension. In fact, the direct involvement of young volunteers allowed them to implement and enrich their theoretical and practical knowledge, enabled the establishment of a new or more solid connection between participants and the natural environments, in particular with the insect species, often less appreciated by young people who are, especially early in their education, more interested in studying vertebrates.

KEY WORDS: Monitoring, Conservation, Citizen Science, Biodiversity, Habitats Directive, Natura 2000 Network, Nature State Reserves.

ORAL PRESENTATION

SESSION III

ECOLOGY AND CONSERVATION

LIFE SPAN Saproxylic Habitat Network: planning and management for European forests (LIFE19 NAT/IT/000104)

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Traditional forest management, focused on the maximization of continuous wood production, has brought the reduction of structural heterogeneity, deadwood volume and microhabitat availability, and consequently flora and fauna diversity. This is even more true for saproxylic biodiversity, i.e. that represented by species that, at least in one stage of their life cycle, are linked to decaying or dead wood or to other saproxylic organisms.

LIFE SPAN project's main aim is to demonstrate that it is possible to combine traditional forest management practices, with the creation of a biodiversity protection network, without compromising the management's economy. Through an innovative forest planning and management approach, always attentive to the economic sustainability of the proposed interventions, management solutions, aimed at the conservation of habitats and species of Community interest linked to dead wood, will be implemented and monitored.

The main tool of the project is the creation of Saproxylic Habitat Sites (SHS) in the productive forest matrix. Intervention will be carried out to increase the presence of deadwood, microhabitat, structural heterogeneity and gaps in 25 SHS in Italy (Cansiglio Orientale Forest) and in 18 SHS in Germany (University Forest Sailershausen). Saproxylic vertebrate (e.g. woodpeckers and bats) and invertebrate (e.g. beetles and true bugs) species will be monitored. The interventions are expected to trigger a rapid evolution of processes beneficial for biodiversity, which will naturally promote the increase in ecosystem services.

KEY WORDS: insects, biodiversity, dead wood.

SESSION III

ECOLOGY AND CONSERVATION

Environmental amelioration of floodplain areas for wild bees (Hymenoptera: Apoidea: Anthophila).

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Habitat fragmentation and intensive agriculture using pesticides and herbicides are considered among the main factors responsible for the pollinator decline (Nieto et al., 2014). The new Common Agricultural Policy (CAP) introduces measures to prevent this decline, such as supporting sustainable farming practices and enhancing non-productive lands for increasing biodiversity, including pollinators. In addition to non-productive agricultural areas, some urban and peri-urban areas can be subjected to management for reaching the same objectives.

To date, several environmental requalification actions to support the pollinator community have been undertaken, but few studies have evaluated their effect using biological monitoring methods (Kremen and M'Gonigle, 2015; Wood et al., 2017; Kanduth et al., 2021).

This research, conducted in collaboration with the Ente Consorzio di Bonifica 3 Medio Valdarno (CBMV), aims at environmental amelioration actions of riparian areas that have been recently subjected to extensive land works for territory hydraulic safety, and the subsequent evaluation of these actions on the pollinators. These types of areas are generally not subjected to renaturation and are flooded on average every thirty years. Considering that the superfamily Apoidea entails the most efficient pollinator species, the improvements were designed primarily for the benefit of this group and consisted of the sowing and handling of two commercial mixtures of self-seeding nectariferous plants on areas of equal shape and surface. In particular, the selected mixtures differed both in the number and type of plant species: the first contained 31 flowering species while the second contained 12. Both mixtures were characterised by native annual species except for *Phacelia tanacetifolia* (Bentham, 1853), which is considered naturalised. The germination and flowering of the different species were assessed at the same time as the monitoring of wild bees, which was completed by means of standard transects and "pan traps".

41.9% of the species of the first mixture germinated and produced an average plot covering of 38%; of the second mixture, 66.7% of the species sprouted and the average cover was 60%. About 80 bee species belonging to 26 different genera were recorded in the study area. 9 species bloomed in the first mixture and 7 in the second and their coverage was respectively 42% and 91% of the total sprouted species of the two parcels. As for the bees observed during the transects, 23 and 31 species respectively were found in the two plots, while only 16 species were observed in non-sown plots, that were dominated by grass species. The results seem to suggest that even a limited number of nectariferous plant species, if present in significant amounts are sufficient for environmental enhancement in favour of pollinating insects.

KEY WORDS: Pollinators, Agricultural Ecology, Floodplain areas.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Influence of soil properties and land use on the invertebrate communities of Po Plain agroecosystems

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The specific impact of agricultural activities on soil organism health is far from being fully understood. Consequently, it is also unclear how soil invertebrates' communities-mediated functions can be affected by land use and soil properties. In this study, the effects of different factors (including agricultural practices and soil properties) on the composition and diversity of soil invertebrate communities were evaluated for representative cropping and semi-natural systems of one of the most important agricultural areas in Europe, the Po Valley. The invertebrate communities were characterized using eDNA metabarcoding approach targeting the Cytochrome oxidase subunit I (COI) and 18S rRNA. The two markers showed differential efficiency in characterizing the soil biota. Specifically, COI had a lower taxa coverage but a higher taxonomic depth than 18S rRNA. The communities substantially differed in terms of composition among the cropping systems, even when higher taxonomic levels were considered. The highest level of diversity was detected in stable meadows; in cornfields the less species-rich communities were found. Annelida, Arthropoda and Nematoda showed differential responses to soil properties and agricultural practices, both in terms of community compositions and diversity. Finally, organic carbon, pH and phosphates levels, porosity and tillage, the use of fertilizers and pesticides resulted to be the most relevant factors affecting the soil invertebrate communities.

KEY WORDS: eDNA metabarcoding, soil invertebrates, agroecosystems.

SESSION III

ECOLOGY AND CONSERVATION

The genetic differentiation of *Anonconotus italoaustriacus* Nadig (Orthoptera Tettigoniidae) in the eastern alps suggests management of populations into Evolutionarily Significant Units (ESUs)

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Anonconotus italoaustriacus is a grassland species historically present in a few disjunct ranges in north-eastern Italy and High Tauern in Austria. The species has recently been included in the Red List of the International Union for Conservation of Nature (IUCN) as an endangered species with a decreasing population trend. Literature reports the presence of only four populations in Italy: the population of the Belluno Dolomites (Vette Feltrine, Busa delle Vette, BL), Sciliar Group (Alpe di Siusi, BZ), the population of San Candido (Monte Elmo, BZ), and the Baldo Group (Monte Altissimo, TN). Knowledge of the genetic structure of endangered insect populations as well as the delineation of evolutionary significant units (ESUs) are useful and integral components of many conservation plans. In this work, the genetic differentiation of five *A. italoaustriacus* populations, covering the entire Italian geographical distribution known for this taxon and partially the Austrian distribution was studied through the analysis of four fragments of the mitochondrial DNA.

Results showed a strong geographical structure among populations and complete absence of gene flow. Divergence time estimation analyses suggested that an ancient separation of bush-cricket populations occurred approximately 1–1.5 Mya. Translocation actions, implying crossing populations, could have deleterious effects and carry risks such as lack of adaptation or outbreeding depression. Since populations of *A. italoaustriacus* represent genetically differentiated entities, conservation efforts should consider each population as a management unit. Conservation actions are therefore proposed, preceded by thorough ecological studies, and focused on monitoring, conserving, and enlarging existing habitat areas.

KEY WORDS: mitochondrial DNA, endangered species, gene flow, conservation, katydid.

SESSION III

ECOLOGY AND CONSERVATION

Evaluation of the potential effects of beaver dams on the aquatic macroinvertebrate communities in Central Italy

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The presence of the Eurasian beaver *Castor fiber* L. has been confirmed in Central Italy since 2020 and, probably, represents the result of an illegal release. Beavers are considered as “ecosystem engineers”, as they may modify the riverine ecosystem where they live. After the discovery of the first beaver dam in the Tevere river (Tuscany, Central Italy), we evaluated the effects of beaver presence on the aquatic macroinvertebrates, with a focus on those more sensitive to environmental stress such as ephemeropterans, plecopterans, and trichopterans (EPT). We used the Italian extended biotic index (IBE) method to quantitatively assess the effects of beavers on macroinvertebrates, sampling both upstream (M) and downstream (V) of the beaver dam (three transects on each side of the dam from one bank to the other, covering all types of microhabitat) using a sampling net (25 cm × 40 cm with 20 mesh per cm²). We also selected a control area (C) upstream of the beaver dam, free of beaver presence (three transects as in M and V). Sampling was conducted in winter, spring, summer, and fall 2022. To standardize data collection, we kicked the river-bottom substrate with the sampling net ten times collecting all the macroinvertebrates. No differences were found in the taxa richness, abundance, and Shannon diversity index among the three areas. However, the multivariate analysis of macroinvertebrates communities revealed a strong and significant effect of season, with distinct assemblages in spring, summer, and autumn sampling, as well as an effect of beaver presence, with samples in the vicinity of the beaver dam significantly differing from control sites. Moreover, the number of EPT taxa in the control area was significantly higher than in upstream and downstream transects.

KEY WORDS: benthic macroinvertebrates, *Castor fiber*, dam, Eurasian beaver.

SESSION III
ECOLOGY AND CONSERVATION

Trophic preferences of the wild bee community (Hymenoptera: Apoidea: Anthophila) in habitats restored from non-productive marginal areas

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The mutualistic relationship between prònubes and plants is among the most fundamental ecosystem service for humans and one of the key factors underlying the functionality of terrestrial ecosystems. Among pollinator insects, wild bees (Hymenoptera: Apoidea: Anthophila) are the most important group, both for their pollination efficiency and for the number of species which, in Italy, is estimated to be over 1017. Wild bees are strongly limited by plants whose nectar is used as their nourishment and on which they forage for pollen, nectar and oils to sustain their offspring. The quality of these resources depends on the plant species and on the environment in which they are found. The synergism of numerous elements, such as the intensive and extensive use of pesticides and herbicides, the habitat fragmentation, and the climate change, has led to a decline in the global biodiversity of wild bees to extinction events at the local scales. Studies on trophic preferences are important to implement knowledge on the resources needed but also to evaluate the effectiveness of measures aimed at the improvement of degraded and marginal environments for conservational purposes.

In this work, we studied the trophic preferences of wild bees in peri-urban floodplain areas undergoing an environmental amelioration project for pollinators conservation. The project, in collaboration with the Consorzio di Bonifica 3 Medio Valdarno (CBMV), is focused on the sowing of different mixtures of annual nectariferous plants and the experimentation of “bee-friendly” management practices in riparian areas that have recently been subject to reshaping to reduce flood risks. In the territory of competence of the CBMV, the areas subject to this kind of engineering intervention are large and only in a few cases they are subject to renaturalization; and a few years after the earthworks, they are mainly covered by grasses.

Trophic preferences were analysed from data from standardised monitoring (transects) and from samples extracted from artificial nests placed in the experimental areas. Our data showed a very rich plant-pollinator network. More than 68% of the specimens preferred to forage on plants belonging to the sown mixtures while only 18% visited flowers of intrusive plants. The most frequently visited sown species were *Phacelia tanacetifolia*, *Lotus corniculatus*, *Medicago sativa* and *Cichorium intybus* while *Verbena officinalis* and *Convolvulus arvensis* were the most frequently chosen among the intrusive species. Analyses revealed strong preferences by some bee genera for specific plant species. Specimens belonging to the genus *Eucera*, which were abundant in the experimental areas, showed a strong preference for the flowers of *Centaurea cyanea* and *Cichorium intybus*, while species belonging to the subgenus *Seladonia* and to the genus *Lasioglossum* showed preferences for the flowers of *Verbena officinalis* and *Convolvulus arvensis*. Equally strong was the preference of species belonging to the family Megachilidae for the flowers of Fabaceae. The information obtained was fundamental for improving the composition of the experimental mixtures, adapting them as much as possible to the preferences of the wild bees community present in our areas.

KEY WORDS: Anthophila, trophic preferences, pollen, marginal areas.

ORAL PRESENTATION

SESSION III

ECOLOGY AND CONSERVATION

The effects of urban agriculture on urban pollinator communities: A case study in the city of Bologna (Italy)

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Insects are responsible for pollinating most wild flora and food crops worldwide along with contributing to many ecosystem services. However, global declines in pollinator populations jeopardise the provisioning of these ecosystem services in both managed and natural ecosystems. Urbanisation has detrimental effects on biodiversity and ecosystem integrity as agricultural and semi-natural habitats are converted into landscapes dominated by built features and impervious ground cover. Urban agricultural sites are a growing component in urban contexts and harness the capacity to increase the sustainability of cities by serving as a source of biodiversity conservation, reducing their ecological footprint and improving the quality of life. However, the amount to which urban agricultural sites contribute towards biodiversity conservation is still poorly understood and requires elaboration. In this study, a faunistic analysis was conducted to survey wild bee (Hymenoptera: Apoidea) and hoverfly (Diptera: Syrphidae) communities present at an urban farm in Bologna, Italy. Two sampling methods were applied; observational plots, which were used to construct plant-pollinator networks, and pan traps, to acquire a broader idea of the diversity of pollinators present at the farm. The structures of plant-pollinator networks were explored to understand the interactions between trophic levels and the ramifications of local pollinator declines. This study involves five years of data, including two years' worth of sampling conducted before the urban farm's establishment. The results underline the necessity for greater focus on non-bee pollinators, in this instance syrphid flies, as providers of pollination services, especially in urban settings where bee declines are drastic. Additionally, syrphids accounted for a third of all flower visitations, suggesting that they can act as safeguards that protect agriculture from bee declines and in some occurrences visit plants not serviced by wild bees. As urban agriculture fosters significant environmental, economic and social value, it is integral to understand the ecosystem services that fundamentally sustain it and determine which conservation strategies to adopt to provide suitable habitats and refuges for pollinators.

KEY WORDS: Apoidea, Syrphidae, urban agriculture, pollination, conservation.



SESSION III

ECOLOGY AND CONSERVATION

Evaluation of the field performance of a research dog for the conservation of the saproxylic beetle *Osmoderma eremita* (Scopoli, 1763) (Coleoptera: Scarabaeidae: Cetoniinae) in relation to the characteristics of its habitat

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Currently, detection dogs are widely used in nature conservation; in relation to insects, they are used to identify pest, alien and harmful species and, more rarely, to safeguard species of high naturalistic value. The ability of dogs to identify their target is usually expressed through the use of descriptors such as accuracy, sensitivity and specificity. In this work, the repeatability of the research results obtained in the field by a conservation detection dog (i.e., a detection dog with a biological target) is tested; moreover, the effect on these results of factors related to the habitat of the target species, the hermit beetle *Osmoderma eremita* (Scopoli, 1763), a protected and priority species included in the annexes of the Habitats Directive, are investigated. The tests were carried out in a protected natural area (Monti Simbruini Natural Regional Park, Lazio, Italy) where the colonization of a large number of trees by this species had previously been ascertained through the work of the dog and where the presence of adults was subsequently confirmed through the use of traps baited with a specific attractant.

Two research repetitions were carried out with the dog on a series of trees randomly selected from those suitable for the presence of *O. eremita* larvae, but in which the possible presence of larvae had not been previously verified. The results of the two tests were compared to verify the degree of repeatability of the performance of the dog; moreover, the possibility of influencing the characteristics of the habitat-trees of the target species was verified (= characteristics of the trees such as the presence and number of cavities, diameter of the tree etc.) also on the obtained accuracy, sensitivity and specificity values.

KEY WORDS: Detection dog, *Osmoderma eremita*, habitat-trees, conservation of saproxylic insects.

SESSION III

ECOLOGY AND CONSERVATION

The Big five: Species Distribution Models from Citizen Science Data as Tool for Preserving the Largest Protected Saproxyllic Beetles in Italy

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Citizen science (CS) projects are defined as scientific research projects that involve the general public in collecting and/or analyzing data. The participation of volunteers in CS projects has increased in recent decades. Indeed, CS has proved to be a powerful tool for facing conservation challenges, from public awareness-raising regarding environmental issues to building scientific knowledge. CS data have been used in quantitative ecology to analyze species ranges by means of species distribution models. Unfortunately, in general, collecting occurrence data on invertebrates, particularly on endangered insect species, can be difficult, due to their life cycle, low detection probability, the great sampling effort needed and, in many cases, the engagement of expert entomologists for species identification. In this regard, citizens' involvement in entomological studies has recently become a pillar of conservation-oriented research.

We selected data on saproxyllic beetle occurrence from two CS projects, LIFE MIPP and InNat, carried out in Italy from 2012 to 2022. Five saproxyllic beetle taxonomic units (TU) were chosen: *Morimus* Brullé, 1832, *Lucanus cervus* (Linnaeus, 1758), *Cerambyx cerdo* Linnaeus, 1758, *Rosalia alpina* (Linnaeus, 1758) and *Osmoderma* Serville, 1828. These five TUs (hereafter the big five) are easily recognizable by the citizen due to their relatively large size and thanks to in-depth sheets for each saproxyllic beetle in the project-related apps and websites. We investigated the Italian distribution of the big five, to describe their niche space, paramount areas for their conservation and conservation gaps.

CS occurrence data from the two CS projects and climate and environmental variables were used to produce Habitat suitability (HS) maps for each species and averaged HS maps. The big five's conservation status was assessed interpolating HS maps with the distribution of protected areas, concomitantly identifying conservation gaps.

The pre-alpine and Apennines arcs, north-eastern Sicily and eastern Sardinia, were identified as conservation's hotspots. Ranking HS levels from minimum to optimal, the extent of conservation gaps decreases as environmental suitability for the big five increases.

For the first time in Italy, CS data have been used to investigate niche space of the largest protected saproxyllic beetles and analyze the distribution of their suitable habitat. The resulting HS raster maps and vector layers, reporting HS value in all Italian protected areas were provided and discussed, reporting an application example for conservation purposes.

KEY WORDS: Cerambycidae, conservation biology, gap analysis, Lucanidae, protected areas, remote sensing, satellite imagery, Scarabaeidae, species distribution model.

POSTER

SESSION III
ECOLOGY AND CONSERVATION

Bumblebees (Hymenoptera, Apidae) as indicators of Key Biodiversity Areas

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Insect populations across the world are being impacted by global change, resulting in declines of various species in different parts of the planet. One way to address this issue is through the implementation of innovative methods like the Key Biodiversity Areas (KBA) approach, which can identify areas with high biodiversity value for insects. This approach involves standardized criteria to identify sites of high biodiversity value, which could accelerate the KBA identification process if applied to numerous species. The knowledge of where vital insect populations reside can strengthen conservation efforts. However, a comprehensive evaluation of the KBA criteria for insect taxa is yet to be conducted, and very little is known about the effectiveness and limitations of this methodology. We applied four KBA criteria to 28 bumblebee species/subspecies in Italy and found potential KBAs for one species and three subspecies. The new KBAs partially overlapped with the existing Italian KBAs and the protected areas network. We also found that the degree of overlap between our identified KBAs and those of vertebrate species was only 12%, suggesting that the KBA network may expand significantly as more species are evaluated. Our findings highlight issues such as data availability and the possibility to apply KBA criteria to subspecies under specific conditions. Large-scale assessments like the one presented in our study will allow a deeper understanding of the true potential of the KBA approach for insect conservation and whether it actually may impede the loss of important units of their remarkable diversity.

KEY WORDS: conservation, bumblebees, Large scale assessment, Key Biodiversity Areas.

SESSION III

ECOLOGY AND CONSERVATION

High inter-individual diversity of the gut microbiota in *Trypocopris pyrenaicus* (Charpentier, 1825) (Coleoptera, Geotrupidae)

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Dung beetles contribute first and foremost to dung removal. Still, they also contribute to an impressive variety of other ecosystem services, and the gut microbiota (i.e., a heterogeneous group including bacteria, archaea and fungi) may be crucial in their role as ecosystem service providers. The taxonomic composition of gut microbiota is shaped by multiple factors including diet, developmental stage and gut morphology, host taxonomy, environment, and mechanism of transmission. These microorganisms play a crucial role in degrading otherwise unpalatable recalcitrant compounds such as cellulose and lignin. However, little is known about the taxonomic diversity and ecological functions these micro symbionts can provide. Moreover, until now, most of the studies focused on lab-reared species, whereas there is a need to study the microbiota of adult individuals in natural conditions.

Here, we describe and compare the taxonomic and functional diversity of bacteria, archaea, and fungi in the gut microbiota of ten wild adult individuals of *Trypocopris pyrenaicus* – a dung beetle species widely distributed across Europe that feeds on faeces of several vertebrate species.

We found marked inter-individual variability in the taxonomic composition of the gut microbiota with multivariate ordination of individuals that changes depending on whether we consider bacteria or fungi. Shared OTUs and families (i.e., core microbiota) are very few. The individuals tested shared 0.25% of the bacterial-archaeal OTUs and 0.50% of the fungal OTUs. They also shared 0.91% and 2.35% of the bacterial-archaeal and fungal families respectively. Among bacteria and archaea, the most represented Phyla are Firmicutes, Proteobacteria, Bacteroidota, Actinobacteriota and Euryarchaeota, although the relative abundances varied between individuals. Among the fungal OTUs, the most abundant phylum is Ascomycota, followed by Basidiomycota.

The most frequently observed bacterial and archaeal functions relate to aerobic chemoheterotrophy, chemoheterotrophy and fermentation, while the fungal trophic categories most found in all individuals are pathotroph-saprotroph and pathotroph-saprotroph-symbiotroph.

The considerable inter-individual variability revealed by our results suggests that when studying the microbiota of dung beetles, many individuals should be considered per species to accurately describe the community and to identify a consistent core microbiota. Furthermore, since fungi or bacteria produce different inter-individual ordinations, we suggest considering both microorganisms in future microbiota studies.

KEY WORDS: dung beetles, gut microbiota, bacteria, archaea, fungi.

POSTER

SESSION III
ECOLOGY AND CONSERVATION

Biodiversity of beetles in urban and peri-urban areas in the city of Gubbio

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In urban environments, intense land management, pollution and the removal of dead or decaying wood often pose a threat to numerous insect species with negative effects on biodiversity. The aim of this work was to examine the diversity of beetles in urban and peri-urban environments in Gubbio (Umbria), with particular reference to the species included in the Red Book of Italian Saproxyllic Beetles.

In particular, four sampling sites were defined to evaluate the different environmental conditions in the urban area. Ranghiasi Park was identified as a reference for the urban environment, two sampling sites, Semonte and Ghigiano, each located close to a cement plant, were identified to evaluate the effect of industrial activities, and a forest area in a rural environment far 7 km from the town and the Semonte site and 15 km from the Ghigiano site.

The capture of adult beetles was performed using 4 window flight traps (WFTs) at each site. The WFTs were positioned approximately 10 m apart from each other. The traps were checked approximately every 30 days for a total of 4 samplings, from June to October 2021.

A total of 605 adult specimens of beetles belonging to 87 species and 27 families were collected. The most abundant species were *Cetonia aurata* and *Protaetia morio* (Cetoniidae), with 94 and 80 specimens respectively. As regards the IUCN risk categories, 4 species are included in the Near Threatened (NT) category, while 21 are in Least Concern (LC). Furthermore, for the species present in the Red Book of Italian Saproxyllic Beetles, the trophic level to which they belong was defined: xylophagous and saproxyllophagous represented 5.7% of the total species sampled, followed by sap-feeder (4%) and predators (2.30%). Two specimens were collected from the mycetophagous category.

The beetle community sampled at the four sites showed differences both in terms of specimens and species collected. In particular, the sites of Semonte and Ghigiano included the majority of specimens collected, while the forest site the largest number of species sampled. The same phenomenon was observed in the Ghigiano site (abandoned oak forest) and in the urban site. In the latter, the trees (mainly holm oaks) allowed the occurrence of a high percentage of species despite the management activities of the urban park. Although direct effects of cement factories were not investigated on insect communities, we hypothesize that the concurrent anthropic activity and release of pollutants have influenced the abundance of individuals and species in the sampling sites. Most of the sampled species, in fact, develop inside the wood of trees and/or in the roots of herbaceous plants which are known accumulators of pollutants.

Thus, native tree species results necessary to promote biodiversity in industrial areas.

KEY WORDS: cement plants, saproxyllic beetles, IUCN, pollution, monitoring.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Exposure, elevation and forest structure predict the abundance of saproxylic beetles' communities in mountain managed beech forests

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The attention of the new EU Forest Strategy for 2030 is focused on developing sustainable strategies to revert biodiversity loss in managed forests. In the managed beech forests of Central Italy (Molise), the diversity of saproxylic species is under threat by intensive management and interventions are needed to revert this negative trend. To evaluate the impact of forestry on the biodiversity of these ecosystems and suggest sustainable management solutions, we analyzed the relationship between abundance of saproxylic beetles and (i) forest stand exposure and elevation, (ii) deadwood availability (coarse woody debris - CWD - and stumps), (iii) abundance of microhabitats. In our study area in the Apennines, four sampling sectors with different altitudes and exposures were identified. Fifteen circular plots (13 m radius) were established in each sector where deadwood and microhabitats were surveyed and saproxylic beetles sampled. We fitted joint species distribution models to quantify the relationship between forest attributes and saproxylic species' abundance, including the interactions with their family and trophic category.

Overall, 2334 specimens, i.e., 64 species of saproxylic beetles, were collected. Both abundance and species richness were higher in the sectors with high elevation (respectively, 55% and 44%) and South exposure (respectively, 28% and 44%). Average deadwood volumes were low (stumps: 7.6 m³ha⁻¹; CWD: 0.3 m³ha⁻¹; snags: 0.4 m³ha⁻¹), and insect galleries were the most abundant microhabitat (380 records over a total of 434). The most important variables affecting abundance were stump characteristics (model deviance = 81.2), elevation (deviance = 64.7), and CWD characteristics (deviance = 58.0).

Our results show that topographical variables and forest structure jointly affect the abundance patterns of saproxylic beetle communities in managed forest ecosystems. These ecological interactions imply that management has different impacts on the saproxylic communities in different topographic conditions. To acknowledge this complexity we advocate for a landscape-level forest management supporting the local beetle diversity maintaining a mosaic of seminatural forest characteristics in different topographic contexts. Our results calls for the application in managed forests of closer-to-nature management targeting deadwood retention, increasing microhabitats and retaining habitat trees in the forest landscape.

KEY WORDS: deadwood, elevation, exposure, forest heterogeneity, Fourth-corner problem, Italy, Joint Species Distribution Models, microhabitats, trophic categories.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

LiDAR data for characterizing forest complexity and beetle and bird diversity in the Vallombrosa Nature Reserve

Francesco Parisi¹, Giovanni D'Amico¹, Elia Vangi¹, Gherardo Chirici¹, Saverio Francini¹, Francesca Giannetti¹, Claudia Coccozza¹, Guglielmo Londi², Costanza Borghi¹, Davide Travaglini¹

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Forest ecosystems with complex structures provide a wide variety of habitats and ecological niches that guarantee high levels of biodiversity.

Remote sensing techniques have been widely used to quantify the heterogeneity and complexity of forest ecosystems. In particular, airborne laser scanning (ALS) by providing three-dimensional information of the forest is the most promising technology for developing accurate models of distribution or diversity of species or groups. This study aims to explore the ability of ALS-derived metrics for monitoring the biodiversity of beetles and birds. The research was carried out in the Nature Reserve of Vallombrosa (Tuscany). Specifically, 47 circular areas with a radius of 13 meters were selected in 2020 in mixed stands of *Abies alba* Mill. and *Fagus sylvatica* L. In each area we carried out beetles monitoring, saproxylic and epixylic microhabitats census, as well as structural surveys. In each area the bird community was also surveyed in a plot of 1.25 ha. The specific richness and the Shannon diversity index were calculated for each area and community. Overall, 10,966 specimens of beetles, belonging to 174 species and 32 families, were sampled in the Reserve. Moreover, 30 species of 15 families of birds were identified. A total of 2573 (1031 ha⁻¹) microhabitats was found. In particular, the saproxylic microhabitats numbered 1820 (729 ha⁻¹), while the epixylic ones 753 (302 ha⁻¹).

The study area was divided into a grid with cells of 23x23 m. In each cell, more than 200 ALS-derived metrics related to the forest structure were extracted. Through the analysis of the correlation between ALS-derived metrics and diversity indices (specific richness and Shannon index), the best predictors for the wall-to-wall estimation of the variables of interest were selected for each community. For each dependent variable, represented by the specific richness and Shannon index for (i) saproxylic and (ii) non-saproxylic beetles, (iii) birds and (iv) microhabitats, a random forest machine learning model was trained with the best ALS-derived metrics. On average the models achieved an RMSE% of 17.5% with an r^2 of 0.22. The models were applied in silver fir and beech forests of the Vallombrosa Reserve for the production of the respective wall-to-wall maps.

KEY WORDS: Biodiversity, Habitat Complexity, Microhabitat, Saproxylic, Remote Sensing.

ORAL PRESENTATION

SESSION III

ECOLOGY AND CONSERVATION

Monitoring *Toumeyella parvicornis* (Hemiptera, Coccidae) infestation in Italian Mediterranean pine forests using Sentinel-2 imagery

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Forests provide many services to society but climate change, biotic, and abiotic forest disturbances are altering ecological systems. Among these, Mediterranean pine forests, distinctive environmental elements of the Italian coastal area for both natural and historical reasons, are particularly susceptible. As evidenced by numerous wind damages, drought stress, and more recently *Toumeyella parvicornis* infestation in central Italy. On the other hand, there is a lack of reliable and spatialized data on the spread of infestations and stress states. Monitoring is crucial for these forests that over time provided goods and services that contributed to the socio-economic development of the coastal area, such as stabilizing dunes, protecting agricultural lands from sea winds, providing wood products, pine nuts, and other non-wood products such as turpentine and truffles. Mediterranean pine forests in Italy cover an area of more than 240 k ha, of which about 65k ha are maritime pine (*Pinus pinaster* Ait.) forests, 48k ha are stone pine (*Pinus pinea* L.) forests, and Aleppo pine (*Pinus halepensis* Mill.) forests account for 115 k ha. In this context of persistent stressors, monitoring forests' health status using all available sources of information is more essential than ever.

In this study, Sentinel-2 optical data are used to monitor the health status and damage that occurred to Mediterranean pine forests in Italy in recent years (2018-2022). The forest disturbance map was constructed exploiting the cloud computing platform Google Earth Engine implementation of the 3I3D unsupervised algorithm, which, through Sentinel-2 photosynthetic activity indices analysis, provides the probability of forest disturbance occurrence for each pixel. In terms of the damaged area, we identified a growing trend over the years (4.5% of Italian Mediterranean pine forests in 2018, 4.0% in 2019, 6.4% in 2020, and 14.6% in 2021), with an abrupt increase in 2022 (24.2%). While our model was calibrated using reference data available for a Mediterranean pine forest study area of about 1000 ha in central Italy and 80% accuracy was reported, more exhaustive reference data should be used for providing solid estimates. On the other hand, Sentinel-2 data proved to be a relevant source of information, pointing to a very serious situation for Mediterranean pine forests.

KEY WORDS: Google Earth Engine, Infestations, Mediterranean basin, Pine forests, Sentinel-2, Tortoise Pine Scale.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Sustainable control of insect pests in Mediterranean agroecosystems

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Agricultural expansion and intensification with the consequent loss of seminatural habitats and the application of agrochemicals, are considered major drivers of biodiversity loss. The reduction in the abundance and diversity of natural enemies inhabiting agricultural landscapes can in fact lead to an ineffective natural control of pests, with potential negative repercussions on production. This study focuses on three important habitats in Mediterranean agroecosystems: vineyards, olive groves and seminatural habitats. The research aims to assess the effect of local habitat and landscape composition on the abundance of natural enemies and on the control of crop pests. We selected 12 agricultural landscapes along a gradient in seminatural habitats. Within each landscape we monitored the three habitats (35 sites in total) measuring the abundance of key phytophagous insects, and the abundance of natural enemies. We also measured predation rates in the field using seed cards and dummy caterpillar. We found both local habitat type and landscape composition to strongly influence biological pest control. High cover of semi-natural habitats in the landscape generally reduced pest occurrence in olive groves, whereas in vineyards the impact of landscape composition depended on pest species. Similarly, habitat and landscape impact on predators depended on taxonomic group. Predation rates were higher in semi-natural habitats compared to vineyards and olive groves, and in landscapes characterized by high cover of seminatural habitats. Our results suggest that supporting biological pest control in Mediterranean agroecosystems necessitates a landscape approach. Moreover, although important for farmland biodiversity, the contribution of semi-natural habitats to biological control might depend on local cropping system, potentially generating trade-offs in heterogeneous landscapes.

KEY WORDS: Vineyard, Olive, Semi-natural habitat, Natural enemies, Pest management, Dummy caterpillar, Seed cards.

ORAL PRESENTATION



SESSION III

ECOLOGY AND CONSERVATION

Diversity of diurnal lepidopterans and coastal dune vegetation: insights from shifting and backdunes of an adriatic protected area (italy).

Micaela del Valle Rasino¹, Andrea Sciarretta¹, Marco Colacci¹, Simone Fattorini², Angela Stanisci¹, Maria Laura Carranza¹

¹ University of Molise, Italy; ² University of Aquila, Italy

Lepidopterans are threatened worldwide by the direct and indirect impacts of human activities. Coastal dune landscapes are characterized by a steep environmental gradient, and many insects are associated with specific sectors of the dune zonation.

We investigated communities of diurnal lepidopterans in two different zones (the shifting dune and the backdune) in the Natura 2000 site Foce Trigno-Marina di Petacciato (N2K IT7228221; Central Adriatic coast, Italy), which is included in the Long-term Ecological Research Network (LTER IT20). Lepidopterans and vascular plants were sampled in three transects perpendicularly to the seashore, from the driftline to the backdune, according with LTER protocols. Lepidopterans were sampled during day-time using entomological nets following the European Butterfly Monitoring Scheme (eBMS). Data were collected every 15 days (from April to October 2022) in the shifting dune and the backdune of each transect. Vegetation sampling was carried out following a random stratified protocol on 4x4m plots (total 30 plots) distributed in the shifting dunes and the backdunes. In each plot, the relative abundance of each plant species was recorded using the Braun-Blanquet scale. We compared Lepidopteran communities of shifting dunes and backdunes by calculating richness, Shannon diversity and Buzas and Gibson's evenness indexes followed by a Mann Whitney test. We also compared the respective species-abundance distributions by using rank abundance curves. Finally, we explored beta diversity (turnover) by using Whittaker index and identified the association between lepidopteran species and dune zonation by cluster analysis. The same analyses were performed for vegetation data.

Our results showed the presence of distinct plant and lepidopteran communities across the dune zonation. Concerning Lepidoptera, we recorded 93 individuals, belonging to 19 species and 6 families. The diversity of diurnal lepidopterans responded to the dune zonation with significantly higher values of richness, Shannon diversity and evenness in the backdunes. The rank abundance curves were adequately fitted by a geometric series, as expected for simple communities in harsh and stressful environments. Some diurnal lepidopterans were sampled exclusively on the shifting dunes (3 taxa) and the backdunes (7 taxa), respectively. For instance, *Gegenes nostradamus*, a xerothermophilous taxon typically associated with well-preserved environments, was exclusively found on the shifting dunes. On the other hand, on the backdunes no typical taxa of Mediterranean maquis were found, but mostly sciophilous species. Concerning vegetation, we also registered a steep zonation, with communities hosting a greater richness on the backdunes and a strong turnover of unique species for each zone.

By exploring the links between plant and lepidopteran communities, this study offers new insights for the prioritization of conservation efforts in these fragile ecosystems.

KEY WORDS: alpha diversity, beta diversity, butterflies, conservation, LTER network, Natura 2000, rank abundance curves.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

The importance of coastal dune mosaics as reservoirs of moth (Lepidoptera, Heterocera) diversity in Central Italy (Molise)

Micaela del Valle Rasino¹, Andrea Sciarretta¹, Marco Colacci¹, Simone Fattorini², Angela Stanisci¹, Maria Laura Carranza¹

¹ University of Molise, Italy; ² University of Aquila, Italy

Lepidoptera are good indicators of environmental variability and play a key role in ecosystem functioning. Although most of lepidopterans have good dispersal capabilities, their larvae tend to have quite restricted food niches, thus establishing strong links with their host plants and habitats. Moth communities are very sensible to variations in local plant communities, yet little information is available on the Lepidoptera - vegetation relationships in coastal dune landscapes.

We explored the relationship between the diversity of nocturnal lepidopterans and coastal dune vegetation in the Natura 2000 site Foce Trigno-Marina di Petacciato (IT7228221), which is located along the Adriatic coast of Central Italy, and is part of the Long-Term Ecological Research network (LTER IT20).

Moth and plant communities were sampled along three transects perpendicular to the seashore from the driftline to the backdune, as specified by the LTER protocol. Moths were sampled using light traps equipped with UV LEDs every 15 days (December 2021 - November 2022) on shifting dunes and backdunes. Vegetation was sampled using a random stratified protocol on the shifting dunes and backdunes. We sampled thirty 4x4m vegetation plots (five for each dune sector and transect). For each plot we recorded the complete list of vascular plants along with their relative abundance according to the Braun-Blanquet scale. For shifting dunes and backdunes we calculated the alpha-diversity (richness and Shannon index) of moths and we compared the different dune sectors using a Mann Whitney test. The same analyses were performed for vegetation data. We explored beta diversity (turnover) by Whittaker index and identified the association between moths and dune zonation by cluster analysis.

Concerning Lepidoptera, a total of 652 individuals belonging to 110 taxa (9 families) were recorded. Noctuidae were the most represented family, with 55 taxa and 300 individuals (46%), followed by Geometridae, with 17 taxa and 96 individuals (15%). Alpha-diversity of moths did not present significant differences between shifting dunes and backdunes. However, moth assemblage composition differed substantially between the two sectors of the dune zonation, with 23% of taxa found only on the shifting dunes and 26% of taxa occurring exclusively in the backdunes. Regarding vegetation, we also evidenced a strong zonation and a high turnover species for each sector. The exclusive presence in shifting dunes of *Mythimna riparia* Rambur, 1829 and other oligophagous species feeding on Poaceae highlights the strong relation of moth communities and plant species distribution due to nutritional niches. On the other hand, species feeding on Mediterranean maquis species (e.g. *Pistacia lentiscus* L. and *Asparagus acutifolius* L.) such as *Zebeeba falsalis* Herrich-Schäffer, 1839 exclusively occur on backdunes, confirming a similar relationship.

The observed characterization of Lepidoptera community in different dune habitats suggests their potential use as bioindicators of habitat conservation status and their important contribution to global biodiversity of these ecosystems.

KEY WORDS: Adriatic coast, bioindicators, dune landscape, Lepidoptera, moth's communities, plant diversity.

POSTER

XXVII ITALIAN NATIONAL CONGRESS OF ENTOMOLOGY

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SESSION III

ECOLOGY AND CONSERVATION

Preliminary investigations on the Orthoptera communities of the Piedmont Apennines xeric meadows

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Orthoptera constitute a key taxonomic group for the study of biodiversity in natural and agricultural environments. Indeed, they respond rapidly to the intensification of agricultural management or habitat change through changes in species diversity and composition and at the same time they provide useful ecosystem services. Although many orthoptera communities of north-western Italy (Piedmont, Liguria, Val d'Aosta) have already been studied (mainly from a taxonomic point of view), there is little knowledge on the communities inhabiting the xeric meadows of the Ligurian-Piedmont Apennines, especially in terms of abundance and distribution. Therefore, in this research we investigated the orthoptera community of the Piedmont Apennines with a particular focus on the species present in the SCI "CALANCHI DI RIGOROSO, SOTTOVALLE E CARROSIO" and in the adjacent areas. The research was conducted in the dry meadows of a hilly area in the Alessandria Province, in two stations (4 sampling areas) in the municipalities of Arquata Scrivia and Gavi Ligure. The samplings were carried out from May to July for 11 sessions along 16 10x2m transects crossed with a grass net. All Orthoptera have been classified at the subfamily level and where possible at the species level. In total, 3500 specimens belonging to 16 subfamilies were collected. Although the Kruskal Wallis test and the Mann-Whitney U test did not showed significant differences in terms of richness and diversity of subfamilies between the stations and the areas investigated, PERMANOVA and the cluster analysis indicated that the composition of the Orthoptera communities based on the abundance of subfamilies is homogeneous within each area with a degree of similarity greater than 80% but varied significantly between the two stations and between the areas of each station. The subfamilies that contribute to the dissimilarity between the two stations investigated are Tettigoniinae, Oecanthinae and Saginae for Arquata and Gomphocerinae for Sottovalle. However, within the Arquata station, the three investigated areas differed significantly in terms of the subfamilies present. Despite the short distance between the three areas of Arquata, each of them is distinguished by the significant abundance of some subfamilies. In one area the subfamilies Saginae and Calliptaminae dominate. In the other two areas, the subfamilies Phaneropterinae and Gomphocerinae respectively dominate. Since the geographical distance does not influence the dissimilarity in terms of subfamilies composition between the investigated areas (geographical distance: $\beta = -2.249$; $p = 0.302$; $R^2_{\text{conditional}} = 0.275$), it is probable that the microclimatic conditions and the surrounding landscape characterized by an extensive wooded areas play a decisive role in the composition of the orthoptera communities of the area.

ORAL PRESENTATION

SESSION III
ECOLOGY AND CONSERVATION

Relative contribution of cultivated and semi-natural environments to the conservation of beta-diversity of moths in an organically managed olive grove (Lepidoptera)

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The presence of a mosaic of diversified habitats contributes to the increase of beta-diversity that is greater as differences among habitats increase. Basically, a diversified landscape inhabits a higher diversity of species. Particularly important is the role of semi-natural patches in agricultural landscape in supporting a high beta-diversity which contributes to the ecosystem functionality. In the case of Lepidoptera, the presence of diversified habitats support the availability of a wide trophic resources for both larvae and adults. However, not all landscape elements contribute equally to beta-diversity. In this paper we investigated the relative contribution of cultivated and semi-natural areas to the diversity of nocturnal lepidoptera observed in an organically managed olive grove. The study area is located in the municipality of Marcellinara, Catanzaro province, at an altitude of about 200 meters. The farm can be subdivided in two areas. One area, situated at a slightly lower altitude, is relatively cooler and more humid because of a small stream around which hygrophilous vegetation develops. The second area, situated at a slightly higher altitude, is relatively dryer and warmer and delimited by an escarpment covered by a degraded Mediterranean maquis and small oak coppices. Four pairs of sampling sites consisting of a semi-natural site and the contiguous olive grove at a distance of about 50 meters were settled-up. In each of them a UV LED light trap was placed from July 2018 to June 2019 and activated simultaneously once per month. Overall, 334 species and 7,566 individuals were sampled. A quantitative Cluster Analysis (Bray-Curtis) showed the presence of two groups of communities which correspond to the semi-natural habitats and to the olive groves. As expected, the greatest diversity and abundance was found in semi-natural areas. In fact, a nestedness analysis showed that in almost all the investigated site pairs communities of olive groves are nested in those of semi-natural habitats. The only exception is due to the lack of a tree cover in a semi-natural site. However, olive groves hosted a significant percentage of exclusive species, i.e. species and individuals not registered in semi-natural sites. Overall, the cultivated areas contributed for a percentage between 8 and 24% to the overall species richness which corresponds to a percentage between 3 and 31% of individuals. We confirmed the fundamental importance of semi-natural areas in supporting the diversity of a group of pollinators such as nocturnal lepidoptera, and highlights a non-negligible contribution of cultivated areas to the whole beta-diversity likely thanks to the organic management of the farm.

KEY WORDS: agro-ecosystem, biodiversity, Lepidoptera.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Areal vs. linear: the effects of landscape configuration and forest structure on composition and diversity of black alder macromoth communities (Lepidoptera)

Stefano Scalercio¹, Federica Fumo¹, Rosario Turco¹, Vincenzo Bernardini¹, Laura Bevacqua¹, Margherita Lombardo²

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The landscape configuration and the forest structure assume more and more importance as determinants of animal communities which respond in a diversified manner according to their biological and ethological characteristics and the types of landscape and forest under consideration. This paper is focused on nocturnal lepidoptera inhabiting eight alder woodlots in the Sila National Park, Calabria region. Woodlots were divided into linear and compact according to their horizontal structure within a short buffer. The aim of the study was to understand the role of forest structure and landscape in determining the composition of nocturnal lepidopteran communities at different observation scales. To test differences of abundance and richness of moth communities between the two types of alder woodlots, we used Mann-Whitney test for medians, Shannon diversity, equitability and Fisher's indices. Furthermore, the nestedness test (NeD) was performed to verify if the linear alder communities were nested in the compact ones. As expected, communities inhabiting linear woodlands have significantly less species and less individuals. Correspondence Analysis based on species abundance showed that nocturnal moth communities are mostly clustered according to small-scale woodland typology. The only exception was a compact woodlot showing a community composition similar to those of linear woodlots due to the lack of tree cover horizontal continuity. *Orthosia incerta* was one of the most abundant species within compact sites, a highly variable species that inhabits mesophilous deciduous forests. On the other hand, *Agrotis cinerea* was very abundant within linear sites, usually considered characteristic of mountain meadows. The study also showed a negative correlation of forest age and tree diameter with the number of species because the largest trees are often those in isolated specimens along the rivers. The correlation of the percentage of forested area with abundance and composition of communities is positive at 50 and 200 m buffers, while it is absent at smaller and larger buffers. To understand how riparian ecosystems work is crucial for their management facing the expected climate change. Our results demonstrated that a width of 50 m may not be sufficient to give proper functionality to the wooded area, at least for nocturnal moth communities, e.g. planning afforestations on larger surfaces.

KEY WORDS: Landscape ecology, biodiversity, Sila National Park.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

***Zeuneriana marmorata* and its conservation: past, present, future (Orthoptera: Tettigoniidae)**

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Zeuneriana marmorata (Fieber, 1853) is listed as EN (Endangered) by IUCN in the Red List of European Orthoptera. Current populations are in NE Adriatic coast in Italy, inland in Slovenia and one recently discovered in Italian inland.

These are assumed to be remnant sub-populations of a larger metapopulation living in the wet habitats of the plains around the North Adriatic Sea. Causes of range fragmentation are supposed and current threats outlined. Regarding Italian populations, study (bioacoustic) or conservation (translocation, area management) actions applied to date and planned for the future are explained.

Above all, the translocation was useful in establishing a new Friulian sub-population in the locality of Schiavetti (Monfalcone).

KEY WORDS: *Zeuneriana*, conservation, translocation, bioacoustics.

SESSION III

ECOLOGY AND CONSERVATION

**Effect of sublethal dose of neonicotinoid insecticide in the behavior of solitary bee
Centris analis(Hymenoptera, Apidae)**

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Pollinator insects, such as bees, are exposed to pesticide residues present in field. Most studies focus on assessing the lethality of these compounds to bees; however, little is known about the sublethal effects and the pesticide consequences on pollination and nesting activities. The present study aimed to evaluate the effect of the neonicotinoid insecticide acetamiprid on the behavior of the solitary bee *Centris analis*. The experiments were conducted under laboratory conditions with 12 males and seven females of *C. analis* per experimental group, namely: i. control, in which the bees received a sucrose solution at 35% m/m; ii. Solvent control, referring to bees fed with sucrose solution containing acetone at the same concentration used to dilute the insecticide; iii. Insecticide, in which the bees consumed a sucrose solution containing 7 ng/μL of acetamiprid. The bees were exposed ad libitum for 48 hours. After this period, all bees were fed with sucrose solution at 35% m/m. For behavioral evaluation, the bees were transferred individually to an arena 30 cm long in Y format containing three stimuli: a light source in the center, a feeder in one arm of Y, and in the other a wooden block to simulate the natural nesting environment. The individuals were filmed for 15 minutes at 48 hours and 96 hours after the beginning of the test. The videos were analyzed using the EthoVision XT software to evaluate the distance moved, speed, frequency and time spent in each zone of the arena. At 48 hours, males exposed to the insecticide were lethargic with speed and distance moved six times lower than non-exposed males ($p = 0.006$). Among the stimuli offered, the light source was the bees' first choice, however, the exposed males took 17 times longer to reach the light source than the control bees ($p = 0.03$). Females were more tolerant to the insecticide than males, showing a nine-fold reduction in average speed ($p = 0.03$) compared to the control, but without changes in other parameters. After 96 hours, bees of both sexes did not show behavioral changes, indicating that within 48 hours after exposure, motor functions were re-established. The obtained results showed that the insecticide in residual concentration can impair the locomotion of the bees. Despite the recovery of symptoms, this experiment was conducted in laboratory conditions, so the bee had an easily accessible food source that may have contributed to its recovery. Under natural conditions, the observed effects may facilitate the predation of these individuals and interfere with the population dynamics of *C. analis*.

KEY WORDS: Locomotion, Acetamiprid, Pollinators.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

fAIR PROJECT: Functional Artificial Intelligence and Robotics for Agro-Ecosystem Services and Food Production Assurance

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¹ University of Naples Federico II, Italy; ² Institute for Sustainable Plant Protection The Italian National Research Council Piazzale Enrico Fermi, Italy

Application of new future technologies based on Artificial Intelligence (AI) and robotics in the management of ecosystem services is essential for obtaining quality of agricultural productions. The importance of pollinators in agriculture and ecology is well known, so much so that it is estimated that the annual worldwide economic value of pollination of agricultural production amounts to around 260 billion euros. To meet the need for pollination services, it is necessary to have healthy bee and bumblebee populations, protecting them from disease, lack of food and parasites. The general aim of this project is to obtain, by integrating the multilayer and multidimensional data with A.I. and robotic approach based, an aggregate index of ecosystem services to develop DSS at farm level. This general objective can be broken-down into the following specific objectives: i) Design and development of a robot (flowerbot) for laboratory based assays to study in detail behavior and interaction of pollinators with specific flowers and Volatile Organic Compounds (VOCs) blends; ii) use and calibration of the commercially available sensors for in vivo agro-ecosystem (open-field and greenhouse) applications; iii) integration of laboratory and in vivo based data to develop and running algorithms for DSS application of specific crop ecosystem services and production. Here, the interaction between *Apis mellifera* L. (Honey Bees) and citrus plantations in open fields in Metaponto (Basilicata) and between *Bombus terrestris* and tomato plants in greenhouses in Torre del Greco (Naples), were studied. In particular, the apiaries involved in this study were equipped with remote sensors to measure, in real time, relevant parameters relating to hive conditions, such as hive weight, bee sounds, temperature, humidity inside the hive and external weather conditions. The integration of the data from the above-mentioned parameters recorded by the sensors thus provides information on the state of the colony, its interaction with its surroundings and the influence of climatic conditions. At present, the research carried out has produced a series of preliminary data with reference to the year 2022.

KEY WORDS: Artificial intelligence, remote sensing, ecosystem services.

SESSION III
ECOLOGY AND CONSERVATION

Frequencies of ant spiders during year round survey from two sites of vineyards in Northern Israel

Ittai Warburg

Haifa University, Israel

In this research, two sites of vineyards in Northern Israel were surveyed for the diversity of invertebrates: Geshur vineyard (32.755°N; 35.714°E; 366m ASL), and Ramat Magshimim vineyard (32.813°N; 35.806°E; 406m ASL). From these vineyards, invertebrates were collected by pitfall-traps during the year of 1998 usually monthly. Those invertebrates were separated to different systematical orders between the years 1998-2000. The Arachnids that were collected in that survey were identified between the years of 1999-2000 in the National Nature Collections at the Hebrew University of Jerusalem, Givat-Ram Campus, Jerusalem. From the genus *Palaestina*, the following frequencies were found during the year of 1998. From Geshur vineyard: in March – 1, in May – 2, in June – 37, in July – 20, in August – 32, in September – 1, in October-November – 2, in December – 1. In Ramat Magshimim vineyard, ant spiders of the genus *Palaestina*, were not found during the same period. From the genus *Zodarion*, the following frequencies were found during the year of 1998. From Geshur vineyard: in May – 5, in June – 7, in July – 10, in October-November - 2. From Ramat Magshimim vineyard: in June – 1, in August – 1, in October-November – 2. It is obvious, that most of the ant spiders in both of those vineyards were found during the summer season. This finding can be related to that the main food of ant spiders are ants, mainly harvester ants, which are active on the ground surface mainly during the summer. The findings, that during summer 1998, the frequencies of ant spiders were prominently bigger from Geshur vineyard than from Ramat Magshimim vineyard, together with that the genera richness of ant spiders was bigger in Geshur vineyard than in Ramat Magshimim vineyard, can be related to that about 100m-200m south of Geshur vineyard there was or is an area of natural Mediterranean shrub, while Ramat Magshimim vineyard was or is located in between cultivated areas. It is known, that vicinity of agricultural fields to natural areas may increase species diversity, species richness or frequencies of animals and plants in those agricultural fields. These natural habitats can be therefore sources of animals, like spiders, which can act as biological controllers to pests of agricultural crops. Therefore, it is recommended to place agricultural fields near natural habitats, in order to control pests and to increase the ecological stability in those areas.

Acknowledgments

Dr. Rakefet Sharon did the field work in this project. I would like also to thank hereby the National Nature Collections at the Hebrew University of Jerusalem in Givat-Ram Campus, Jerusalem, for identifying the spiders that were caught in that project. This work was funded between the years 1998-2000 by a grant from the Foundation of Regional Research and Development of the Ministry of Science and Technology of the State of Israel.

KEY WORDS: Ant spiders, Northern Israel, vineyards.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

Frequencies of harvesters (Arachnida: Opiliones) during year round survey from two sites of vineyards in Northern Israel

Ittai Warburg

Haifa University, Israel

In this research, two sites of vineyards in Northern Israel were surveyed for the diversity of invertebrates: Geshur vineyard (32.755°N; 35.714°E; 366m ASL), and Ramat Magshimim vineyard (32.813°N; 35.806°E; 406m ASL). The average annual precipitation in Geshur vineyard is 500mm-600mm; in Ramat Magshimim vineyard: 600mm-700mm (according to Israel precipitation map: lib.cet.ac.il). From these vineyards, invertebrates were collected by pitfall-traps during the year of 1998 usually monthly. Those invertebrates were separated to different systematical orders between the years 1998-2000. The Arachnids that were collected in that survey were identified between the years of 1999-2000 in the National Nature Collections at the Hebrew University of Jerusalem, Givat-Ram Campus, Jerusalem. From Geshur vineyard the following frequencies of harvesters were found during the year of 1998. In January >13, in February >25, in March - 2, in May - 4, in October-November >5, in December >3. From Ramat Magshimim vineyard the following frequencies of harvesters were found during the year of 1998. In January - 19, in February - 10, in March - 1, in May - 8, in October-November >10, in December >5. It is obvious, that most of the harvesters in both of those vineyards were found during the rainy season. This finding can be related to that the main food of harvesters are springtails, which need humidity for their activity, and therefore are active on ground surface mainly during the winter. The finding that during winter 1998, the frequencies of harvesters were bigger from Geshur vineyard than from Ramat Magshimim vineyard, can be related to that about 100m-200m south of Geshur vineyard there was or is an area of natural Mediterranean shrub, while Ramat Magshimim vineyard was or is located in between cultivated areas. It is known, that vicinity of agricultural fields to natural areas may increase species diversity, species richness or frequencies of animals and plants in those agricultural fields. These natural habitats can be therefore sources of animals, like harvesters, which can act as biological controllers to pests of agricultural crops. Therefore, it is recommended to place agricultural fields near natural habitats, in order to control pests and to increase the ecological stability in those areas.

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KEY WORDS: Arachnids, harvesters, vineyards.

POSTER

SESSION III

ECOLOGY AND CONSERVATION

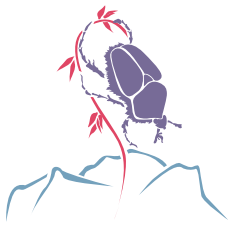
Long term monitoring of nocturnal macrolepidoptera in a suburban area of south Italy: results from 2019 to 2021

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Long-term monitoring programs (LTMPs) are increasingly demanded to facilitate early detection of ecosystem changes as a result of habitat alteration and to study their relationship to climate change. Most active LTMPs focus on measuring abiotic and vegetation parameters, but continuous fauna monitoring can highlight changes occurring in species composition and abundances of indicator taxa providing information on habitat alterations at varying spatial and temporal scales. In addition, long-term monitoring programs provide data useful to assess abrupt changes in animal communities, but also changes in communities occurring as a response to slow ecological trends. Detection of phenological changes is facilitated by daily sampling, as weekly or monthly sampling cannot show small shifts. In addition, daily monitoring facilitates the detection of rare species of conservation interest, the observation of migratory events, and the detection of alien species. In 2019, two light traps for nocturnal macrolepidoptera monitoring were installed at the experimental farm of the Research Center for Forestry and Wood in Rende, Italy. The traps were placed in a site located in the middle of the Mediterranean Basin, where climate warming is stronger than elsewhere, and in a suburban area with increasing anthropogenic pressure. They were activated every night, with few exceptions, and after two years a total of 12,164 individuals belonging to 297 taxa were detected. Qualitative-quantitative analysis of communities showed high similarity with those observed in other cultivated areas in Calabria with a Mediterranean climate. The presence of small remnants of riparian forests contributed significantly to the community composition, as the finding of hygrophilous species demonstrated. Interesting was the presence of *Xanthia ocellaris* and *Leucania obsoleta*, never found in Calabria before, and *Utheteisa pulchella*, which periodically migrates to Southern Europe from North Africa. The timespan encompassed during this study was enough to add 177 species to the Crati Valley fauna, but was too short to capture population changes in response to environmental changes. Nevertheless, our results provided a solid basis for studying ecological dynamics occurring in an area subjected to anthropogenic pressures and climate change acting in the short and long term, respectively.

KEY WORDS: Lepidoptera, Calabria, Climate change.



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Session IV

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Current knowledge and new data on the Pterophoridae (Lepidoptera) of the Calabria region (Italy)

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(CREA) Council for Agricultural Research and Economics, Research. Centre for Forestry and Wood, Italy

In the large grouping of microlepidoptera, the Pterophoridae are easily recognizable due to numerous peculiar morphological characters that characterize the members of this family. The Italian pterophorid fauna includes just over one hundred species, fairly well distributed in the different regions from north to south, but with large areas of the territory still to be investigated, especially in the southern portion.

This contribution reports the current knowledge on the Pterophoridae of the Calabria region and adds further faunistic data from recent field surveys carried out in the years 2013-2020. In particular, the surveys concerned the mountains of the Pollino, Sila, Serre and Catena Costiera Paolana.

The species of pterophorids currently known for Calabria are 21; these were 13 until the revision of Prola & Racheli (1984), but in the last twenty years the number has increased with numerous species reported in contributions made for the knowledge of the regional fauna (Baldizzone & Scalerchio, 2018; Bonelli et al., 2021). In particular, data of significant faunistic and biogeographical interest are those of *Merrifeldia garrigae* Bigot & Picard, 1989 (new for Italy), *Stenoptilodes taprobanes* (Felder & Rogenhofer, 1875) (new for southern Italy), *Agdistis tamaricis* (Zeller, 1847) and *Agdistis satanas* Millière, 1875 (first records for Calabria).

Among the unpublished data presented here on the recently found Pterophoridae in Calabria, several species belonging to the genera *Agdistis*, *Amblyptilia*, *Calyciphora*, *Capperia*, *Oxyptilus*, and *Pterophorus* which confirm single records from the middle of the last century, or which increase the diffusion of those species reported only recently for this region. Furthermore, we report the first data for mainland Italy of *Lantanophaga pusillidactyla* (Walker, 1864), an allochthonous species of neotropical origin whose larva has *Lantana camara* L. (Verbenaceae) as main host plant, reported exclusively in Sicily (Bella & Marchese, 2007), and expanding in the Mediterranean area.

KEY WORDS: Lepidoptera Pterophoridae, new data, Calabria region.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Contribution to the knowledge of South Italian Gelechiidae (Lepidoptera: Gelechioidea)

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The microlepidopteran fauna of southern Italy is poorly explored and the number of known species has been significantly increased only in recent years. For example, the family Gelechiidae has about 359 species in Italy and only 35 of these are known from Calabria. The highly fragmented faunistic and taxonomic knowledge needs a great sampling effort and specific studies. This work aimed to partially fill the gaps in this family by providing unpublished data that significantly enlarged the known range of many of the considered species. The study is based on 79 specimens belonging to 39 species collected in Calabria and Basilicata and deposited in the collections of the Bavarian State Collection of Zoology, Germany, and in the Laboratory of Wildlife Management and Forest Biodiversity of the Research Centre for Forestry and Wood, Italy. In Calabria sampling was carried out using light traps equipped with UV LED light, while in Basilicata the used light sources were different as the collection techniques. Identifications were carried out using available iconography and dissection of genitalia in more difficult cases. The DNA barcoding technique allowed us to confirm species identifications in most cases providing the first sequences from southern Italy, and to establish how some populations are divergent from others found in Europe and northern Italy. Such are the cases of *Mirificarma cytisella* and *M. eburnella*, which are divided into five and two Barcode Index Numbers, respectively, in their European range. From a faunistic point of view, 23 species are new for the investigated regions, and six for southern Italy: *Mirificarma maculatella*, *Scrobipalpa acuminatella*, *Caryocolum tischeriella*, *Caryocolum herwigvanstaai*, *Teleiodes italica* and *Carpatolechia decorella*. Very interesting were the findings of *Aproarema cinctelloides*, new for the Italian fauna, and *Cosmardia moritzella*, new for the peninsular Italy and known for very few alpine localities. In addition, *Aroga pascuicola*, *Oxypteryx immaculatella* and *Helcystogramma lamprostoma* are new for the fauna of continental Italy.

KEY WORDS: biodiversity, fauna, microlepidoptera, DNA barcoding.



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Integrative taxonomy of the soil-dwelling harvestman genus *Scotolemon*, with the discovery of a new endemic species from Sardinia (Opiliones: Phalangodidae)

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Scotolemon Lucas, 1861 is a genus of soil-dwelling harvestmen inhabiting the deciduous forests of Mediterranean Europe and Northwestern Africa. In Italy, it is represented by two species, *Scotolemon doriae* Pavesi, 1878, which seems to be widespread in the country (including Sicily and Sardinia), and *Scotolemon terricola* Simon, 1872, recorded from Sardinia. Nevertheless, a thorough investigation of the taxonomy, distribution, life cycle and ecology of these harvestmen has never been conducted. In the perspective of such a revision, we started sampling specimens from the lesser explored regions of the country and to investigate species boundaries within an integrative taxonomy framework. This approach considers the external and internal morphology (including male genitalia), molecular data (DNA barcoding) and chemical analyses of glandular secretions by gas chromatography coupled with mass spectrometry (GC-MS). The preliminary results include the discovery of a new putative endemic species from Sardinia, which shows affinities with *Scotolemon lespesi* Lucas, 1861 from Eastern Pyrenees. In addition to the traditional lines of evidence, the delimitation of these two taxa is supported by chemical analyses, which show differences in the molecular structure of the main alkylphenol characterizing the secretions.

ORAL PRESENTATION

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

***Exaireta spinigera* (Wiedmann, 1830) (Diptera: Stratiomyidae) in Calabria**

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Exaireta spinigera (Wiedemann, 1830) (Diptera, Stratiomyidae), an alien species native to Australia, introduced in Hawaii and North America, is rapidly spreading throughout Europe also due to anthropic activities. In the European territory, it was found for the first time in 2008 in France and later in Belgium, Italy (Lazio and Calabria) and Russia. Information on the biology of *E. spinigera* is limited, although the species is often found in association with another invasive species belonging to the family Stratiomyidae, *Hermetia illucens* (Linnaeus, 1758). The two species are both saprophagous and their diffusion is connected to human activities, mainly to the presence of decomposing organic matter. Contrary to what reported in the literature about the distribution of the species, considered restricted to urban environments, in Calabria *E. spinigera* has been found in both urban and natural environments, from the coastal level up to about 1200 meters above sea level, in beech and pine forests. The preliminary results of the study on the distribution of *E. spinigera* in Calabria provide additional data in comparison to those reported on the “Citizen Science” platform (<http://www.naturamediterraneo.com>), and highlight a plasticity in the choice of habitat by the species, which also includes mountain natural environments.

KEY WORDS: natural environments, urban environments, Calabria, Stratiomyidae.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

First record of *Zelus renardii* (Kolenati, 1856) (Hemiptera: Reduviidae) from Calabria

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The species *Zelus renardii* (Kolenati, 1856) (Hemiptera: Reduviidae) was found for the first time in Calabria on the Campus of the University of Calabria (Rende, Cosenza). The discovery occurred on January 2023 at the Laboratory of General and Applied Entomology of the Department of Biology, Ecology and Earth Sciences. The species was reported for the first time in Italy in 2013 in Lazio and subsequently in Puglia, Sardinia, Campania, Liguria and Sicily. *Zelus renardii* is a Nearctic species, native to North and Central America, which in recent decades has shown a clear and rapid expansion in other biogeographical areas of the world, including the entire American continent, Asia and Oceania. The species is entomophagous and considered a generalist predator, although some data demonstrate its stenophagy on a small group of insects; it has been reported in agricultural environments but also in urban and peri-urban areas. *Zelus renardii* has been observed actively preying on phytophagous species, including *Philaenus spumarius* L. 1758 (Rhyncota: Aphrophoridae) and *Drosophila suzukii* Matsumura, 1931 (Diptera: Drosophilidae).

The feeding plasticity characterized by stenophagy in some environments and by generalist predation in others makes this species a model of interest in the field of biocontrol, possibly also in the management of important pests of the olive tree, such as *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae). Moreover, considering the tendency of *Z. renardii* to prey on *P. spumarius*, it appears an interesting species for reduction of infections by *Xylella fastidiosa* Wells et al., 1987 (Xanthomonadales: Xanthomonadaceae).

KEY WORDS: biological control, Calabria, entomophagous predator, Reduviidae.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Mitochondrial markers applied to the study the genetic of populations of the Antarctic springtail *Kaylathalia klovstadi*

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Previous studies have identified glaciers as a major dispersal barrier for springtails in Antarctica, with high genetic divergence between populations separated by glaciers. In Victoria Land, Tucker Glacier is a very important dispersal barrier, as observed for *Friesea propria* and *F. gratae* (Carapelli, 2020). Tucker Glacier itself was identified as an important dispersal barrier for *Kaylathalia klovstadi* by gene structure analysis based on the mitochondrial marker *cox2* (Frati, 2001). In a 2019 paper, Collins analysed the genetic diversity using the mitochondrial marker *cox1* of three *Kaylathalia klovstadi* populations northern of the Tucker IceGlacier, paving the way for future analyses of the DNA barcoding fragment southern of this icegeographical barrier. Our analyses tested the effectiveness of the Tucker Glacier as a biogeographic barrier, as well as Whitehall Glacier and the sea (nomemareCOI)marine channel between Coulman Island and the Hallett Peninsula. Specimens identified as *K. klovstadi* were collected during the Italian National Antarctic Programme (PNRA) from six sites in Terra Victoria. The whole genome was extracted from at least 10 specimens from each of the six populations sampled, for a total of 62 individuals. Two mitochondrial fragments (*cox1* and *atp6*) were analysed and combined into a supermatrix. The resulting sequences were collapsed into haplotypes. The haplotypes were subjected to phylogenetic analyses leading to the visualisation of a phylogenetic tree and analyses of genetic variability by p-distance, haplotype network, haplotype and nucleotide diversity, pairwise differences (θ_P) and segregating sites (θ_S). To assess the weight of individual biogeographic barriers in the analysis of molecular variance (AMOVA), populations were forced into groups according to three hypotheses: I) Northern and Southern Tucker Glacier; II) Northern and Southern Whitehall Glacier; III) Closeness of populations in the haplotype network and phylogenetic tree. Comparison of the percentages of variance among groups and among populations, both among themselves and between the different hypotheses, shows a greater value of diversity among groups in hypothesis III) than in I); it would therefore appear that Tucker Glacier is not the main barrier to dispersal of this species. Carapelli, Antonio, et al. "Molecular comparison among three Antarctic endemic springtail species and description of the mitochondrial genome of *Friesea gretae* (Hexapoda, Collembola)." *Diversity* 12.12 (2020): 450.. Frati, F., Spinsanti, G., and Dallai, R. (2001). Genetic variation of mtCOII gene sequences in the collembolan *Isotoma klovstadi* from Victoria Land, Antarctica: evidence for population differentiation. *Polar Biol.* 24, 934–940. doi: 10.1007/s003000100302

KEY WORDS: *Kaylathalia klovstadi*, collembola, springtail, *cox1*, *atp6*, population genetic, dispersal barrier, genetic divergence.

POSTER



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Synopsis and novelties on the Heteropterofauna of Sardinia

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Due to the rich diversity of its insects and the great variety of its natural environments Sardinia has always attracted the attention of Italian entomologists. The history of our knowledge of its Heteroptera, commonly called true bugs, has spanned for almost two centuries, since the description of *Pachycoris caudatus* Burmeister, 1835 (Scutelleridae). The most relevant contributors were M. Spinola (1837, 1840) who reported 29 species, describing 13 as new, A. Costa (various papers between 1864 and 1886) who listed 203 additional species, Ferrari (1878, 1888) who added 76 more taxa, Singer & Mancini (1938) and Singer (1940) who mentioned 88 new records, Servadei (1952, 1967) who recorded 88 species as new for the island, Tamanini (various papers between 1947 and 1980) who added 8 species describing six as new, Carapezza (1977, 1984) who listed 12 additional species, Cianferoni (2011) who provided new data and a checklist of Gerromorpha, Nepomorpha and Leptopodomorpha. The knowledge accumulated so far was summarized in the most recent Checklist of Italian Heteroptera (compiled by Carapezza, Cianferoni and Faraci in 2021) which lists 557 species from Sardinia, in addition to 38 doubtful species; the previous Checklist of Italian Heteroptera (Faraci & Rizzotti Vlach listed 534 species and 45 doubtful records. Recent field research carried out on the island by A. Carapezza and L. Loru, and the study of specimens preserved in private and public collections led to the identification of 55 species never recorded from Sardinia, 6 of which proved to be undescribed species (4 belonging to Miridae and 2 to Rhyparochromidae). New records and descriptions of new species will be published soon in a series of papers in preparation. In the light of these results the total number of Heteroptera known to live in Sardinia rises to 589.

Considering also still unpublished data, Sardinia hosts 19 endemic/subendemic Heteroptera (species/subspecies): 12 Sardinian endemic taxa (SARD) of which 5 not yet described, 6 Sardo-Corsican endemic taxa (SACO), and 1 Sardo-Corso-Balearic endemic taxon (SCBA).

In particular: 1 SACO in Veliidae (Gerromorpha); 2 SACO in Corixidae, 1 SCBA in Micronectidae, and 1 SACO in Nepidae (Nepomorpha); 1 SARD in Saldidae (Leptopodomorpha); 9 SARD (3 of which are undescribed) and 1 SACO in Miridae (Cimicomorpha); 2 SARD (undescribed) in Rhyparochromidae and 1 SACO in Cydnidae (Pentatomomorpha).

An interesting comparison can be made with Sicily, an island of comparable size (25,832 km² vs. 24,100 km² of Sardinia). Considering only the endemic species of each island (a subspecies for Sicily is here excluded) and also including the smaller islands, we know 9 endemic species of true bugs for Sicily and 12 (considering also those being described) for Sardinia. Therefore, Sardinia has 33% more endemic species than Sicily (which has even smaller islands which are larger in surface and even more distant from the main island compared to Sardinia). Most of the endemic species belong to the family Miridae (7 spp. in Sicily vs. 9 spp. in Sardinia).

KEY WORDS: Endemisms, first records, Hemiptera, Heteroptera, insular fauna, new species.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

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

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Ticks are obligate hematophagous ectoparasites feeding on vertebrate hosts, that are widely distributed around the World. From an ecological point of view, ticks play a crucial role in population dynamics and their distribution is influenced by a wide range of ecological parameters. To date, research regarding ticks feeding on wildlife in Italy is scarce, especially concerning species with little or no medical or veterinary relevance. Aim of this three-year project is improving knowledge concerning tick species present in Latium (Italy) for the creation of an Atlas of diversity, distribution and host range. Here we present the preliminary data collected during the first sampling season from May to September 2022. Twenty-three Natural Reserves and three CRAS (Centro Recupero Fauna Selvatica) in the Region were visited, where ticks were collected both from the field and directly on wild hosts. Field sampling was carried out using a white wool blanket both on herbaceous vegetation (“dragging method”) and shrub vegetation (“flagging method”), collected ticks were preserved in 70% Ethanol. For the collection of specimens for this project, in addition to the sampling activity, we were supported by the collaboration of the staff from natural reserves, CRAS, veterinary doctors, local farmers and hunters. Ticks were identified to species by morphology according to different keys for Italian and European species. In total 208 specimens were collected, belonging to 5 genera, and 8 species, namely *Dermacentor marginatus*, *Hyalomma detritum*, *H. marginatum*, *Haemaphysalis punctata*, *Rhipicephalus bursa*, *R. sanguineus*, *R. turanicus*, and *Ixodes ricinus*.

KEY WORDS: Ticks, Checklist, Latium, Italy.

POSTER

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Importance of the naturalistic collections for investigations on environmental changes: the Odoardo Pirazzoli entomological collection as case study

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Naturalistic collections are very important for the study of the environmental changes that have occurred over time. For the Imola area, the Coleoptera collection by Odoardo Pirazzoli (1815-1884), conserved, and partly on display at the "Giuseppe Scarabelli" Museum of Imola, is of considerable interest, also from this point of view. The collection, started in 1840 and carried on by Pirazzoli for over forty years, is one of the largest in Italy at the time. It comprises 27,698 dry specimens, divided into 152 boxes and almost all determined. The collection also includes the lectotype of *Leptomastax hypogea* (Coleoptera, Staphylinidae, Scydmaeninae), a new genus and a new species described in 1855 by Pirazzoli on samples collected in the surroundings of Imola near the bank of the Santerno river.

Pirazzoli's is the only historical entomological collection in Romagna and, while not lacking in specimens from other Italian and foreign areas, it largely includes samples collected in Imola and its surroundings. A comparison with the current situation is thus possible. The first surveys were carried out in the Acque Minerali Park, which extends between the right bank of the Santerno river and the hillsides and is one of the places in Imola most closely connected to the history of the city. These surveys seemed to indicate a decrease in the most demanding species in terms of environmental quality. For example, among carabids (which are present in 32 boxes and constitute a conspicuous part of the collection), *Nebria psammodes* (P. Rossi) (of which there are 4 specimens in box 13) was found by Pirazzoli along the Acque Minerali Stream, as reported in his unpublished notes, kept in the municipal historical archive of Imola: "Luoghi umidi ed arenosi sul Rio delle Acque. Imola 12 aprile" (= "Wet and sandy places on the Acque Stream. Imola 12 April..."). This species has no longer been found in this place, probably due to environmental changes, i.e., the disappearance of sand and gravel from the riverbed and water pollution. A similar impoverishment concerns cerambycids, such as *Cerambyx welensii* (Küster) (= *C. velutinus* Brullé) (box 135), collected in Imola (again based on Pirazzoli's notes) in 1858 in trunks of *Quercus robur* L. (= *Quercus pedunculata* Ehrh.). The species, once common in the Acque Minerali Park and in the Tozzoni Park of Imola, no longer seems to be present in these areas, as well as in other zones. In fact, it is included in the list of the species protected by the Regional Law 15/2006 "Provisions for the protection of the minor fauna in Emilia-Romagna", as it is threatened by the reduction of its living environments.

Investigations on other specimens of the collection and on the rich Pirazzoli's correspondence (which largely includes unpublished material) are currently in progress. The aim is to find useful information for the study of entomological and environmental continuity in the Acque Minerali Park and, in general, in the Imola area.

KEY WORDS: entomological collections, Odoardo Pirazzoli, environmental changes, Imola area.

POSTER



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Microevolutionary processes in a species introduced for the biological control of grasshoppers: the case of *Mylabris variabilis* (Pallas, 1782) (Coleoptera, Meloidae) in Sardinia and Corsica

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Mylabris variabilis is a beetle of the family Meloidae, tribe Mylabrini, widely distributed from the Iberian Peninsula to Central Asia. The hypermetabolic development takes place inside the oothecae of Acrididae Orthoptera. For this biological feature, in 1946 the Italian Ministry of Agriculture promoted the introduction of *M. variabilis* in Sardinia aiming to reduce the swarm of grasshoppers that was devastating the local crops. 7000 individuals were collected from a population of Latium (Santa Maria di Galeria), northwest of Rome, and were then released in several sites around the island. Ten years later the same procedure was carried out in Corsica with individuals from Provence. Since then, *M. variabilis* has successfully colonized both islands enlarging its range of distribution, but more importantly there hasn't been a massive grasshopper invasion ever since, except some less widely spread phenomenon. Since 2019, the problem has resurfaced in central Sardinia causing great damages to the local agriculture. Having many detailed information about the introduction of this species (year of release, number of individuals collected, areas of capture and release) it would be interesting to investigate the presence of microevolutionary processes that might have led, in these almost 80 years, to the significant accumulation of genetic mutations in the introduced populations. Furthermore, it might be possible to study whether the expansion pattern and the demographic trend of the parasitoid could be related to grasshopper swarms. For this purpose, samples of Sardinian and Corsican populations of *M. variabilis* were collected and genetically compared with samples from Latium and Provence. Preliminary analyses were conducted with the mitochondrial gene COI (Cytochrome Oxidase I), which, however, does not show significant differences between the native populations and those introduced. The COI marker is widely used in population genetics studies but doesn't seem to be adequate to highlight the presence of possible polymorphisms in such a short period of time. Future analyses will therefore be directed towards the use of NGS (Next Generation Sequencing) techniques which, at a finer scale, will allow to amplify the genome more consistently, to identify polymorphisms in regions with a higher mutation rate.

KEY WORDS: blister beetles, parasitoidism, Orthoptera, COI.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

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

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The presence of stink bugs (Hemiptera: Pentatomoidea) in South Tyrol has increased in recent years, not only in cultivated areas, but also in urban areas. These species are often phytophagous, very mobile and polyphagous, feeding on cultivated, wild and ornamental plants. Among the main natural enemies there are egg parasitoids, which in some cases can reach high levels of parasitization, favoring the regulation of stink bug populations. South Tyrol is a mountainous region, rich in woods and where apple production covers the main valleys up to 1,100 m a.s.l. To better understand the abundance and distribution of these species and their parasitoids, an extensive monitoring program was carried out in 27 sites, different in terms of habitat type and altitude range. Monitoring methods included visual inspection and beating sheet for adults and nymphs, and further visual inspection for egg mass collection. Field surveys were monthly performed at each site, from April to September.

During field surveys, nearly 600 stink bug adults and nymphs were recorded, while 164 egg masses were collected. Greater diversity and abundance of species were found in forests and urban areas compared to orchards, with up to 10 different bug species at one site. Overall, similar numbers of individuals were observed at different altitudes, but species abundance varied with both altitude and habitat type. The two predominant species found during the investigations were *Pentatoma rufipes* and *Halyomorpha halys*, an exotic species that has recently invaded areas of northern Italy and quickly spread all over the country. The first one was more abundant in urban areas and in woods at higher altitudes, while the second one was more present in urban areas and in orchards in the valley, at lower altitudes. Stink bug egg masses were mainly found in urban areas, while only a few were collected in orchards. Eight species of parasitoids belonging to the Scelionidae, Eupelmidae and Pteromalidae families emerged from the collected eggs. Parasitization rate was higher in forests and urban areas, where the abundance of individuals was also higher.

The results of this study add important information on the distribution of stink bugs and their egg parasitoids in South Tyrol. Moreover, they can be usefully exploited for planning the management of crops present in the area in the event of stink bug population outbreaks.

KEY WORDS: Hemiptera Pentatomoidea, field survey, parasitoids.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Genetic structure of *Stenostoma rostratum* (Fabricius, 1767) (Coleoptera, Oedemeridae), a specialist of coastal dune ecosystems.

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Stenostoma rostratum is a beetle of the family Oedemeridae, whose genus includes three other species: *S. cossyrense*, *S. lowei*, *S. melitense*. While the latter are all generalist, *S. rostratum* is a specialist, halophile species and strictly related to dunal ecosystems. At the same time, it has a wide and naturally fragmented range, including almost all the Mediterranean coasts, and some areas along the North Atlantic coasts (in Morocco, Portugal, Spain and France). It has been hypothesized that *S. rostratum* has a transmarine dispersion. While it's not clear if oviposition and larval development occurs on roots and stems of dune plants, they have been demonstrated to take place in driftwoods stranded on the shoreline. As a result, the wide distribution of *S. rostratum* could be related to this peculiar reproductive strategy, relying on woods transported by the currents, acting as floating rafts promoting the dispersion towards new habitats. In this work we reconstruct the phylogenetic relations between some Western Mediterranean and Atlantic populations of *S. rostratum*. The main objective of this research is to define if the fragmented distribution is reflected in the genetic structure of the species, with genetic drift events and loss genetic variability typical of small and isolated populations; or if the assumed transmarine dispersion can contrast isolation, promoting genetic homogeneity. Some preliminary analyses have been conducted using the mitochondrial marker COI (Cytochrome Oxidase I). Overall, most of the observed haplotypes were shared among populations, even those geographically distant, and no signal of genetic isolation was recovered. This result suggests the presence of gene flow between populations, supporting the transmarine dispersion hypothesis. *S. rostratum* does not seem to be exposed to a genetic impoverishment, even if strictly related to a highly threatened ecosystem. In order to confirm these results, we will perform more thorough analyses using genomic data, with an enlarged sampling including also Eastern Mediterranean populations of *S. rostratum*.

KEY WORDS: Phylogeography, Transmarine dispersion, Mediterranean, conservation.



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

The genus *Chelidura* Latreille, 1825 in the Alps, between complexity and climatic risk (Dermaptera, Forficulidae, Anechurinae).

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Up to recent years, the genus *Chelidura* Latreille, 1825, has been treated, as far as Western Europe is considered, in a very confused and contradictory way. The composition of the genus itself, which according to many authors also includes the species now assigned to the genera *Chelidurella* Verhoeff, 1902, and *Mesochelidura* Verhoeff, 1902, has only recently been defined thanks to the revision, mainly on a molecular basis, of the genus *Chelidurella*. Regarding the species attributed to the genus *Chelidura* of western Europe, the Italian entomologists who had dealt with them (Felice Capra and Augusto Vigna Taglianti above all) had a fairly clear picture, assigning the populations of the Alps (France, Italy and Switzerland) to *Chelidura aptera* (Megerle in Charpentier, 1825), those of the Pyrenees (France and Spain) to *Chelidura pyrenaica* (Bonelli in Gené, 1832), and those of the Massif Central (France) to *C. pyrenaica arverna* David & Van Herrewege, 1973. Nevertheless, the European bibliography has very often confused these species and above all their distribution. The main problem for a systematic study on the genus *Chelidura* is represented by the fact that the type species of the genus, *C. aptera*, had a rather vague *Locus typicus*, namely Europe, and that no type material is preserved. The recent designation of a Neotypus for *C. aptera* and molecular-based analyses have finally allowed to conduct a more detailed study of this genus in Western Europe, demonstrating that it is composed of a single species in the Pyrenees (*C. pyrenaica*) and another, independent one on the Massif Central (*C. arverna*), confirming the previous approach of the Italian authors. Regarding the Alpine populations, these studies highlighted a much more complex composition. Some populations are therefore in an advanced stage of description as new species or will be re-evaluated, based on both molecular and morphometric analyses. The application of morphometric analyses, besides a more reliable interpretation of molecular data, is needed because the extraction of DNA from collection specimens is not always possible and the collection of new specimens from populations known from the literature or from collection material is difficult. Recent, intense, and repeated research carried out in well-known localities has in fact given negative results, suggesting an effect of recent climatic changes which could have considerably modified the high-altitude habitats to which the alpine species of the genus *Chelidura* have always been associated.

KEY WORDS: Dermaptera, Alps, taxonomy, climate change.

ORAL PRESENTATION



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

The wild bees of Rome: biodiversity and pollination networks in the Roman nature reserves

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Italy is a biodiversity hotspot for wild bees (Hymenoptera, Apoidea). In fact, more than 1000 species grouped in 6 families are reported in our country, about half of the species reported in all of Europe. These insects play multiple ecological roles, but the best known is certainly their crucial role in pollination. Large urban green areas, with their peculiar characteristics compared to environments immersed in more natural matrices, delivers a reservoir of biodiversity for this important group of insects.

The first results obtained during the first year of sampling of this project focused on the study of pollinator insects in Rome are described here. The objectives of this study are: 1) to describe the wild bee communities within the city of Rome in terms of species richness, composition and morphological and functional diversity; 2) to compare the pollination networks between urban sites and the natural areas surrounding the city to evaluate their alimentary preferences in an urban environment; 3) to produce an updated checklist of Apoidea and their phenology for Rome and to verify if this city represents a biodiversity hotspot for wild bees; 4) to contribute to the creation of a reference collection of Apoidea for the city of Rome. The samplings were carried out from March to October 2022, once every month; a total of 15 fixed linear transects were carried out distributed in 7 nature reserves belonging to the RomaNatura network within the urban area of Rome and in 3 sites in the Appia Antica Regional Park. All samples were collected using an entomological net, prepared dry and determined on the bases of their morphological characters. This first phase of the project led to the identification of 39 genera of Apoidea and the reporting of a new species for Italy. All the approximately 2000 wild bees sampled were collected during their foraging phase on the wild flowers along the transects. All plants visited by bees at the time of capture were also determined, for a total of more than 80 genera of plant species. By cross-referencing the floristic data and those deriving from the identification of wild bees, it will be possible to understand the food preferences of the different species of Apoidea and draw a profile of the pollination networks in urban nature reserves. This information can also be used to provide guidelines on urban green management in order to protect or even increase the diversity of Apoidea in large cities.

KEY WORDS: wild bees, pollination networks, urban ecology.

POSTER



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

World-wide invasion of *Popillia japonica* reconstructed based on complete mitochondrial genomes and nuclear snp markers

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Popillia japonica is a coleopteran of substantial economic interest, as it can attack numerous wild and cultivated plant species. Native to Japan, the pest has invaded large areas of the USA, Canada, Azores, Northern Italy and Ticino, and it is considered a priority for control in the European Union. We determined the complete sequence of the mitochondrial genome in 86 individuals covering the entire distribution of the species and developed a panel of 295'396 biallelic unlinked nuclear single nucleotide polymorphisms (snps) in 83 individuals based on full genome resequencing. Phylogenetic analysis supports a primary division between South Japan and Central/North Japan, with all invasive samples coming from the latter. The origin of invasive USA samples is incompatible, time-wise, with the introduction of a single lineage, with multiple Japanese lineages having been introduced and only one accounting for most of the population expansion. Colonization to Italy and the Azores are supported as two separate invasions stemming from the USA population, and samples from Ticino appear as an expansion from the Italian population. Within major areas, snp analysis identifies some substructure in Japan and between islands in the Azores, whereas the USA and Italian+Ticino populations are characterized by extensive mixing and no substructure. Demographic analysis identifies a population expansion followed by a period of contraction prior to the invasion. The arising scenario is compatible with previous reports and adds time and demographic dimensions to available reconstructions. Full results of the mitochondrial datasets and preliminary analyses of the nuclear snp dataset are presented.

KEY WORDS: *Popillia japonica*, colonization, invasion, mitochondrial genome, snps

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Heroic viticulture and arthropod biodiversity in Capraia Island (Tuscany, Italy)

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Heroic vineyards describe traditional landscape management in typical impervious mountain or small island areas, characterized by steep slopes, impediments to mechanization, and subjected to hydrogeological risk. Capraia Island has a long viticulture background started since ancient Romans (i.e., 'palmenti'), but it has been only recently recovered through the restoration of dry-stone walls of the historical terracing and the establishment of new vineyards with various cultivar (e.g., Cannonau, Vermentino, and Ansonica). The risks of unsustainable soil management practices can limit the contribution of soil organisms to ecosystem services (e.g., nutrient cycles, pH alteration, low humidity, thus affecting vine health and productivity). Within the framework of the 'European Directive on the Conservation of Biodiversity', this study is included in a System Action of 5 Italian National Parks to evaluate the level of biodiversity of the terraced agroecosystem by using a set of environmental indicators.

In October 2022, to evaluate soil biodiversity of Capraia Island vineyards, 10 organic vineyards were investigated in comparison with 1 abandoned terraced vineyard with Mediterranean shrub vegetation (used as control). In each investigated sites, three soil samples were collected in the inter-row at 10 cm depth, considering inter-row tillage, cultivar, altitude, and vineyard age. Soil biodiversity was studied by edaphic microarthropod indices and soil biological quality by QBSar index. The total abundances of microarthropods were correlated with the main soil physico-chemical parameters (e.g., total organic carbon, TOC; pH; electrical conductivity; total nitrogen; assimilable phosphorus; texture; and moisture). The year 2022 was particularly dry, with rainfalls 55% lower than the average climate (350 mm vs. 640 mm).

The investigated soils are particularly rich in sand component (>50%) and texture varies from Loam (L) to Sandy-Clay Loam (SCL). In tilled inter-rows, soil moisture and TOC are very low (<2%), except in the control site.

Over 9400 microarthropods were collected. The most abundant group is mites (74%), followed by springtails (17%). The other arthropods identified constitute low relative frequency (8%). The highest QBSar value (189) was recorded in the control, followed by irrigated vineyards and those covered with grasses in the inter-rows. The total abundance of microarthropods were positively correlated with TOC and negatively with the silty soil component ($p < 0.05$). Conversely, soil moisture does not significantly influence arthropods which are largely composed by xerophilic or mesophilic species. Moreover, in two sites, the recent new plantation (2 years) negatively impacted the abundances and biodiversity.

These preliminary results contribute to better understand the impact of restoration terraced vineyards on soil properties to adopt heroic agriculture model, based on the maintenance of natural soil fertility and the reduction of chemical inputs. Improving soil functional biodiversity in areas particularly dry could favor this sustainable agriculture model based on integrated approach focused on site-specific agro-ecological and regenerative strategies, that preserve the soil physical, chemical, and biological interactions and the ecosystem services of the terraced landscape.

KEY WORDS: terraced vineyards, QBSar, agro-ecological and regenerative strategies, micro-habitat.

ORAL PRESENTATION



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Contribution to the knowledge of the water beetle fauna of the “Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere e Gorgo del Drago” Nature Reserve (Coleoptera)

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The Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago Nature Reserve extends for 7,399 hectares in the municipalities of Corleone, Mezzojuso, Godrano, Marineo, and Monreale, province of Palermo (north-western Sicily). The protected area includes the Rocca Busambra mountain chain and the forest areas of Bosco del Cappelliere and Bosco della Ficuzza. Wetlands are mainly characterized by streams, ponds, and lakes, mostly seasonals.

The aim of the project is to collect data on the occurrence, distribution, habitats, and state of conservation of aquatic beetles, considering the lack of knowledge and the obsolescence of several data in the literature. During the field investigations, 32 species belonging to 7 families of water beetles were recorded; four genera and 10 species were not known for the protected area, increasing the species currently known from 59 to 69. The faunistic and ecological data collected so far represent a basis for defining conservation measures for species and habitats, some of the latter protected under the Habitats Directive 92/43 EEC.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

**Contribution to the knowledge of the Apoidea of the Pollino National Park
(Hymenoptera: Apoidea)**

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Apoidea, like all pollinator insects, are in decline due to continuous exposure to pesticides, habitat destruction and climate change, which often act synergistically, amplifying their effects. However, knowledge about their diversity, distribution and conservation status are still poor in much of Europe. The aim of this study was to expand the basic knowledge of Apoidea in the Pollino Massif, which is representative of many of the environments found in Calabria and Basilicata regions. The study area is entirely included in the Pollino National Park. Sampling was carried out from July to October 2022, along 66 transects, two-thirds of which in semi-natural environments and the remainder in biological and conventionally managed agricultural areas, following the ISPRA-University of Turin protocol. Transects were 250 metres long and were located over an altitudinal gradient ranging from 200 to 2,200 metres. During sampling, the abundances of Apoidea were assessed dividing them in 3 groups: *Apis mellifera*, *Bombus* and other Apoidea. We collected 186 specimens, identifying 41 species of which 22 species were never recorded for the Calabrian and/or Lucanian fauna. Particularly interesting was *Nomada argentata*, included in the IUCN Red List of Threatened Italian Bees. The number of new records for the two regions was high, also considering the very short sampling period. New species for the Lucanian fauna are: *Bombus campestris*, *Nomada argentata*, *Colletes abeillei*, *Hylaeus angustatus*, *H. imparilis*, *Lasioglossum leucozonium*, *L. morio*, *Heriades crenulatus* and *H. rubicola*. New species for the Calabrian fauna are: *Andrena pellucens*, *Hylaeus dilatatus*, *Halictus langobardicus*, *Lasioglossum albipes*, *L. calceatum*, *L. corvinum*, *L. cupromicans*, *L. laeve*, *L. morio*, *L. nigripes*, *L. xanthopus*, *Lithurgus cornutus*, and *Megachile versicolor*. Collected data, although partial, represent an important starting point to delineate the diversity of Apoidea in the Pollino National Park and suggest how much more remains to be discovered in the area.

KEY WORDS: Bees, Pollinators, Calabria, Basilicata, Conservations, Distributions.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

The spreading of two alien predators in Sardinia (Italy): *Zelus renardii* and *Nagusta goedelii* (Hemiptera Heteroptera Reduviidae)

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Zelus renardii is a species of assassin bug (Hemiptera Heteroptera Reduviidae) native to Central and North America and spread in many areas of the world, including Europe, where it is rapidly expanding especially in the Mediterranean part. In Italy this species was recorded for the first time in 2013 and since then it has spread to almost the entire peninsula, including the islands. It was reported in the southern part of Sardinia in 2020.

Nagusta goedelii is another species of reduviid occurring from Central Asia to South-eastern Europe. At least from a century it has been spreading westwards, in Central and Southern Europe. Also in Italy this species is constantly expanding since its first report in 2007. The species was recorded in 2020 in the northwestern part of Sardinia.

Both species have been found in 2022 in Sassari (northern Sardinia) in sites monitored for the invasive alien bug *Halyomorpha halys* within the project “USEit — Utilizzo di sinergie operative per lo studio e la gestione integrata di specie aliene invasive in Italia” financed by the National Research Council of Italy (CNR). The occurrence of *Z. renardii* is reported for the first time for northern Sardinia and that of *N. goedelii* confirmed in the same area through new collected material.

In the north of the island the two reduviids, both generalist predators, were observed in co-occurrence with the pentatomid *H. halys*. In fact, *N. goedelii* is potentially capable of preying even the eggs and first instar nymphs of *H. halys* and it is not excluded the same for *Z. renardii*.

If this interaction was confirmed, the consequences need to be carefully evaluated. Even if predation on allochthonous invasive species may appear positive at first sight, the bioceneic relationships that two generalist alien predators will establish with the native species are still unpredictable.

KEY WORDS: alien species, distribution, introduction, invasive species, Mediterranean.

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Bioacoustics in Orthoptera research: environmental recorders and the emblematic case of *Acheta pantescus*

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During some research on pelagic birds in the circumsicilian islands in 2022, environmental recorders were used to record the sounds emitted by the pelagic birds throughout the night. During this research in some coastal locations on the island of Pantelleria, the sound of a hitherto unknown cricket was recorded on several occasions, which turned out to be a new species, *Acheta pantescus* Massa, Cusimano, Fontana & Brizio, 2022. The interesting aspect was not only the randomness of the finding, but also the method used to intercept the cricket, which made it possible, on an island much explored from an entomological point of view, to discover a new species with truly elusive habits that is very difficult to detect during the day. It is therefore proposed that these environmental recorders be used to carry out nocturnal searches in particular environments or to try to rediscover species of orthopterans with nocturnal or even diurnal habits that were thought to have disappeared or were only known to have been found by chance in remote times. The recorders are controlled by an application on a smartphone that allows recording only at night or only during the day or at defined times (e.g. half an hour every hour from sunset to sunrise).

KEY WORDS: Bioacoustics, Orthoptera, Pantelleria, cryptic species.

POSTER

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Biodiversity in agroecosystems in Calabria

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Biodiversity needs to be conserve in both natural and agricultural ecosystems, since it plays a key functioning role, such as pollination or pest control. The decline of biodiversity in agroecosystems is largely determined by intensive agricultural techniques but also by the introduction of non-native species and the use of chemical pollutants. With respect to insect, the intensification of agriculture is the main factor leading to declines in diversity and abundance: the progressive intensification of agroecosystems, the emergence of monoculture with the elimination of hedges and wooded areas and the use of pesticides are some of the factors that have reduced insect diversity and abundance. For all these reasons is important to monitor biodiversity and to protect and enhance the presence of insects in agroecosystems. The agroecosystems of Calabria are set in peculiar landscape contexts dominated by ecotones of natural and semi-natural vegetation, creating a mosaic of natural habitats intermixed with cultivated areas. They represent a unique traditional, seminatural agroecosystem to be valued and protected.

A research project is currently in progress and its aim is studying and safeguarding entomological diversity in this agroecosystem located in the Sila plateau (Calabria, Southern Italy). The agroecosystems intended for the cultivation of the Sila IGP potatoes are investigated for the first time in order to identify species of insects of agronomic interest, phytophagous, antagonists of harmful insects and to verify the state of health of pollinator populations linked to the Sila potatoes production. The investigated sites are all inside a transect at about 1200 m a.l. and there is a biological agroecosystem of Sila potatoes IGP, an ecotonal grazing area and a further agroecosystem of Sila potatoes IGP with integrated potatoes management. The sampling of insects and other arthropods is still ongoing and it is carried out in each area with the help of indirect methods: pitfall traps, pan traps with different colours and Malaise traps. Insects are identified on a morphological basis with the use of specialized dichotomous keys. The focus is to create the first checklist of arthropod species of agricultural interest within the Sila potatoes agroecosystem and to identify the main potato pests and evaluate the density of their population.

There are several groups currently investigated and the most abundant genera are *Hirticomus* Pic, 1894 (Coleoptera, Anthicidae), *Calathus* Bonelli, 1810 (Coleoptera, Carabidae), *Oedemera* Olivier, 1789 (Coleoptera, Oedemeridae), *Bibio* Geoffroy, 1762 (Diptera, Bibionidae), *Drosophila* Fallen, 1823 (Diptera, Drosophilidae), *Empis* Linnaeus, 1758 (Diptera, Empididae), *Deraeocoris* Kirshbaum, 1856 (Hemiptera, Miridae), *Camponotus* Mayr, 1861 and *Tapinoma* Forster, 1850 (Hymenoptera, Formicidae), *Andrena* Fabricius, 1775 (Hymenoptera, Andrenidae), *Bombus* Latreille, 1802 (Hymenoptera, Apidae) and *Halictus* Latreille, 1804 and *Lasioglossum* Curtis, 1833 (Hymenoptera, Halictidae).

KEY WORDS: Agroecosystem, Conservation, Coleoptera, Diptera, Hemiptera, Hymenoptera.

POSTER



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Contribution to the faunal study of the Neuropterida of Monte Prinzerà
(Apennines, Province of Parma)

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During research on the lepidoptera fauna of the Monte Prinzerà Oriented Nature Reserve, carried out above all using light traps in the years 2003-2005, a few dozen specimens of Neuropterida were also collected, the study of which was completed only recently. The resulting faunal outline, object of this work, aims to provide an initial contribution to the census of the neuropteroid insects of the area. Monte Prinzerà is located on the northern side of the Tuscan-Emilian Apennines. The protected area, included in the municipal territories of Fornovo di Taro and Terenzo (Province of Parma), is located along the ridge that separates the valley of the Taro river from that of the Sporzana stream, its right tributary, and covers varying altitudes of between 290 and 736 m a.s.l. The naturalistic peculiarity of the district consists particularly in the presence of two ophiolitic outcrops accompanied by an impermeable belt of scaly clays: the larger of the two outcrops constitutes Monte Prinzerà proper, while the other, much smaller, is Prinzerolo. Alongside a prevalence of arid environments linked to the characteristics of the ophiolitic rocks, at the foot of the outcrops there are wetlands. There is therefore considerable variety in the biotopes and microhabitats, with a great diversity in the vegetation: from barren stony ground to high grasslands, from mesophilous meadows to woods of downy oak. The result is a high level of biodiversity, including that of insects. The choice of four collection stations took into account the different herbaceous, shrubby and tree plant formations which characterize the different environments. Most of the Neuropterida were collected with light traps; only a few specimens were collected using other methods (net; direct search for larvae). Given non-optimal conservation conditions, the identification of some specimens captured with traps was limited to the genus. The collected material included the two orders Raphidioptera and Neuroptera, respectively with 1 family and 1 genus, and with 6 families and 17 genera: Raphidiidae (*Dichrostigma*); Coniopterygidae (*Semidalis*, *Coniopteryx*); Mantispidae (*Mantispa*); Hemerobiidae (*Drepanopteryx*, *Megalomus*, *Wesmaelius*, *Hemerobius*, *Micromus*); Chrysopidae (*Nothochrysa*, *Chrysopa*, *Chrysoperla*, *Pseudomallada*); Myrmeleontidae (*Myrmeleon*, *Euroleon*, *Macronemurus*, *Neuroleon*); Ascalaphidae (*Libelloides*). Among the finds of greatest faunal or biogeographical interest - partly due to the current scarcity of data for Italy as a whole or for peninsular Italy - we note: *Coniopteryx (Holoconiopteryx) renate* Rausch & H. Aspöck, 1977, E-Mediterranean xero-thermophilic species, linked to the genus *Quercus*, already known as present in Italy in Basilicata, Calabria and Sicily; *Mantispa aphavexelte* U. Aspöck & H. Aspöck, 1994, Central Asian-Mediterranean xero-thermophilic species, which seems to find its northern limit of Italian distribution in the Tuscan-Emilian Apennines; *Drepanopteryx phalaenoides* (Linnaeus, 1758), Asian-European species with wide ecological valence, associated above all with shrubby and arboreal broad-leaved trees, and which on the contrary seems to have in the Tuscan-Emilian Apennines its southern limit of distribution in Italy. Further research, to be carried out above all with the usual collection methods with a net (mowing; shaking-beating of the fronds), as well as by sight, will allow us to expand the overall picture outlined above.

KEY WORDS: Tuscan-Emilian Apennines, Province of Parma, Raphidioptera, Neuroptera, geographical distribution, light traps.

POSTER

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

**Increasing the knowledge on the butterflies of the Pollino National Park
(Lepidoptera: Hesperioidea, Papilionoidea)**

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The pollinators play a key role as support to the reproduction of many angiosperms, which in turn support a high biodiversity both directly and indirectly. In addition, flowering plants are fundamental for the production of many foodstuffs for human needs.

In recent years the health status of pollinator lepidoptera populations has deteriorated dramatically. The factors contributing to this decline are many: changes in land use, environmental pollution, invasive exotic species, pathogens and climate change. The synergic effect of these factors amplifies their negative consequences on pollinators, which find themselves “attacked on several fronts”, decreasing their adaptive capacity and resilience. These pressure factors reach their maximum on isolated populations at the limit of the geographical and ecological distribution of species, as is often the case for relict populations in mountain areas. The Pollino Massif is the southernmost refuge area for many species of Lepidoptera. The Ministry of the Environment and Energy Security has launched a research programme aimed at increasing knowledge of Apoidea and Lepidoptera in National Parks. In this poster we report some data that increases knowledge on the distribution of butterflies in the Pollino National Park by sampling 66 transects from June to October 2022. Transects cover several types of natural habitats along a very strong altitudinal gradient ranging from 200 to 2,200 meters. One third of them covered cultivated fields and within each crop type an organic and a conventional transect was settled-up. We used the ISPRA protocol 330/2020. Butterflies were sampled along 500 meters long transects, walking slowly and with a constant going and recording individuals flying inside an imaginary box 5x5m large in front of the operator. We found 91 species, three included in the Habitat Directive annexes (*Phengaris arion*, *Parnassius mnemosyne*, *Melanargia arge*) and one included in IUCN Red List of Italian butterflies (*Polyommatus galloi*). Additionally, we confirmed the strong reduction of *Erebia cassioides* distribution, now restricted to cacuminal grasslands, and found even at 1,400 meters of altitude 48 years ago. This monitoring program increased the knowledge on the butterfly fauna thanks to the sampling of geographic areas never surveyed before.

KEY WORDS: biodiversity, pollinators, conservation

POSTER

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

A critical review of the Sicilian Odonatan checklist and bibliography

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The impetus of new field research has allowed a greater knowledge of the Odonata of Sicily, thus laying the foundations for the creation of the first regional atlas of this group, under the auspices of the National Biodiversity Future Center (NBFC – Spoke 3). In this direction, the necessary first step is that of a critical review of the literature. This contribution presents a reasoned check-list of the Odonata of Sicily based on the papers published between 1840 and 2022. Overall, 61 species have been confirmed (of which 6 remain in the list even if with doubts) among those present today and in the past in Sicily. These represent 64% of the species recorded in Italy and 43% of the species recorded in Europe. A series of factors (lack of specimens in the collections, taxonomic gaps from the past, etc.) interlace, making it difficult to ascertain the presence of different entities. Among other things, to the observations of naturalists who until the 1970s reported taxa that are now dubious or synonymised by modern literature, is added the ever greater destruction of the natural aquatic habitats of Sicily. Some species reported in ancient times could simply have become extinct, or could be the result of transitory colonizations in a variety of biotopes that are now highly degraded or lost (e.g. interdunal ponds). We suppose this is the case of *Calopteryx splendens* sensu lato, of *Coenagrion castellani* or of the unmistakable *Libellula quadrimaculata* and of *Lestes macrostigma*; the presence of *Calopteryx virgo* and *Boyeria irene* is also to be considered highly doubtful. However, these are species reported with few records often from the nineteenth century, and not yet found in specific research after 2000. In addition to these 6 problematic cases, both the presence and distribution of *Ischnura pumilio*, observed with certainty only once after 2000, and of *Chacolestes parvidens*, of which there are also reports of hybrids with *C. viridis*, need to be investigated through specific researches. Recent decades have seen the arrival of a new wave of species, often of Afrotropical origin. The first reports, dating back to the 70s-80s of the last century, concern species such as *Brachythemis impartita* and *Zygonyx torridus*, at first considered accidental, which then founded stable populations in Sicily or Pantelleria (*Ischnura fountainei*). Two other species (*Brachytron pratense*, *Lindenia tetraphylla*) would have colonized the island only in recent years. *Orthetrum chrysostigma*, *Sympetrum sinaiticum*, *Diplacodes lefebvrii*, *Tramea basilaris* and *Trithemis kirby*, have been found mainly in the islands of Sicily Channel, and more episodically in Sicily during dispersal movements, and are for now to be considered accidental. The new arrivals also include the localized colonization (or possible recolonization) in the extreme eastern tip of the island (Messina) by *Platycnemis pennipes*, a species already mentioned in the past and then excluded from recent check-lists. Finally, on the basis of biogeographic and ecological considerations, we deemed it appropriate to exclude the presence of *Sympetrum depressiusculum*, *S. flaveolum*, *S. vulgatum* and *Somathoclora* sp.

KEY WORDS: Odonata, dragonflies, Sicily, check-list review.

ORAL PRESENTATION

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Odonatans of the historic public gardens of Palermo

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Urban environments represent an open frontier in the study of biodiversity and offer promising but generally neglected opportunities for its management and conservation, particularly in a country like Italy where 7.6% of the territory is urbanised.

The urban territory of Palermo includes several public gardens with fountains and basins, artificial and usually permanent lentic environments which constitute a habitat for various aquatic organisms, including the Odonatans. In the period April-October 2021 and 2022, visual censuses of the Odonatan communities of 7 public gardens in Palermo were carried out, focused on adult insects in a reproductive attitude, with occasional visits also in the rest of the year. The research highlighted the presence of 14 species; the community is typically Mediterranean and thermophilic, strongly dominated by some ubiquitous species with an Afro-Mediterranean distribution (*Crocothemis erythraea*, *Trithemis annulata*, *Anax imperator*) with a variable retinue of other species. The period of greatest activity is the summer and early autumn, in line with the phenology of the species of still waters, but flying adults can also be found in the winter months, characterized by the dominance of *Sympetrum striolatum*, the most mesophilous of the species present. The presence of *Orthetrum trinacria* is noteworthy, another species of Afrotropical origin currently expanding towards the North, widespread in Sicily but previously not attested in the Palermo area. Most of the sites show a similar specific richness ($S = 7$), with the Botanical Garden which stands out for the highest presence of 10 species and Villa Trabia with the minimum presence of 2 species. Further studies could give a more complete picture of the urban diversity of this group; as well as the relationship between factors such as vegetation, the effect of allochthonous predators (e.g. alien pond turtles) and maintenance regimes with the richness and abundance of populations. Lastly, the Odonata could act as a flag species, acting as catalysts for a naturalistic management of these environments that maintains favourable ecological conditions also for other organisms, first of all the *Discoglossus*.

KEY WORDS: Dragonflies, Zygoptera, Anisoptera, Urban fauna, Historic gardens.

POSTER



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

An Alpine-Appennine endemism: *Anonconotus* Camerano, 1878 (Orthoptera, Tettigoniidae). Biogeography, bioacoustics and conservation.

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The genus *Anonconotus* Camerano, 1878 is composed of 10 species of which 8 in the Alps (*A. alpinus* (Yersin, 1858), *A. baracunensis* Nadig, 1987, *A. ghiliani* Camerano, 1878, *A. italoaustriacus* Nadig, 1987; *A. ligustinus* Galvagni, 2002; *A. mercantouri* Galvagni & Fontana, 2003; *A. occidentalis* Carron & Wermeille, 2002; *A. pusillus* Carron & Sardet, 2002) and 2 on the Apennines (*A. apenninigenus* (Targioni-Tozzetti, 1881), *A. sibyllinus* Galvagni, 2002). All species live in high-altitude meadow environments, between low grasses and the ground, where they are active between August and September. The characterization on a morphological basis is not certain for all species, in fact the taxonomy of the genus has been greatly revised in recent years. Some populations are isolated, even rarefied and this leads to considerations on the possible protection of some species, e.g. *A. italoaustriacus* is listed as endangered in the IUCN Red List of Orthoptera of Europe. Genetic and bioacoustic studies are underway to characterize species and populations.

KEY WORDS: *Anonconotus*, Alps, Appennine.

ORAL PRESENTATION



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

**Re-discovery of *Limnebius asperatus* KNISCH, 1922, a century after its description
(Coleoptera: Hydraenidae)**

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Among the Hydraenidae of the European fauna, *Limnebius asperatus* KNISCH, 1922, is certainly one of the most mysterious taxa. Described more than hundred years ago on a couple of exemplares labelled “Italia” with no other data available, this beetle has no longer found, and no further information were known, with the exception that it is supposed to occur somewhere in Italy. A modern diagnosis of the species was given by JÄCH (1993), based on the two syntypes kept in the Zoologische Staatssammlung of Munich (Germany) with the designation of a lectotype and the illustration of the aedeagus. A century after its description, in July 2020 a large population of this insect was discovered by investigating a hygropetric environment at a well-known waterfall in the north-eastern Apennines, in the province of Arezzo, on the border between Tuscany, Emilia-Romagna and Marche. This find provides the first known data on the distribution and biology of this Italian endemism, as well as new data on its morphology. Concerns are also expressed about the conservation of this population, so far the only one known for the species.

KEY WORDS: coleotteri acquatici, nuova scoperta, endemismi italiani, distribuzione, ecologia, ambienti igropetrici, conservazione.



SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Argasidae ticks on migratory birds from Africa: molecular identification of a novel Argas genotype and further record of *Argas (Persicargas) persicus* Oken, 1818 in Italy

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In spring, migratory birds reach Europe, mainly from sub-Saharan Africa or from northern African countries. In 2021, on Ventotene Island (Latium region, Italy) within a project focused on the potential incoming pathogens via migratory birds from Africa, we found two larvae of *Argas* sp. on *Phoenicurus phoenicurus* that shared morphological features with the African *Argas (Argas) africanus*. Comparison of the tested larval DNA sequences to the adult reference sequences showed the highest identity with homologous sequences of *A. africanus*. In 2022 in the same site on Ventotene, we collected one larva on the bird *Sylvia communis*, molecularly identified as *Argas (Persicargas) persicus*.

Specimens found in 2021 were morphologically identified based on the available keys and literature (Hoogstraal et al., 1975; Manilla, 1986). Sequence analysis showed that the two specimens collected on Ventotene displayed an identical sequence haplotype for two molecular targets analysed; the mitochondrial 12S rRNA, and 16S rRNA gene. The BLAST searches using the obtained sequences exhibited the highest identity (> 92%) with homologous sequences of *A. africanus*. Phylogenetic analyses focused on the 16S rRNA gene, which has the potential for resolving phylogenetic relationships among closely related species in Argasidae (Burger et al., 2014). Phylogenetic tree showed close relatedness between the two specimens collected on Ventotene island with sequences of *A. africanus*. The molecular analysis did not allow a definite identification of the species, however, in accordance with the morphological identification, the two larvae were genetically closely related to *A. africanus*. The divergence of around 7% in the 16S rRNA sequences, that we observed between specimens from Ventotene and African *A. africanus*, is a level of divergence that was also observed in populations of soft ticks morphologically similar and nevertheless considered as conspecific (Hornok et al., 2017). Such variety of potentially identifiable genotypes within the same species does not allow us to understand whether in our case we are dealing with a new genotype belonging to the species *A. africanus* or whether this taxa is a complex of morphologically similar cryptic species.

The larva specimen collected in 2022 was morphologically identified as *Argas* genus and processed for molecular identification. Sequence analysis for 12S rRNA and COX1 showed >99% identity of obtained sequences with reference sequences of *A. persicus*. In conclusion, as no other study recorded the presence of this tick in Italy, this note represents the first detection of novel genotype related to *A. africanus* in Italy, confirming the role of migratory birds as real entry route for alien tick species and their pathogens. Moreover, the finding of *A. persicus* noteworthy for Italy where this species is reported as very rare (Pantaleoni et al. 2010). These findings deserve further study and attention also in the light of the globally ongoing climate change.

This research was in the frame of the project 'Emerging and re-emerging zoonoses along the routes of migratory birds. An integrated approach to investigate the potential route of introduction and spread' (IZS AM 04/19 RC) funded by the Italian Ministry of Health

KEY WORDS: Argasidae, ticks, migratory birds, Italy, *Argas africanus*, *Argas persicus*.

POSTER

XXVII ITALIAN NATIONAL CONGRESS OF ENTOMOLOGY

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SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

***Amblyomma marmoreum* group (Acari: Ixodidae) in Italy: new records on migratory birds from Africa**

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Migratory birds play an important role in the transportation of ixodid ticks and tick-borne pathogens among continents. In spring, migratory birds reach Europe, mainly from sub-Saharan Africa or from northern African countries but not much is known about the diversity and ecology of the tick species dispersed by them. From 2017 to 2022, in the framework of two consecutive projects focused on sampling migratory birds from Africa to Europe, a total of 28 immature *Amblyomma* specimens were collected from 7 migratory bird species, during bird-ringing activities on Ventotene Island, an important stop-over site in the Mediterranean Sea. In the absence of adult specimens, morphological identification was limited to assign these ticks to the *Amblyomma* genus.

In this study, sequencing and comparative analysis of five molecular markers (12S rDNA, 16S rDNA, COI, 18S rDNA and 28S rDNA) were performed on immature specimens of ticks with the aim to achieve the taxonomic identification. All sequences from the analysed individuals showed very high identity (range 95.2-99.9%) with the respective homologous sequences of *Amblyomma marmoreum* group available in GenBank. Likewise, the phylogenetic trees based on 12S, 16S and COI sequences consistently indicated that the all specimens from Ventotene clustered with *A. marmoreum* group.

Amblyomma marmoreum (Koch 1844), known as the South African tortoise tick, is quite common and widely distributed across South Africa (Horak et al. 2006). This species is a polytropic tick with a triphasic life cycle. In general, males and females of these species feed on any large reptile, turtles, large lizards and varanids (Hoogstraal and Aeschlimann 1982). Although the adults exclusively feed on reptiles, the immature stages feed on a wide range of vertebrate hosts, both domestic (cattle, sheep, goats, dogs) and wild animals, such as ground-feeding birds, ungulates, canids, felids and lagomorphs (Walker 1991). The first record of the species in Italy occurred in the spring of 2020, when a specimen of *A. marmoreum* was found on Ponza island, positive for *Rickettsia aeschlimannii*, (Battisti et al. 2020) and the second in Maltese Archipelago (Hornok et al. 2022) while other specimens of *Amblyomma* sp. were collected on migratory birds in 2021 (Rollins et al. 2021). In conclusion, this study confirms that immature ticks belonging to *A. marmoreum* group reached for six years the Pontine Islands and probably this happens each year. The entry of alien tick species and their potential in pathogens spreading deserves further study also in the light of the globally ongoing climate change.

KEY WORDS: *Amblyomma marmoreum*, migratory birds, ticks.

POSTER

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

Faunistic notes on Lepidoptera Tortricidae from Sicily

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Over time, the Lepidoptera fauna of Sicily has received the attention of Italian and foreign entomologists, especially for the zoogeographic interest of the Island, for the richness of biotopes and for tourism interest. The main contributions referring to the family Tortricidae can be found in the work of Minà-Palumbo and Failla-Tedaldi (1889) in which the authors listed 113 tortricids. After the catalog of Enrico Ragusa (1905) dedicated to the moths of Sicily, in 1938 Mariani published a new catalog, 184 species were assigned to the tortricids to which with delay, in a note appeared in 1948, due to “the serious events that have engulfed our country”. 61 years after the publication of Curò’s catalog of Lepidoptera d’Italia (1880), Mariani (1940-41) prints his Fauna Lepidopterorum Italiae, among the best studied areas of Italy there is also Sicily (with 184 taxa). Mariani and Klimesch (1957) decided to collaborate to draw up an addenda with 10 new Tortricidae. Until the 1990s, there is a lack of overall faunistic specific contributions. In 1999, 15 species of Tortricidae were listed from Pantano Longarini (Trematerra et al., 1999), with *Cydia multistriana* (Chrétien) new to Europe. Trematerra (1999) reports *Aethes rubiginana* (Walsingham) collected in Taormina and Lipari as new for Italy. In the same year, studying the material collected by Friedrich von Hartig, especially from Etna Mount, was described *Cnephasia etnana* Razowski and Trematerra. From the Pantani di Vendicari was discovered *Pelochrista cannatana* Trematerra, 2000. In the Catalog of “Lepidoptera Tortricidae della fauna italiana”, composed by 633 species, 246 taxa are listed for Sicily (Trematerra 2003). A few years later from Etna, was described *Pammene castanicola* Trematerra (Trematerra and Clausi, 2009). By checking Fauna Europaea (Aarvik, 2013) and from a further survey of the international papers, other species collected in Sicily can be traced, some of which are endemic, so much to bring the fauna of the Tortricidae of the Island to 250 entities, one of the richest regions in Italy. Recently, following the study results of the material collected on Etna Natural Park, Iblei Mountains, Nebrodi Natural Park, and Peloritani Mountains a list of 155 species of Tortricidae were reported by Trematerra et al. (2021), among the others *Fulvoclysia forsteri* (Osthelder) and *Tortricodes selma* Kocak were recorded for first time in Europe.

KEY WORDS: Lepidoptera Tortricidae, Fauna, Sicily.

POSTER

SESSION IV

FAUNISTICS, BIOGEOGRAPHY AND INSULARITY

***Amphipyra cinnamomea* and *Boudinotiana notha*: new records from southern Italy (Lepidoptera: Geometridae, Noctuidae)**

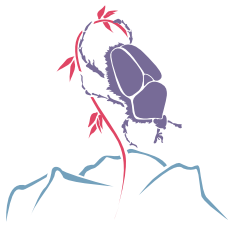
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Faunal studies carried out during the last decade have significantly implemented the knowledge of southern Italy lepidopteran fauna. Exploration of different habitats almost inevitably leads to the discovery of species new for this area. It is not uncommon to find faunistic novelties even in low-natural environments such as agricultural or suburban areas. During research aimed at studying nocturnal macrolepidoptera communities in olive groves and adjacent semi-natural areas of the Crati Valley, two specimens of *Amphipyra (Pyrois) cinnamomea* (Goeze, 1781) (Noctuidae) were found in July 2022. The species is known in Europe for Andorra, Austria, Bosnia and Herzegovina, Bulgaria, Corsica, Croatia, France, Germany, Greece, Hungary, Italy, North Macedonia, Romania, Sardinia, Slovenia, Spain, Switzerland, Yugoslavia, and doubtfully for Slovakia. It now appears to be extinct in Germany, where the last reports for some regions date back to 1881. Also in Switzerland its range has shrunk, being reported only in the extreme southwest of the central plateau, and all records after 1960 are in the surroundings of the Rhone Valais. In Italy isolated specimens were found in Valle d'Aosta, Piedmont, Emilia, Tuscany, Umbria, Latium, Abruzzo, and Apulia. Sardinian citations deserve confirmation. The larvae are polyphagous on some thermophilic trees and shrubs. *Boudinotiana notha* (Hübner, 1803) (Geometridae) was another finding of relevant faunistic interest. It was observed in March 2022 within a suburban area near Cosenza. It is known from central and northern Europe, with a very discontinuous distribution in Mediterranean countries. In Italy it is usually found across Alpine regions, with sporadic records from the Peninsula where it is known only in Tuscany, Latium, and Apulia. The larvae feed on some poplar species, and adults fly in proximity of larval foodplants.

KEY WORDS: Fauna, Heterocera, Calabria.

POSTER



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12-16 June 2023

Session V

SESSION V

AGRICULTURAL ENTOMOLOGY

Qualitative analysis of host plants infested by *Bactrocera dorsalis* (Diptera: Tephritidae) in fruit orchards of the Campania Region

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The oriental fruit fly *Bactrocera dorsalis* (Hendel) belongs to a complex of highly invasive Tephritidae. In the countries of origin, *B. dorsalis* is a multivoltine species that reaches up to 10 generations per year and each female can lay up to 3000 eggs during its entire life cycle. The most significant damages are caused by the larval trophic activity on ripening fruits. The gregarious larvae cause a rapid decay and early fall of the infested fruits. Due to its high rate of polyphagy and the extensive damage caused by the larvae, *B. dorsalis* is reported among the 20 priority quarantine pests in the Commission Delegated Regulation (EU) 2019/1702. Since the end of the 2022 summer, the monitoring activities, carried out by CNR-IPSP in collaboration with the Plant Health service of the Campania Region (URCoFi project), led to collect several specimens of *B. dorsalis* in traps in the provinces of Naples and Salerno. Some of these areas also included sites where *B. dorsalis* was intercepted during the years 2018/2019. *B. dorsalis* was probably introduced through the plant material in the luggage of passengers from Third Countries. *B. dorsalis* has a remarkable ability for ecological adaptation and it can easily settle and spread in the Italian territories, due to the favorable climatic conditions. Furthermore, the literature reports that *B. dorsalis* can attack more than 300 plant species, most of which are of agricultural interest (citrus fruits, pome fruits, stone fruits, and vegetable species). The wide range of suitable host plants is uncertain and not well-defined, as *B. dorsalis* is part of a complex of cryptic species. In addition, the list of susceptible plants is constantly updated also because *B. dorsalis* shows a high ability to adapt to other botanical species not yet confirmed as hosts. A preliminary study on susceptible plant species was carried out given the harmful impact that the establishment of *B. dorsalis* could have on the agricultural economy in Campania and Italy. For this purpose, several samples of symptomatic ripe fruits were collected from the infested areas. The samples were isolated in BugDorm at CNR-IPSP laboratories; when necessary, the larvae were transferred to an artificial diet, and the pupae were isolated in Petri dishes until the emergence of the adults. Then, the specimens were identified through integrative approaches (morpho-molecular analysis) that confirmed their belonging to the *B. dorsalis* complex. A qualitative analysis of the samples revealed an initial assessment of the plant hosts on which *B. dorsalis* completed its life cycle: *Citrus sinensis*, *Citrus aurantium*, *Citrus reticulata*, *Diospyros kaki*, and *Prunus persica*. As expected, and as reported in the literature, this preliminary investigation would seem to suggest that so far the highest emergency rate of adults has occurred from fruits produced by species belonging to the genus *Citrus*.

KEY WORDS: *Bactrocera*, field infestation, host plants, invasive species, oriental fruit fly.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Essential oils as repellents for *Halyomorpha halys*: olfactometric and laboratory choice tests

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The brown marmorated stink bug, *Halyomorpha halys* Stål 1855 (Heteroptera: Pentatomidae), originally from Northeast Asia, is an extremely invasive and harmful insect, first reported in Italy in 2012 and quickly becoming the key phytophagous pest in numerous agroecosystems, causing significant economic damage to many tree crops. In this study, essential oils from hemp (*Cannabis sativa*), artemisia (*Artemisia vulgaris*), cajeput (*Melaleuca cajuputi*), "origano di Spagna" (*Thymbra capitata*), peppermint (*Mentha piperita*), and geranium (*Pelargonium graveolens*) were evaluated for their potential as repellents in integrated pest management strategies (e.g., push and pull) against the insect, particularly in pear orchards. The essential oils were tested at different concentrations and in different blends using a Y-tube olfactometer. Once the most effective oils and blends as repellents were selected, "in vivo" tests were carried out on pear fruits in specially designed experimental arenas. The oils used in these tests were chemically characterized using gas chromatography with flame ionization detector (GC-FID). The results showed the high efficacy of some essential oil blends, particularly cajeput+"origano di Spagna" and peppermint+geranium, as repellents against *H. halys*. However, efficacy is strongly influenced by relative concentrations and time elapsed since treatment, with the latter being an important factor due to the high volatility of essential oils. The experiments described in this study offer promising possibilities for the use of essential oil blends as repellents for the brown marmorated stink bug in integrated pest management strategies (e.g., "push and pull"), but their actual potential needs confirmation in subsequent field experiments.

KEY WORDS: semiochemicals, brown marmorated stink bug, natural control, olfactometer, hemp essential oil, artemisia essential oil, geranium essential oil, cajeput essential oil.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

The genesis of the invasive process of *Bactrocera dorsalis* in Italy. Is it an inescapable event?

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A recent study analyzed data on invasive species worldwide over the period 1950-2010 showing that Italy and France emerged as central hubs of the most invasive insect processes. Moreover, these countries resulted to be the places where alien insect records occur most early and frequently.

The invasive species found in recent years in these countries are numerous, but the record of specimens belonging to the *Bactrocera dorsalis* complex has aroused the greatest alarm due to the high threat that this species represents for the European agriculture. The *B. dorsalis* complex includes several extremely polyphagous and multivoltine cryptic species native to Asia.

The worldwide invasive process of the *B. dorsalis* complex is in continuous and progressive evolution and it has had a further acceleration since 2003 when the species invaded the sub-Saharan countries. After this event, some sporadic records occurred in Europe, but generally in market areas or near airports. In 2018 there were the first catches in traps placed in fields in Italy and these findings led to an increase in monitoring activities and a progressive increase in catches in various European countries. Subsequently, the captures of the 2018-2019 period were classified with the status of incursion. The arrival of the Covid pandemic in the two-year 2020-2021 led to the zeroing of the catches. In 2022, with the increase in monitoring activity related to the recovery of the pre-pandemic level of volume of trade in goods and passengers, the situation changed radically.

Since June 2022, catches have been increasingly frequent and, although mainly concentrated in a couple of municipalities in Campania (98% of catches), further specimens have been recorded in other regions.

Following these new catches, a series of research activities have begun in Campania, to evaluate the extension and delimitation of the infested areas, and to improve the bio-ecological knowledge of the species and/or population present in Italy.

About 300 traps of different typologies, activated with diverse attractants, were placed, and samplings of fruits with symptoms imputable to the tephritid attack have been started. This intense monitoring allowed, in August 2022, the first capture of *B. dorsalis* females, and later, in September, the finding of the first active infestation in Europe.

Following this last record, the catches and the areas with active infestations, despite the numerous containment measures adopted by the personnel of the Campanian Plant Health Service, grew progressively.

However, the captures of females in the traps took place only in the territory of Palma Campania which is one of the two municipalities already affected by the first catches in the period 2018-2019. From the end of October, a progressive decrease in the number of catches was recorded, however, the catches stopped only after the first decade of January. The delayed arrest of the pest activity could be due to the extraordinary climatic conditions recorded in Campania in 2022.

The monitoring activities of *B. dorsalis* are still ongoing to evaluate the effective establishment of the species in Italy and the evolution of its diffusion.

KEY WORDS: active infestation, Campania, fruit fly, invasive species, male lure, species complex, traps.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Occurrence of potential psyllid vectors of '*Candidatus Liberibacter solanacearum*' in carrot and potato fields in Italy

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Psyllids in the genera *Trioza* and *Bactericera* (Hemiptera: Triozidae) represent a potential threat for the cultivation of solanaceous and apiaceous crops worldwide, mainly as vectors of the phloem-restricted bacterium '*Candidatus Liberibacter solanacearum*' (Lso). Different haplotypes of Lso are transmitted by different psyllid species depending on host plants and geographical regions. The Lso haplotypes A and B and their vector *Bactericera cockerelli* (Sulc) are EU-quarantine pests of solanaceous crops. The haplotypes D and E are widespread in the Mediterranean basin, where they affect apiaceous crops and are mainly transmitted by *Bactericera trigonica* (Hodkinson). *Bactericera nigricornis* (Förster) is another worthy candidate in Lso transmission since it has recently proved to efficiently transmit the haplotype E from carrot to carrot in experimental conditions. Moreover, this species is known to feed and reproduce on potato, rising concerns for Lso-D and -E transmission to and within this crop.

Monitoring psyllid populations on both carrot and potato crops can provide important information on the potential risk of Lso outbreaks in Italy, where few data on both pathogen and vectors are currently available. Since 2021, surveys were carried out in carrot fields in Abruzzo and Latium regions (Central Italy), having a winter and summer cultivation cycle respectively, and in potato fields in Calabria region (Southern Italy). In Abruzzo, the first surveys performed in June and July 2021 revealed the presence of abundant populations of *B. trigonica*. In 2022, two carrot fields were selected in the same area, one cultivating carrots and potatoes in two adjacent plots and one cultivating carrot only. Psyllid populations were constantly surveyed from May to November, and the population peaks occurred between the crop emergence in June to the carrot harvest in September. Adults of *B. trigonica* were collected also in the potato field but at a lower level of population density, confirming that potato is only an occasional host for this species. A carrot field was surveyed in 2022 in Latium throughout the year to potentially intercept psyllid at the edge of the winter cultivation period and to identify alternative host plants. Few adults of *B. trigonica* were collected only since October at carrot emergence whereas no specimens were found in absence of the host crops. In Calabria, the presence of psyllids was inspected from July to September 2021 and 2022 in four potato fields located at different altitudes. The surveys allowed to identify abundant populations of *B. nigricornis* throughout the summer seasons.

The field surveys confirmed the presence of potential Lso vectors in Central and Southern Italy, namely *B. trigonica* and *B. nigricornis* that showed to settle preferably on carrot and potato, respectively. The presence of the two species appears to be strictly correlated to the crop availability. Indeed, in Lazio the winter carrot cultivation did not allow the settlement of abundant populations of *B. trigonica* throughout the year, and in Abruzzo the population density sharply decreased at the end of the summer after carrot harvest. The surveys are still ongoing to increase knowledge on psyllid phenology and demography in the inspected Italian areas.

This research was supported by Proteggo project 1.3 and 1.4 founded by MiPAAF, and was carried out in the frame of Euphresco PhyLib III project.

KEY WORDS: *Bactericera trigonica*, *Bactericera nigricornis*, Hemiptera: Triozidae.

POSTER

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SESSION V

AGRICULTURAL ENTOMOLOGY

Spittlebugs in vineyards: influence of management strategies and plant community characteristics on the abundance of spittlebugs

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The bacterium *Xylella fastidiosa* Wells (*Xf*), native to America, has been identified in Europe for the first time in 2013. In Southern Italy, a strain of *X. fastidiosa* subsp. *pauca* is the causal agent of the Olive Quick Decline Syndrome (OQDS) on olive, a destructive disease that killed millions of trees and it is still spreading in the Region. *Xylella fastidiosa* is also a potential major threat to the European wine sector, as it is known as the causal agent of Pierce's disease of grapevine in USA, recently identified also in the Balearic Islands. The key insect vectors of this bacterium in Europe, i.e. spittlebugs (Hemiptera: Aphrophoridae), can reach high populations in vineyards, hence the risk of *X. fastidiosa* outbreaks in wine-producing areas is relevant in case of introduction of the bacterium. The aim of this study was to investigate the influence of management strategy and vegetation community characteristics on the presence and abundance of spittlebug nymphal and adult stages in the vineyard agroecosystem. A two-year field survey of spittlebug populations was carried out in six vineyards (three organic and three IPM) located in different wine-producing areas in northwestern Italy. Nymphal samplings were carried on herbaceous cover of inter-rows within randomly selected sampling units (n = 15, 0.25 m² each). The specific composition and characteristics of vegetation were recorded for each sampling unit. Adult stage was sampled by sweeping net in June and September from three vegetation compartments of the vineyard agroecosystem: i) grapevine plants, ii) herbaceous cover of inter-rows, and iii) spontaneous woody plants. Our results show that the presence of spittlebug nymphs is influenced by management strategies, as sample units in organic vineyards are more likely to host spittlebug nymphs. Also, the density of the more abundant spittlebug species (*Philaenus spumarius* L.) significantly decreases in plots with vegetation characterized by i) high nutrient availability (i.e., mean Ellenberg indicator value of nutrients) and ii) high herbaceous biomass, while *Neophilaenus campestris* Fallén presence is correlated to high cover percentage of monocot plants. Both presence and abundance of spittlebugs was however highly variable among wine-producing areas. The population levels of adults of *P. spumarius* in the vineyard are relatively low, but spittlebug densities on grapevine in summer are similar to those observed on other vegetation compartments (0.05–0.1 adults/sweep). The highest density of adult spittlebugs was observed on herbaceous cover in autumn. Our study shows the presence of the key *X. fastidiosa* vector in Europe on grapevine plants and provide new insights on the role of management strategies and plant community characteristics on density level of spittlebugs in the vineyard agroecosystem. These results help to design environmentally-sound strategies to lower vector populations in *X. fastidiosa* susceptible agroecosystems, e.g. by promoting herbaceous cover not suitable to spittlebug nymphal development or by developing trap cropping systems at the plant community level.

KEY WORDS: *Philaenus spumarius*, grapevine, vegetation management, biological control, insect vectors, *Xylella fastidiosa*.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Reproductive biology of *Philaenus spumarius* (Hemiptera Aphrophoridae)

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The meadow spittlebug *Philaenus spumarius* is the main vector of *Xylella fastidiosa* Wells in Europe and, namely, of *X. fastidiosa* subsp. *pauca*, causal agent of the Olive Quick Decline Syndrome (OQDS) in the Apulia Region of Italy. The phenology and host plant association of *P. spumarius* nymphs and adults in different geographical areas have been described. However, the reproductive biology is still largely unknown and must be studied in depth to design efficient control strategies for this vector species in the areas where *X. fastidiosa* is present. To fill the existing knowledge gaps, different studies were carried out with insect rearings and laboratory analyses. Fecundity was investigated under controlled conditions by counting the egg numbers and of nymph progeny, using both single couples and groups of insects. The ovarian diapause length was studied by ovarian egg counting and analysis of the expression level of the Vitellogenin gene (Vg) during different phases of female adult life. Finally, several spittlebug populations from different areas and environmental conditions were screened for the presence of *Wolbachia*. Fecundity was evaluated for females collected in different sites of Northern Italy and Apulia (Southern Italy). The average number of eggs produced by single females ranged from 18 to 196, highly variable according to the geographic origin of the population and the methodology used for the trials. The ovarian diapause was confirmed for all the spittlebug populations, although with important differences due to altitude and latitude. The females of Alpine populations, collected above 1,500 m a.s.l. showed early development of ovarian eggs and increased levels of Vg expression as early as 2-3 weeks after their emergence. The spittlebugs collected in lowlands of Northern Italy and Apulia Region, started to develop eggs in the ovaries, and showed increased level of Vg expression from the beginning of September and October, respectively. Therefore, females started to mature eggs 4-5 months after their emergence. The expression of Vg was a very reliable marker of ovarian development and could be used to estimate the end of the diapause and the beginning of egg maturation and laying phases. The spittlebug specimens collected at the alpine sites were infected at high percentage (67%) by *Wolbachia*, a much higher frequency compared to that of one population collected at lowlands. Overall, the results indicate that egg production is strongly influenced by environmental conditions and by the period when diapause is overcome. Thus, *P. spumarius* populations were regulated by environmental conditions. The role of *Wolbachia* in the biology of the spittlebug is still unknown, but these symbionts may play an important role in the adaptation of the insect to cold areas, with very short warm seasons required for reproduction. These findings support the role of *Wolbachia* as reproduction manipulators.

KEY WORDS: spittlebug, vector, *Xylella fastidiosa*, fecundity, diapause, *Wolbachia*.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Evaluation of the specificity of pheromones used in the monitoring of some lepidopterans of agricultural interest currently on the market

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The use of pheromone-activated traps is often the basis for the control of phytophagous insects.

The in-depth knowledge of the different components of pheromones and the evaluation of their specificity for target insects is essential to plan a correct pest management.

The literature reports numerous records of non-target species caught in traps activated with commercialized pheromones. These catches make sometimes the identification process very hard, often needing the support of an expert. Indeed, in the absence of an unquestionable identification, non-specific catches can cause several problems, among which the incorrect evaluation of the pest flight curves is undoubtedly the most serious. Therefore, in addition to the safe and complete range of pests that can be caught with these substances, the knowledge of the morphology of the captured insects is crucial.

However, this last aspect could be insufficient to allow a good identification, because samples are often collected in poor conditions due to exposure to atmospheric agents or the effect of the glue on the sticky panels.

To help the work of non-specialized operators, we evaluated the set of different Lepidoptera species caught in traps activated with sex pheromones sampled during the activities carried out in the URCoFi project. The emerging and/or invasive species are: *Apomyelois ceratoniae*, *Cryptoblabes gnidiella*, *Grapholita molesta* and *Thaumatotibia leucotreta*. The molecules used were respectively: (Z,E)-7,9,11-Dodecatrien-1-yl (two trials with and without stabilizer), (Z)-13-octadecenal, (Z)-8-dodecenyl acetate, (Z,E)-8-dodecenilacetato.

The monitoring was carried out using two typologies of traps, Pagoda and Delta, placed in different orchards of the Campania region. The dispensers were replaced as indicated by the manufacturer, while the monitoring activities were carried out weekly. The specimens on the sticky panels (12x20 cm) were transferred to the lab, counted, and the most frequently found species were identified. In some cases, a molecular analysis based on mitochondrial barcoding was performed.

All the traps have caught, also or exclusively, specimens of species morphologically similar to the target species, and they were often difficult to distinguish without in-depth analyses.

In particular, the species trapped were respectively: *Anarsia lineatella*, *Grapholita molesta*, and *Cydia pomonella* on *A. ceratoniae* traps; *Duponchelia fovealis* and *Lobesia botrana* on *C. gnidiella* traps; *Pammene* spp., *Grapholita funebrana*, and *Notocelia uddmaniana* on *G. molesta* traps; *Epiblema scutulana* and *Grapholita funebrana* on *T. leucotreta* traps.

The simple counting of adults caught in the traps or an imprecise identification can lead to an incorrect assessment of the population density of the target pest, resulting in an improper estimation of the damage risk and of the evaluation of the treatment period.

The results show that both the absence of precise information on the species that can be captured with a pheromone and a careless choice of one over another makes the difference in integrated pest management.

KEY WORDS: *Apomyelois ceratoniae*, *Cryptoblabes gnidiella*, *Grapholita molesta*, *Thaumatotibia leucotreta*.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

***Dasineura oleae* (Angelini, 1831): damage and chemical ecology in a tritrophic context**

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Cecidomyiid outbreaks generally last few years, which are preceded and followed by long periods of latency. During the outbreak, the severity of midge attacks may involve crops production, interfering with yield quantity and quality. However, knowing the causes of this phenomenon is generally very complicated, because except during the period of strong infestation, the relations among midge, host plant, and natural enemies are not much investigated. This research focuses on the relationship between *Dasineura oleae* (Diptera: Cecidomyiidae) and its unique host, *Olea europaea*, in a tritrophic scenario. *Dasineura oleae*, known as the olive leaf gall midge, feeds on tender leaflets at larval stages causing malformation and swellings and complete tree defoliation when the infestation is strong. In this work, the damage caused by the midge has been studied from different points of view, starting from the origin of the gall-inducing stimulus. As a consequence of the larval feeding action, the olive leaves showed modifications of the leaf blade shape, that became 6-fold greater because of structural alterations of the spongy parenchymal tissue. In turn, damages on leaf physiology, such as minor efficacy concerning some photosynthetic parameters, were recorded (stomatal conductance (-28%) and net photosynthesis (-35%)). Parenchymal alteration may have a role in inducing plant defense response, as the emission of herbivore-induced plant volatiles (HIPVs), which play an important role in the recruitment of herbivore natural enemies. Here, the first characterization of HIPVs released from infested olive leaves is proposed. A blend of 12 volatiles was emitted from olive trees infested by *D. oleae*. Following the insect attack, the emission amount of some volatiles increased significantly (germacrene-D, (E,E)- α -farnesene, and (Z,E)- α -farnesene, α -copaene, DMNT, (E)- β -guaiene and heptadecane), while other HIPVs were not present in control plants and appeared only after the infestation (β -copaene, β -ocimene, cosmene, unknown 1 and unknown 3). This work aims to improve the knowledge of the relationship between *D. oleae* and its natural enemies, in order to lay the foundation for eco-friendly control methods. Furthermore, since insect cuticular hydrocarbons (CHCs) can be involved in chemical communication, *D. oleae* cuticular extracts were investigated as the first work on a cecidomyiid. *Dasineura oleae* males and females at different ages from emergence (0-12 h; 12-24 h; 24-36 h) and distinct mating conditions (virgin and mated) were considered. A total of 49 compounds were detected, and among them, 18 compounds showed significant differences between groups, inducing to suppose a potential role in intraspecific communication. Even if further behavioral tests are required to assign ecological roles both to HIPVs and cuticular extracts, the obtained preliminary results encourage new outlooks for the Integrated Pest Management (IPM) of *D. oleae*.

KEY WORDS: *Dasineura oleae*, gall, HIPVS, *Olea europaea*, tritrophic relationships.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Evaluation of the role of fruitlets as resources for the first generation of the Mediterranean fruit fly, *Ceratitis capitata*.

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The Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), is considered an important pest due to its high polyphagy and the considerable economic damage that it causes. Largely widespread in the Mediterranean basin and in many tropical and sub-tropical regions, in Italy the infestations are frequent in the southern and central regions where it completes 5-6 generations per year, while in the northern regions the infestations are mainly targeted at fruit crops that ripen at the end of the summer, completing a maximum of 3 generations. During the winter months, the pest population is affected by high mortality due to low temperatures, which reduces the number of overwintering individuals. The host plants present in spring are of crucial importance for the *C. capitata* population dynamics at the beginning of the season. The few species with fruits ripening in spring-early summer are considered key hosts for oviposition, as they determine the development of the first seasonal generation. The fruitlets, which are dropped or designated to drop, could serve as a resource for the *C. capitata* population when other available fruits are not abundant. In this paper, in order to assess the possible role of fruitlets as resources for the first generation of the *C. capitata*, the fruitlets infestation level in the field was estimated and the suitability of fruitlets for the oviposition and the further development of immature stage were examined. Five cultivars of the following species were evaluated: apricot (Bergeron), peach (Alitop nectarine and Suncrest peach), pear (Bella di Pirovano) and apple (Rosa mantovana). For each cultivar, the surveys were carried out on two types of fruitlets: dropped or on-plant (but late in development and destined to drop). The infestation level in the field was assessed on 1000 fruitlets, per cultivar and of each type, which were incubated for 30 days in ventilated boxes. Trials to determine the suitability of fruitlets for the oviposition and the further development of immature stage were carried out in laboratory by placing 5 fruitlets of each type and 20 mated females of *C. capitata* in cages (5 replications for each type). For each type of fruitlet, some physicochemical parameters (size, weight, pH, EP volume, acidity, and Brix degrees) were analysed. In the field infestation assessment, no pupae were found in the boxes while, in the laboratory trials, all the tested cultivars received oviposition stings, with the exception of the dropped peach samples. The number of stings per fruitlet ranged from 0.08 to 8.84, recorded on the fruitlets of dropped apricot and on-trees apple, respectively. Complete larval development was verified in nectarine and pear samples. Adults emerged only from the fruitlets collected on-plant. Based on our laboratory trials, fruitlets of nectarines and pears can be considered as potential hosts of *C. capitata* and therefore important resources for the development of its first generation in the season. These results need to be included in the *C. capitata* Integrated Pest Management programmes.

KEY WORDS: fruitlets, host suitability, oviposition stings, larval development.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Acaricide resistance in *Tetranychus urticae*: the situation in the province of Piacenza

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In open-field tomato crops in the province of Piacenza, the problem of losses caused by the tetranychid mite *Tetranychus urticae* Koch has increased significantly over the years and, in the period between 2017 and 2019, significant damages have been observed in several plots. The adoption of more strict guidelines and increased awareness of farmers and technicians partially reduced the damages in the following years. However, the situation remains worthy of attention. In addition to environmental and agronomic conditions that favoured the infestations, it was thought that the situation could also be attributed to the presence of populations with a more or less reduced sensitivity towards the acaricides used. In fact, it is internationally known that *T. urticae* is one of the pests with the highest number of reported cases of resistance to pesticides. Therefore, in the areas of the province of Piacenza intended for tomato cultivation, investigations to highlight the phenomenon have been carried out since the first reports of damage. The level of sensitivity of various populations collected directly from the field was estimated by means of laboratory bioassays. At first (2017-2021), the sensitivity towards the acaricides with an adulticidal action, authorized by the Emilia-Romagna region integrated production guidelines as abamectin, bifenazate, fenpiroximate and ciflumetofen was evaluated. The sensitivity towards mixtures of substances with a potential synergistic action (potassium salts of fatty acids; piperonyl butoxide) was also evaluated. Later (2021) the efficacy of products with ovicidal action (clofentezine) was investigated. With the exception of the more modern active ingredient (cyflumetofen), the various adult products have shown more or less serious deficiencies and in many cases very limited efficacy. The use of PBO and above all of the potassium salts of fatty acids has allowed, in laboratory conditions, to restore the efficacy above all of fenpiroximate. Sensitivity towards ovicides was found to be particularly compromised and about two-thirds of the populations investigated were found to be particularly resistant to clofentezine with efficacy levels close to zero even when a double dose compared to the recommended label field rate was applied. Resistance to this group of products is linked to the presence of a point mutation in the gene encoding chitin synthase 1 (CHS1) which results in the amino acid modification I1017F. This mutation has been found in many populations of *T. urticae* collected in various European countries such as Belgium, Greece and France but till now no cases had been reported in Italy. The mutation in the CHS1 gene was found both in heterozygous and homozygous states in individuals taken from Piacenza populations with reduced or zero mortality of the treated eggs. Likely the mutation has been present for some time in populations of *T. urticae* in Piacenza Province and the critical issues that have emerged in recent years in the province of Piacenza for the control of this mite in tomato fields are attributable, to a significant extent, to the consequent resistance towards ovicides which are a key element in pest management strategies against this mite.

KEY WORDS: tomato, CHS1, chitin synthesis inhibitors.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

“PestFinder”: a Marie Curie Postdoctoral Fellowships project on mathematical models applied to entomology.

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Population dynamics models are a powerful tool for an integrated management of both agriculture and forest pest species. Classical monitoring, in fact, is not more sufficient to cope with the need to reduce the inputs in cultivated fields. “PestFinder - Model-Based Estimation and Control of Agricultural Infestations Through Abiotic Changes” is a two-year project funded by the European Commission, call HORIZON-MSCA-2022-PF-01. This project originates from the need of better exploring and improving the theoretical aspects behind the decision support systems. The goal of the project is to contribute to the “precision agriculture” paradigm by developing model-based control strategies with a high multidisciplinary approach between entomologists (Dr. Luca Rossini, funded postdoc and main proponent) and control engineers (Prof. Emanuele Garone, project supervisor) at Université Libre de Bruxelles, host institution. As the first objective, we aim to develop models and methodologies capable of driving in an optimal way the agronomic decisions (both in terms of monitoring and control actions) on pest control. To pursue this aim, the first part of the project will concern the development and validation of models capable of describing the spatial diffusion and the temporal evolution of insect populations in cultivated fields. These models will be parametrized w.r.t. environmental parameters (temperature and humidity) and control inputs (e.g., pesticides). The focus will be then shifted on how to use these models for pest monitoring, and in particular on the interplay between quality of the estimation and data collection, in order to develop optimal data collection policies. In the last part of the project, we will use the developed results to develop model-based optimal pest control policies. Although we aim to obtain a theoretical framework that is more general as possible, two species of high agricultural interest (*Drosophila suzukii* Matsumura and *Halyomorpha halys* Stål) will be considered to validate the various steps of the theory. This research project has a highly innovative approach and has the ambition at large to promote a new philosophy on pest management that goes beyond qualitative analysis and decision making, proposing a quantitative model-based approach to pest management. The research objectives are highly original and focus on aspects of pest management that are highly relevant both from the applied and the research viewpoint. We believe that the research and the tools that will be developed in this research project will have a major impact on both the scientific and the farmers community.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Phytosanitary management and hazelnut varietal diversity as factors that act on the incidence of *Phytoptus avellanae* in Latium Region

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Province of Viterbo is one of the most important nut fruits growing regions in Italy, with an area of 24,000 hectares cultivated with hazelnuts. Hazelnut plants in this area are constantly exposed to biotic stress which originates mainly from phytophagous arthropods. Such adversities often result in a quantitative and qualitative reduction of the harvested product. One of the main representatives of arthropods is the hazelnut bud mite *Phytoptus avellanae* Nalepa (Acari: Eriophyoidea). The colonization of buds and reproduction of mites inside the buds cause distinctive enlargement of plant tissues and disrupt their normal development, resulting in loss of bud viability. Monitoring of this pest is usually conducted in winter and consists of gall counting on randomly selected branches. The usage of plant protection products is not recommended if the infestation of buds is not exceeding 15 percent, which is referred to as an economically significant threshold. In cases where the occurrence of the infested buds is higher than the referred threshold, usage of sulphur-based products is advisable, when the mite starts the migration from galls to healthy buds. Differences in mite susceptibility are observed among the hazelnut varieties which suggests that in the future more resistant cultivars should be planted in new orchards.

The main aims, of this preliminary study, is to investigate whether (i) in the Viterbo province area, phytosanitary management of hazelnut orchard (integrated, organic, or no management) has an impact on the occurrence of gall mites and whether (ii) varieties cultivated in a collection present in the province of Viterbo show differences in susceptibility to mite activity.

All activities were conducted in the Viterbo hazelnut district in the winter 2023. The assessment of gall mite incidence was conducted in 3 farms managed with IPM methods, in 3 farms managed with organic, and in 3 localities, selected within the "Valle del Treja" Regional Natural Park, where hazelnut cultivation stopped in the 1990s and which are now in the advanced stage of renaturalization. Ten plants were selected, and on 5 branches of these, 50 distal buds were counted and the number of healthy and infested buds was noted, at each of the nine selected localities. All hazelnut plants checked during the activities belonged to the Tonda Gentile Romana variety.

The same counting methodology was conducted in a hazelnut variety collection located in the same area, in the municipality of Caprarola (VT), consisting of 48 cultivars from around the world, each represented by three plants.

The preliminary results show significant differences, both among phytosanitary management and among the cultivars considered in the varietal collection, opening the possibility for several considerations useful for more effective management of the mite.

KEY WORDS: Mites, big bud, susceptibility, IPM, organic.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

The YOLO framework for early detection of insect and mite pests on hazelnut production

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Several biotic threats, in particular insect and mite pests, cause significant reduction of hazelnut production throughout the world and in Italy as well. In the integrated pest management (IPM), which is of fundamental importance to obtain a quantitatively and qualitatively valuable production, pest monitoring is getting more and more attention. Monitoring aims to check the presence and consistency of pest agents, to set up opportune control strategies but it is, at the same time, a time-consuming activity and involve expert technicians. Given these preconditions, within the EU Horizon 2020 Pantheon Project (Precision Farming of Hazelnut Orchards) we developed a data-driven framework based on Convolutional Neural Networks (CNNs), the YOLOv4 (You Only Look Once) architecture, for early detection of damage agents in an orchard located in the Viterbo hazelnut district, in Central Italy. This innovative system has been tested and validated for the detection of *Palomena prasina* L. (Heteroptera: Pentatomidae) specimens by pheromone traps deployed in the field and for the detection of galls induced by the mite *Phytoptus avellanae* Nalepa (Acari: Eriophyoidea) on the plants. The YOLO system was able to identify the true bug target, distinguishing it with a high level of accuracy from other insects and objects that got accidentally in the traps. The YOLO technology confirmed the capability to identify, with a good level of accuracy also the presence of mite induced galls on the plants. All these aspects, as well as the implications of these findings, are discussed.

KEY WORDS: *Phytoptus avellanae*, *Palomena prasina*, monitoring strategies.

SESSION V

AGRICULTURAL ENTOMOLOGY

The effect of grape vine cultivar Petit Verdot on *Lobesia botrana* (Denis & Schiff.) attractiveness

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Lobesia botrana (Denis & Schiffermuller) (Lepidoptera: Tortricidae) is considered the most destructive grapevine pests in the Palearctic Region. Knowledge of plant susceptibility to insect pests is of primary importance for management programs. Among the causes of susceptibility, the attractiveness of the cultivar greatly affects the infestation level of *L. botrana*. Although Petit Verdot is a widespread cultivar in the Center of Italy, to date no study has investigated on it. Therefore, in this work, the ability of Petit Verdot cultivar to attract *L. botrana* was evaluated for the first time. To reach this aim, the cultivar was compared with Sangiovese, a notoriously attractive cultivar for this tortricid, in a vineyard located in Loreto (AN), Central Italy. With this aim, the host choice by females of *L. botrana* was evaluated by analyzing the infestation index and the infestation severity index. These indices were calculated by monitoring the damage caused by the moth immature stages during the three generations, in June on flower clusters, in July and in September on bunches. Furthermore, the population dynamics of *L. botrana* was studied by trapping males in pheromone traps. The results showed that, although the traps captured more males in Petit Verdot, the presence of larval activity on this cultivar was statistically lower than that on Sangiovese, highlighting a lower attractiveness of Petit Verdot towards *L. botrana*. Understanding the preference of females in choosing the host within the vineyard could ensure a more efficient monitoring system. In this context, in the light of our results, Petit Verdot, due of its low attractiveness to female oviposition, cannot be considered a good early monitoring indicator in fields with mixed vineyard cultivar. However, the obtained results could be useful to further studies on cultivar susceptibility and provide useful information for future vineyard management strategies.

KEY WORDS: *Lobesia botrana*, Petit Verdot, attractiveness.

SESSION V

AGRICULTURAL ENTOMOLOGY

Diffusion of *Orientus ishidae* (Hemiptera: Cicadellidae) in Northern Italy apple orchards and study on host preference

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Flavescence dorée (FD) is a serious grapevine disease associated with phytoplasmas belonging to the ribosomal subgroup 16SrV. The disease is widespread in the major European grape-growing areas where it causes considerable economic losses and is transmitted from grapevine to grapevine by the alien leafhopper *Scaphoideus titanus* Ball.

Others potential vectors have been identified over the last few decades (e.g. *Dictyophara europaea*, *Allygus* spp.) though they seem to play a minor role in the FD epidemiology, acting more as a reservoir of phytoplasma in the environment. One of these species included a non-European leafhopper, *Orientus ishidae* (Matsumura), found positive to FD in different European countries, both in vineyards and wild hosts. Moreover, laboratory experiments have shown that it is capable of transmitting FD from bean to vine or from wild plant to wild plant (e.g. alder to alder).

For this reason, the species has been monitored in Trentino (North-East Italy) vineyards since 2015, but so far the populations found have always been scarce. Unexpectedly, since 2019 abundant populations of the mosaic leafhopper have been reported within apple orchards in Trentino province. In 2022, the monitoring activities of the adult flight activity using yellow sticky traps was extended to other North Italian orchards confirming the widespread presence of *O. ishidae*. A comparison between different management regimes showed higher populations of the leafhoppers in organic orchards (82.1 ± 78 , means \pm SD) compared to conventional orchards (8.4 ± 10.5 , means \pm SD).

Furthermore, to explain the discrepancy between the monitoring data in vineyards and the high catches in apple orchards, we set up a laboratory experiment to investigate whether there was an insect preference between different host plants. The parameters taken into account were mortality and the 'nymph-adult' development time in two cultivated hosts (apple and grapevine) and two wild plants (hazelnut and hornbeam). On apple trees, mortality was not statistically different from wild hosts, whereas on vines a very high value was found (97% of individuals dead by the end of the experiment). Finally, the apple tree turned out to be the host on which the shortest development time to the adult stage was recorded compared to the other substrates (nymph-adult in 26.2 ± 2.2 days).

These data show that *O. ishidae* is better adapted and more widespread on apple trees than on grapevines in Italy, although there is still no certain information on its danger in apple orchards (e.g. its role as a vector of phytoplasma). Further research is ongoing to define these aspects.

KEY WORDS: alien species, leafhopper, development dynamic.

SESSION V

AGRICULTURAL ENTOMOLOGY

Relationship between *Halyomorpha halys* (Stål) population density, its damage, and phenology of three hazelnut cultivars (*Corylus avellana* L.)

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Brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive species accidentally introduced in Northern Italy in 2012 and becoming widespread in our country. This highly polyphagous species represents a serious threat to hazelnut production, even in Campania region, where 22.4% of the national yield is produced. The favorable pedo-climatic conditions of Campania allow the cultivation of a wide range of varieties that differ in quality and earliness. However, these conditions also favor the presence of pests; in fact, the damage caused by *H. halys* adds up to the already well-known hazelnut stink bugs. BMSB trophic activity causes different damages that vary according to the fruit development phase in which the bite occurs. Previous studies showed that, if the trophic activity occurs during the shell and embryo expansion, seed growth stops, causing "traumatic abortion" often followed by early drop. Differently, if the bite occurs during the kernel expansion, this can become either "shriveled" or "cimiciato". The "cimiciato" damage develops also during fruit maturation and is characterized by deformations and necrotic tissue. In addition to the aesthetic damage, the "cimiciato" hazelnut has an unpleasant flavor and is unmarketable. In this work, the relationship between *H. halys* population density, fruit phenology of three hazelnut cultivars, and bug damage was analyzed. The trial was conducted in 2022 in an orchard in Teano (CE). Contemporary with the BMSB monitoring through pheromone traps, the fruit development was studied in the following cultivars: San Giovanni, Tonda Romana (cultivated both in the absence of insecticides and in integrated pest management), and Mortarella (integrated pest management). Furthermore, to evaluate the response of hazelnuts to injury in the different stages of development, *H. halys* specimens were introduced in cages in the fields on the varieties grown without chemical treatments. The "cimiciato" damage recorded on untreated cultivars reached 40% for San Giovanni and 23% for Tonda Romana. The application of pesticides reduces the incidence of "cimiciato", and also removes the differences in damage between the cultivars. The relationship between the density of the BMSB population and the fruit phenology has shown that the "cimiciato" damage is significantly higher in the cv San Giovanni in the absence of insecticide treatments. In fact, in the period of the greatest occurrence of the insect, San Giovanni cv, the earliest cultivar, was in the kernel expansion phase.

The damage is lower on Tonda Romana, a late cultivar, which was in the phases of shell expansion and presence of the embryo in the same period. Finally, the tests with cages confirmed that also in the studied cultivars the BMSB trophic activity, which occurred at the fruit early stage, can cause high percentages of traumatic abortion and fruit drop. The results indicate that the integration between pest monitoring and phenology is the only way to optimize the efficiency of insecticide treatments and confirm the high harmfulness of BMSB in the absence of chemical treatments on hazelnuts.

KEY WORDS: Brown marmorated stink bug, Campania, damage, dried fruit, invasive species.

POSTER

SESSION V
AGRICULTURAL ENTOMOLOGY

The population outbreak of *Ectomyelois ceratoniae*, its polyphagy, and genetic variability

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The carob moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae), is a polyphagous pest native to the Mediterranean area. Currently, the moth is present in many countries of the world where it is reported to be particularly harmful to the production of several fruits in Iran (pomegranates), Tunisia (citrus fruits and dates), and Australia (almonds). The moth damages both fresh fruits in the field and stored dried fruits (walnuts, almonds, dates, carobs, dried figs, and peanuts). Recently, *E. ceratoniae* causes great injuries in Campanian walnut orchards. Walnut fruit sampling showed that 93.8% of the Lepidoptera found inside the fruits were *E. ceratoniae*, while the remaining 6.2% were other species, including the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). To discover more about moth biology, in 2021 some monitoring activities were carried out. The weekly monitoring occurred in three promiscuous orchards (walnuts and citrus plants), located in the province of Naples, by using delta traps activated with sexual pheromones. Furthermore, delta traps for monitoring the codling moth were also set to analyze how the carob moth is taking over *C. pomonella*. Simultaneously, because *E. ceratoniae* seems to prefer different host plants in different countries, a study of the mitochondrial haplotypes was conducted to evaluate the possible alimentary specialization of the populations. Therefore, specimens from different fruits (walnuts, oranges, carobs, almonds) and Campania and Sicily were then analyzed.

The data collected during monitoring confirmed that *E. ceratoniae* is a multivoltine species, with several generations partially overlapping each other. The numerous captures of carob moth confirmed the high percentage of damage found in the past; also, the small number of catches of codling moth confirmed its limited harmfulness to walnut orchards. The two species have life cycles characterized by flight peaks at different periods of the year and a dissimilar number of generations. The second generation peak of the carob moth occurs one month before the peak of the codling moth, explaining the greater harmfulness of *E. ceratoniae* compared to *C. pomonella*.

Actually, in many Campanian walnut orchards, monitoring and control activities focus exclusively on the codling moth. Therefore, the chemical treatments are carried out in the wrong periods, when the carob moth larvae are already in the fruits. The low number of codling moth catches, far from the intervention threshold, erroneously suggests not to treat. In the absence of focused treatments, carob moth develops without limitation, damaging the fruits in the field and the warehouse. Finally, the preliminary molecular results show the presence of only four haplotypes in the studied *E. ceratoniae* specimens, highlighting a very low genetic variability for an autochthonous species. One of these haplotypes is more widespread and frequent and infests all the studied hosts in different localities of Campania and Sicily. This haplotype is also present in other countries worldwide (Australia, Greece, Iran, and Tunisia). The results indicate the absence of food specialization of *E. ceratoniae*, confirm its dangerousness and highlight the existence of an invasive phenomenon of one haplotype at least.

KEY WORDS: Campania, Carob moth, haplotype, Lepidoptera, walnut.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Monitoring *Scaphoideus titanus* populations and pest control strategies in fragmented viticultural areas

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Flavescence dorée (FD) is a serious grapevine disease for European vineyards. The phytoplasma associated with FD is transmitted mainly by the leafhopper *Scaphoideus titanus* Ball (Hemiptera Cicadellidae). Monitoring of *S. titanus* populations is fundamental to define pest phenology and estimate its densities. Moreover, monitoring can provide information on the timing of insecticide applications, and the efficacy of control strategies. *Scaphoideus titanus* populations were monitored during 2021 and 2022 in a hilly area comprised in the territory of the Consortium Conegliano Valdobbiadene Prosecco Superiore DOCG (Treviso district, Veneto Region), where severe FD outbreaks have been recently reported. The spatial and temporal distribution of nymphs and adults was studied in four fragmented areas characterized by a high incidence of FD, using SADIE. The nymph population sizes in the two years were comparable whereas a huge decline in adult populations was observed in 2022. This result is likely due to the use of pyrethroids instead of systemic insecticides against nymphs. Spatial distribution analyses showed patches and gaps of both nymphs and adults in the four areas. Nymph and adult patches were overlapping in 2021, when insecticides were only partially effective, whereas patches were partially related in 2022. Adult patches in late summer of 2021 and nymph patches in spring of 2022 were significantly related. Control strategies adopted in 2022 were successful in three out of four areas. Results confirmed that FD and the related vectors should be managed at a territory level.

KEY WORDS: Flavescence dorée, *Scaphoideus titanus*, monitoring, spatio-temporal distribution, control.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Spatio-temporal distribution of grapevine leafhoppers (Hemiptera Cicadellidae) and their egg parasitoids (Hymenoptera Mymaridae) in vineyards in north-eastern Italy

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The leafhoppers *Empoasca vitis* (Göthe), *Zygina rhamni* Ferrari, and *Erasmoneura vulnerata* (Fitch) (Hemiptera Cicadellidae) are commonly detected in vineyards located in north-eastern Italy. Semi-natural areas contiguous to cultivated plots could play a role in the distribution of these pests inside vineyards. Woody vegetation outside vineyards is also crucial for the persistence of leafhopper parasitoids (e.g., Hymenoptera Mymaridae). In this work we investigated the effect of woody vegetation on the spatial and temporal distribution of the three leafhopper species and egg parasitoids potentially associated with them. Observations were carried out in four vineyards inhabited by the three leafhopper species and located in the Veneto region. Vineyard colonization by *E. vulnerata* adults was strongly affected by woody vegetation, while this phenomenon was less important for the other leafhopper species. Woody vegetation also influenced the vineyard colonization by egg parasitoids (*Anagrus atomus*). In some vineyards, the distributions of *A. atomus* and *E. vulnerata* were significantly associated. Sentinel eggs of both *E. vulnerata* and *Z. rhamni*, exposed in vineyards, were parasitized by *A. atomus*. The results obtained here can be used in the definition of habitat management practices for sustainable viticultural systems.

KEY WORDS: Hemiptera Cicadellidae, Hymenoptera Mymaridae, vineyards, woody vegetation, spatio-temporal distribution.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Effects of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *Macrolophus pygmaeus* (Hemiptera: Miridae) on morpho-physiological traits of plants

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Whiteflies (Hemiptera: Aleyrodidae) are considered critical pests able to attack a wide range of cultivated and ornamental plants in almost all areas of the world. In particular, *Bemisia tabaci* (Gennadius), defined today as a complex of several genetically different species, is a global pest that causes significant losses to a wide variety of crops. Indeed, it can affect the physiology and development of infested plants and can rapidly acquire resistance to insecticides, making its control problematic. In order to reduce *B. tabaci* infestations, the release of natural enemies has become increasingly important as an ecologically safe and effective biological control method. The mirid bug *Macrolophus pygmaeus* (Rambur) plays an important role in the natural control of *B. tabaci* but, due to its zoophytophagy, its overall effects on plants are not totally understood so far. More generally, a thorough understanding of the role of zoophytophagous insects within agroecosystems is needed to comprehend the evolutionary processes, the forces regulating multitrophic interactions, and the impact that different trophic levels might have on food production. In the present work, the impact on eggplant and tomato plants has been assessed comparatively in systems composed by “plant + *B. tabaci*” and “plant + *B. tabaci* + *M. pygmaeus*”, respectively. Main morphological and physiological traits of the plants (e.g. height, dry weight, leaf area and indirect chlorophyll content) have been considered. The study showed how the presence of *M. pygmaeus* can significantly reduce the negative effects caused by *B. tabaci* to host plants, strengthening the role that this zoophytophagous insect plays in the multitrophic system considered.

KEY WORDS: Whitefly, predator, zoophytophagy, trophic interactions, plant morphology, plant physiology.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Use of different attractants to improve the catches of *Bactrocera dorsalis* females

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The preventive pest monitoring plays a crucial role in the study of insect populations and in biological and integrated control programs. The monitoring of *Bactrocera dorsalis* is based on the use of traps activated with methyl-eugenol, a male lure which occasionally can catch some female specimens. Protein activated traps are used to catch females of tephritids, which exploit the need to take essential elements to complete egg development.

In 2022, the catches of *B. dorsalis* in Italy were recorded again about two years after the first findings. The catches occurred mainly in Campania region, in the province of Naples and, to a lesser extent, in the Salerno province and Emilia Romagna region. The monitoring activity carried out in 2022, using traps activated with different attractants, confirmed that the use of torula yeast as a food attractant ensures the catches of a low number of females of *B. dorsalis* (around 1% of the total) highlighting, therefore, the need for research additional attractive molecules to be used for both monitoring and the mass capture of the pest.

Laboratory tests, reported in literature, showed the attractive effects of 1-butanol for sexually mature *B. dorsalis* females. The objective of this work was to test the attractive efficacy of 1-butanol in field conditions. We conducted the attractiveness tests in five mixed orchards with a predominance of plant species of the *Citrus* and *Diospyros* genera. We performed two tests using 1-butanol respectively in agarose gel and in polyethylene (PE) bags of different thickness (90, 120 µm). In a first test, we compared the attractive power of Rebell Amarillo sticky traps (n=5) activated with the following substances in glass vials: 1-butanol gel, 1-butanol gel and torula, 1-butanol gel and yeast, only torula, and methyl-eugenol as control. In a second test, yeast was excluded, and 1-butanol gel vials were replaced with PE dispensers of different thickness. In both tests, traps were monitored weekly. Traps activated only with 1-butanol and 1-butanol in other combinations did not show greater attractiveness than traps activated only with torula. On the other hand, there was a substantial increase in the catching of other tephritids. However, the traps activated with methyl-eugenol confirmed a significant presence of *B. dorsalis* in the studied areas.

The results seem to exclude the possibility of improving the attractiveness of traps by the use of 1-butanol, at least for the populations of *B. dorsalis* so far found in Italy.

KEY WORDS: butanol, Campania, food attractants, methyl-eugenol, oriental fruit fly, torula.



SESSION V

AGRICULTURAL ENTOMOLOGY

Effects of food and pheromonal attractants and their combination on mass trapping of *Aromia bungii* (Faldermann) (Coleoptera: Cerambycidae) in fruit orchards in Campania - Italy

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Aromia bungii (Faldermann) is a wood borer native to Eastern Asia. The species is now established in two regions of Italy (Campania and Lombardy). *A. bungii* is regulated in EU as quarantine priority pest and targeted by eradication measures. This polyphagous pest causes considerable damage in fruit orchards and, to a lesser extent, to ornamental species belonging to the genus *Prunus* spp., boring larval galleries in tree trunks and main branches. Phytosanitary surveys are mainly based on field monitoring of infestation signs. In Campania, reproduction starts in the last decade of May with adults mainly active during June and July. The possibility to employ effective chemical attractants to trap adults could enhance phytosanitary field monitoring. With this aim during 2022 food and sex attractants have been used singly or in combination to bait Mastrap L[®] (Isagro, Italy) and Novatrap (Novapher, Italy), the latter slightly modified to facilitate the catches of *A. bungii*, in apricot orchards of Campania region during the flight period of the pest. Overall, the highest percentage of trapped beetles (72%) were obtained using both food and sex attractant as a lure. The modified Novatrap was more effective when triggered with the combination of food and sex attractants, while the Mastrap was more effective when activated only with the sex attractant alone. The low level of trapped adults has been ascribed to patchy field populations of *A. bungii* affected by the eradication measures. Further field trials are needed to validate the trend observed so far.

KEY WORDS: invasive species, monitoring, mass trapping red-necked longhorn beetle.

SESSION V

AGRICULTURAL ENTOMOLOGY

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

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The impact of climate change on insect populations can significantly affect future pest management strategies. Herbivorous insects are highly sensitive to temperature increases, especially those with low thermal thresholds. This makes it likely that crop losses due to insect outbreaks will increase substantially. To address these challenges, it is necessary to use successful forecasting models and a range of control methods in combination with appropriate digital tools for monitoring insect populations, as well as reliable and immediate crop damage data. Optimizing the monitoring efforts is essential for site-specific Integrated Pest Management (IPM) over vast areas, and the demarcation of monitoring zones based on varying levels of risk over time can play a significant role in this regard.

Bactrocera oleae Rossi (Diptera: Tephritidae) is a monophagous pest of *Olea* spp. in a large number of European countries, Asia, and Africa, and was accidentally introduced to North America. It is expected to expand its range due to global warming, colonizing areas at higher latitudes and altitudes. Increased temperatures, particularly in autumn, could extend the oviposition period of *B. oleae*, resulting in high yield losses.

In this study, we analysed *B. oleae* infestation over seven years (2015-2021) across 85 olive farms in Umbria, Italy. We evaluated the relationship between infestation indexes and a set of agroclimatic variables for modelling purposes. Given the strong relationship between olive fly development and weather data, the analysis was based on weather data (maximum and minimum temperature and rainfall data), morphometric and environmental data, and on data obtained from infestation surveys. *Bactrocera oleae* infestation (1% attack) was negatively affected by altitude and spring mean daily temperatures, while it was positively influenced by winter mean daily temperatures.

Concerning the day of first attacks, an increase in altitude delayed the occurrence of attacks in the July-August period. Conversely, high winter and spring temperatures had a positive effect on anticipating the attacks.

Infestation occurring in September-October was negatively affected by increasing altitude and high spring temperatures, but positively by high winter temperatures and temperatures 7 days before the monitoring day. For the September-October period, precipitation delayed *B. oleae* attacks, while high temperatures 7 days before the monitoring day anticipated them.

Our data would be helpful for the development of predictive models and for increasing the reliability of Decision Support Systems in olive orchards.

KEY WORDS: Agro-climate variables, olive orchards, monitoring.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

A physiologically-based population model for the phenology of *Philaenus spumarius*

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The meadow spittlebug, *Philaenus spumarius* L. (1758) (Hemiptera: Aphrophoridae), is the main vector of the plant pathogen bacterium *Xylella fastidiosa* (*Xf*) in Europe. *Xf* is the causal agent of the severe disease of olive quick decline syndrome (OQDS) in Apulia. Uprooting infected plants and control of the vectors are the pillars of the containment strategies of this devastating disease. The main objective of vector control is to prevent colonization of olive canopy by newly-emerged adults. Therefore, IPM strategy focuses on suppressing juveniles in the herbaceous cover and protecting olive trees from surviving adults. In this frame, the development of accurate tools to describe and predict the phenology of *P. spumarius* populations in agroecosystems is fundamental to support rational decision-making. A properly calibrated phenological model allows management actions to be planned correctly, greatly increasing their effectiveness and reducing both the costs of implementation and the resulting environmental impacts.

We developed a temperature-driven physiological-based model to predict the phenology of *P. spumarius*. We estimated the parameters of bio-demographic functions used in the population model describing the processes of diapause termination and age-distribution of overwintering individuals, and the temperature-dependent development and mortality rates by integrating lab experiments and literature evidence. The model has been calibrated and validated with field data collected in the Liguria and Apulia regions (Northern and Southern Italy, respectively).

The model can be used for proper design and implementation of Integrated Pest Management strategies to control *P. spumarius* populations and thus support *Xf* containment.

KEY WORDS: Meadow spittlebug, *Xylella fastidiosa*, Physiologically-based model, Integrated Pest Management, Pest phenology.

SESSION V

AGRICULTURAL ENTOMOLOGY

Assessment of biological soil quality in organic farming systems in the Caserta area

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Edaphic fauna is involved in numerous processes that ensure soil functionality and structure, as well as nutrient availability to plants. The Soil Biological Quality Index based on the microarthropod community (QBS-ar) is a qualitative index exploiting the presence/absence of different edaphic groups, more or less adapted to hypogean life, to assess soil biological quality. The QBS-ar index assumes that microarthropod groups, in particular those adapted to underground life, can be linked to stable and less anthropized ecosystems. Therefore, the QBS-ar index can be used to highlight the impact of different agronomic soil management on soil fertility.

This study compared different organic systems (vineyard, hazelnut grove, non-productive chestnut grove) in the Caserta area during the spring period. The three systems had different soil management, involving specific tillage. The non-productive chestnut grove underwent almost no soil management. On the other hand, the hazelnut grove was subjected to frequent cutting (monthly), while the vineyard had an alternate management, with naturally grassed inter-rows (cut occasionally: 2-3 per season) and inter-rows on which a green manure mixture had been sown and then buried (early April), and therefore the soil was uncovered by turf during the experiments.

For every grove (forest-chestnut grove, hazel grove, grassed vineyard, ungrassed vineyard) 6 replicates (each one including 3 sub-replicates) were provided. The samples were transported in refrigerated containers to the laboratory and subjected to extraction in Berlese funnels for 10 days. The recovered arthropods were identified and their QBS-ar indices calculated by summing the EMI values of every identified groups. The surveys covered the spring season involving two different sampling periods: the first in mid-spring (April 20-May 6) and the second in late spring (May 25-June 10).

The results of the activities showed significant differences according to both sampling period ($P < 0.05$) and arboretum type ($P < 0.0001$), but not due to the interaction between the two factors. In general, QBS-ar values were significantly lower in the second sampling period than in the previous one, showing that higher temperatures tend to reduce soil moisture and consequently the animal component. Among groves, the largest differences were found between the forest-chestnut grove (average QBS-ar: 219.7 ± 16.4) and the two productive tree systems (QBS-ars ranging from 151.2 - 86.7). In addition, the QBS-ar of the vineyard's grassed inter-rows was significantly higher ($T1 = 151.2 \pm 3.9$; $T2 = 133.3 \pm 27.7$) than that of the ungrassed inter-rows ($T1 = 131.8 \pm 3.0$; $T2 = 86.7 \pm 15.6$) and the hazelnut grove ($T1: 120.6 \pm 7.96$; $T2: 110.7 \pm 10.2$), indicating that soil tillage and frequent mechanical activity can negatively affect soil biological fertility.

KEY WORDS: QBS-ar; soil fertility; arthropods; edaphic fauna; hazelnut; vineyards.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Computerized management of quarantine pest surveys in the context of the new EU regulatory framework

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The use of computerized techniques is now essential for the management of data relating to EU relevant quarantine pest surveys (EU Regulation 2016/2031) and, even more so, when the surveys are referred to the 20 priority pests (EU Regulation 2019/1702).

The Plant Protection Service of the Campania Region has for more than a decade been using an information technology (IT) support developed in collaboration with Ariespace (a spin-off of the "Federico II" University of Naples) called SIMFito (Information System for Phytosanitary Monitoring).

SIMFito is a Web application, the result of a decade long evolutionary update, which allows online entry of data relating to the investigations carried out by the inspection staff, researchers and technicians of the Regional Phytosanitary Coordination Unit - URCoFi, which ensures the traceability of data relating to field and laboratory investigations for all the harmful organisms to be investigated.

As a first step, the surveyor identifies the survey site, characterises it by filling in a format that allows, among other things, geolocation within the same application. After entering the general data of the site, the field activities are entered, which begin with the selection of plant species present at the site and being monitored. Automatically, harmful organisms that the EPPO has associated with the plant in question are selected. A regional database has also been implemented for all those specific cases not covered by the aforementioned EPPO database.

Furthermore, the detector enters data concerning the areas, the number of plants viewed, the traps positioned/checked, any samples taken with production of the relative code in order to guarantee traceability also in the subsequent phase of sample management by the laboratory. Thus, with a single observation it is possible to investigate even multiple pests by tracking them, separately (multisurvey). The platform ensures the management and archiving of laboratory analysis conducted on the samples, including those taken in the traps.

Complete data acquisition enables the production of official investigation reports, including laboratory test reports. The system ensures the traceability of the analyses carried out with the production of the test report, which contains the data relating to the botanical species or other matrix, the pest sought and found, the method used, the conditions of the sample upon acceptance, etc. Thanks also to this IT support, the phytopathological laboratory of the Campania Region has been accredited according to the EN ISO/IEC 17025 standard and therefore recognized as an official laboratory under Regulation (EU) 2017/625 and pertaining to the national laboratory network.

With the georeferencing of the sites monitored with traps, the database allows the export of reports in tabular and cartographic format, aggregating the data on a spatial and temporal scale. An infected /infested zone and a buffer zone can be defined around sites found positive for a given pest, according to spatial parameters chosen on the basis of the risk of diffusion of the single pest. At the same time, the system makes it possible to provide phytosanitary advice to citizens by freely accessing the phytosanitary bulletin section.

POSTER



SESSION V

AGRICULTURAL ENTOMOLOGY

The action plan to counter the spread of *Bactrocera dorsalis* in the Campania region

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The phytosanitary monitoring activity coordinated by the Campania Region Phytosanitary Service (SFR) in agreement with the partners of the Regional Phytosanitary Coordination Unit (URCoFi), allowed to detect in June 2022 the presence, in a trap placed in the territory of Palma Campania (Na), of a male of *Bactrocera dorsalis*. This fly for its characteristics is among the 20 quarantine pests considered a priority for the EU (Regulation (EU) 2019/1702) whose monitoring is mandatory. With the official confirmation of the finding, the SFR has included in the timeline provided by Article 29 of Legislative Decree 19/2021, the information required by the European electronic notification system - Europhyt so that the Ministry can notify the EU Commission. With the succession of new catches, it became necessary to take the first official measures, activate the Territorial Unit for Phytosanitary Emergencies, and define the Delimited Area (DRD 176/2022).

The main official measures immediately taken by the SFR were: preparation of reinforced monitoring in the delimited area; phytosanitary treatments at sites with captures; applications of "Attract & Kill"; prohibition of the movement of fruits of host species from production sites where traps with captures were placed; and removal of fallen fruits on the ground from the same sites. Despite the adoption of the first phytosanitary measures, the findings of tephritid continued also in the autumn-winter period, so all procedures were activated, which led to the approval of the Action Plan for *B. dorsalis* by the National Phytosanitary Committee (at the meeting of November 28, 2022) and the Campania Region (Regional Council Resolution No. 714 of 20.12.2022).

The Action Plan was developed in line with the provisions of Article 27 of Regulation (EU) 2016/2031, and therefore the urgently needed phytosanitary measures were identified to mitigate the risk caused by *B. dorsalis* from the affected area having two main strategic objectives: that of preventing the dispersal of the insect into new areas and that of putting in place actions tending to contain or eradicate the insect in the short term.

The first objective includes blocking movement for:

- the fruits of host species
- the soil from production fields of host species
- the planting plants with soil
- the planting plants of host species with hanging fruit

The phytosanitary measures more closely related to eradication have included:

- application of insecticides
- selective insecticidal bait treatments
- male annihilation technique
- soil treatment
- harvesting and total destruction of symptomatic fruits or those present on the plants where the traps that have caught in the last two months have been placed and on neighboring plants located within a 10-m radius
- increasing the number of traps.

As an exception to the block of the movement of fruit from the infested area, the movement of fruit that originates from an uninfected production site recognized as such under ISPM - FAO No. 10 and previously approved by the SFR in compliance with a whole series of phytosanitary requirements was allowed.

KEY WORDS: *Bactrocera dorsalis* , Action plan.

POSTER



SESSION V

AGRICULTURAL ENTOMOLOGY

National Plant Pest Survey Program

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The increase in the spread of plant pests (ON) with all the economic, social and ecological consequences brought the European Union to adopt drastic measures to make the surveillance of quarantine organisms more extensive and effective in the whole European territory. The new Phytosanitary regime is defined by Reg. (EU) 2016/2031, is applied in its entirety from December 2019 and establishes protective measures against pests of plants. At the national level, a "Working group for the national survey program on harmful plant organisms" (WG) has been established with the purpose of coordinating and conforming the activities inherent to the National Phytosanitary Monitory Programmes. This system works thanks to the collaboration between the Masaf - Ministry for Agricultural, Food and Forestry Policies, the CREA-DC Defence Research and Certification (which is recognized as the National Reference Institute for Plant Protection with Legislative Decree no. 19 of 2 February 2021), the Regional Phytosanitary Services and the National Phytosanitary Committee (CFN). Between the different tasks, the WG prepares, on the basis of proposals received from the regional Phytosanitary Services and autonomous provincial, the National Survey Program, the Multi-Annual Programs, and the National Phytosanitary Program. The WG defines objectives and investigation criteria based on the risk assessment of harmful organisms indicated in the Implementing Regulation 2019/2072 and subsequent amendments, it draws up the technical sheets for the investigation procedures, it defines the documentation relating to the annual reporting of the technical data for the activity carried out and for the participation and reporting in the Phytosanitary Programs financed by the EU. The WG makes use of and manages the development of IT tools for the management and collection of investigation data (i.e. MORGANA). The purpose of this contribution is to describe the national organizational system and its many activities, already started in 2015, with the aim of intensification, harmonization and appropriate assessments of effectiveness for Phytosanitary surveillance according to European standards.

KEY WORDS: EU Reg. 2016/2031; survey; pest; plant protection.

SESSION V

AGRICULTURAL ENTOMOLOGY

***Halyomorpha halys* in Mediterranean areas: invasion drivers, genetic diversity, and potential biological control**

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An early understanding of the introduction pathways of invasive species in newly invaded areas is crucial to plan efficient strategies to prevent pest outbreaks. The brown marmorated stink bug (*Halyomorpha halys*) is an East Asian invasive species currently established across Europe and North America, where it caused important economic losses for growers. Information regarding the occurrence, genetic diversity of the pest and the factors shaping its population dynamics in Southern Italy remains scarce. In the current study, we monitored *H. halys* in three different habitats potentially important for the spread and the establishment of *H. halys* (i.e., crop fields, urban gardens, fruits warehouses) selected along a gradient in urban habitats in the landscape. Then, in crop fields and urban gardens we measured the potential biological control by natural enemies with sentinel egg masses. Finally, we carried out a genetic characterization of the specimens collected in Apulia region using multiple markers to identify the potential pathways of entry in the region. We found that high proportions of urban habitats in the landscape generally supported a higher occurrence and abundance of *H. halys* in crop fields, whereas high cover of semi-natural habitats increased parasitism and predation rates. Moreover, the parasitoids *Anastatus bifasciatus* and *Ooencyrtus* sp., important control agents of *H. halys* in areas where the pest is already established, were the most common species observed. Our findings suggest that the composition of agricultural landscapes affects both the occurrence and the biological control of *H. halys* in newly invaded areas, potentially influencing the establishment probability of local populations. Finally, the finding of several host and symbiont haplotypes within the Apulia region, and high similarity with the Northern Italy *H. halys* populations, suggested continuous events of introduction probably occurred from the Northern part of the peninsula. Future monitoring programs focused on both urban and cultivated areas will be fundamental to track the spread of *H. halys* in this region in the future.

KEY WORDS: alien insect, brown marmorated stink bug, invasive pest, landscape composition, parasitism, predation.

SESSION V

AGRICULTURAL ENTOMOLOGY

Contact toxicity of *Punica granatum* (L.) fruit peel extracts against the granary weevil *Sitophilus granarius* (L.) (Coleoptera, Curculionidae)

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The pomegranate, *Punica granatum* (L.) (Punicaceae), is an important fruit plant adapted to a wide range of agroclimatic conditions. The peel, which represents about 40–50% of the total fruit weight, is considered a by-product of industrial processing.

In this study, contact toxicity of fruit peel extracts of two commercial pomegranate cultivars, Ako (early maturing) and Wonderful (late maturing), was investigated against *Sitophilus granarius* (L.) adults.

Fruits of both cultivars were collected from rural areas near Foggia (Apulia region, Italy). Pomegranate peels were manually removed, dried (27°C x 15 days), grinded, and sieved (Ø 0.5 mm). Aliquots of the dried powders (100 g) were extracted for 24 h at room temperature with solvents of decreasing polarity: acetone, diethyl ether and hexane (300 mL). Colouring of powders and extracts was determined (L*a*b*, digital colorimeter Apple). Each crude extract was centrifuged (40,000 x g, 10 min, T 10°C), filtered (Whatman No. 113) and dried under vacuum in a rotary evaporator (40° C, 200 mb). Dried crude extracts (150 mg) were resuspended in the corresponding solvent (1 mL) and two-fold serial dilutions (150-18.75 µg/µL) were prepared for topical application. One microliter of each extract was applied on the pronotum of a *S. granarius* adult in thanatosis. For each extract, 3 groups of 10 insects were treated. An equal number of individuals tested with the respective solvent was used as control. Mortality of adults maintained in the dark at 27±1°C and 55±5% r.h. was recorded 1, 24, 48, and 72 h after treatment.

The extracts of Ako and Wonderful cultivars showed different pigmentation. The highest mortality percentages (70-90%) were registered for the hexane and acetone extracts of both cultivars, while diethyl ether extracts showed a low toxicity. Twenty-four hours after treatment, mortality of adults treated with hexane and acetone extracts of both cultivars was significantly higher than control starting from the 37.50 µg/adult dose. For all the extracts, mortality did not vary significantly from 24 to 72 h after treatment. The 24-h lethal dose 50 (LD50) values calculated for the hexane and acetone extracts were respectively 81.14 and 81.86 µg/adult for the Ako and 129.67 and 47.75 µg/adult for the Wonderful cultivars. The 24-h LD90 values of the same extracts were 306.37 and 231.76 µg/adult for the Ako and 221.49 and 141.40 µg/adult for the Wonderful cultivars. Overall, the highest adult contact toxicity was found for the acetone extract from the Wonderful fruit peel.

The results of this study strongly suggest that pomegranate fruit peels, even in a circular economy approach, are potential sources of bioactive compounds that could represent an ecological alternative for integrated pest management of storage pests.

KEY WORDS: pomegranate, contact toxicity, storage pests, plant extracts, botanicals, bioinsecticides.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Tabby knot-horn moth of olive in the North-East Italy: monitoring and investigation of the control techniques

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Euzophera pinguis (Haworth, 1811) and *Euzophera bigella* (Zeller, 1848) (Lepidoptera: Pyralidae), also called Tabby knot-horn moth, are harmful to many cultivated and spontaneous trees. These two species have been observed causing damage to olive trees in Spain, Greece, and Lebanon.

The presence of these Tabby knot-horn moths was known in Northern Italy, but only recently damages were observed on olive groves. The losses were caused by the larval trophic activity what causes branches desiccations.

In 2022, in the Garda Lake area (northern Italy) a study to evaluate the flight curves and the efficacy of two different devices used for the Mass trapping to control *Euzophera* species was performed.

The monitoring was carried out using the pagoda trap (ISAGRO[®]) lured by two specific sexual pheromones (ECONEX[®]), to evaluate the Mass trapping control two different traps polillero and pagoda (ECONEX[®], ISAGRO[®]) lured with the same specific pheromones were compared.

The monitoring showed that *E. pinguis* was the more present Tabby knot-horn moth in the study area. *E. pinguis* held two generation per year, with the flight peaks in late spring and in autumn. About *E. bigella* the low catches didn't allow to perform the population dynamics study.

The Mass trapping study showed that the pagoda traps were the more efficient to intercept *E. pinguis*. Further analysis to understand the suitable numbers of traps per hectare to control the species will be necessary.

KEY WORDS: *Euzophera pinguis*, *Euzophera bigella*, olive tree.

SESSION V

AGRICULTURAL ENTOMOLOGY

The role of the National Reference Institute for Plant Protection (CREA-DC) and of the National Laboratory Network in support to the Italian phytosanitary organization

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In December 2019, the new phytosanitary regime defined by Regulation (EU) 2016/2031 came into force, establishing rules for determining phytosanitary risks and measures to reduce them to an acceptable level. In addition, the Commission with Regulation (EU) 2017/625 has introduced a single system of controls for human, animal, and plant health (One Health approach). At national level, the Legislative Decree n.19 on 2021/02/02 redefined the new national phytosanitary system introducing with the art. 8 the National Reference Institute for Plant Protection (CREA-DC) to support the Italian phytosanitary system. The priority of the new regime is to reduce the environmental and social-economic consequences of the introduction of relevant and prior quarantine pests for EU. The new regulatory framework confers great importance on diagnostics by defining the roles of European and National Reference Laboratories and, as an absolute novelty, the obligation to operate under the EN ISO/IEC 17025 accreditation system for those laboratories that carry out analyses in support of official controls. In this context, the Legislative Decree n.19/2021 establishes the national laboratory network, which identifies official laboratories as well as other designed ones that can participate in the achievement of new regulatory framework objectives. Finally, the latest Ministerial Decree n.169819 on 2022/04/13 defines and illustrates the "Characteristics, areas of competence, structures and modality of identification of laboratories operating on plant protection" to which all the different kinds of laboratories must comply. This contribution aims to illustrate the role of the National Reference Institute for Plant Protection and the structure of the National Network of laboratories with the aim of highlighting all the potential of this organization for territorial surveillance and phytosanitary diagnosis in entomological field.

KEY WORDS: accreditation ISO/IEC 17025 system, harmful organism, plant protection, National Reference Institute, National Reference Laboratory, laboratory network.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Effects of natural insecticides on the green peach aphid *Myzus persicae* (Sulzer) and its natural enemies *Propylea quatuordecimpunctata* (L.) and *Aphidius colemani* Viereck

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Natural insecticides are frequently proposed as an environmentally safer alternative to synthetic insecticides. The efficacy and selectivity of these plant protection products, however, are often only partially supported by empirical evidence.

In this study, we tested the effectiveness of 4 natural pesticides belonging to different categories on the green peach aphid *Myzus persicae* (Sulzer), as well as their selectiveness towards two of its natural enemies, the ladybird *Propylea quatuordecimpunctata* (L.) and the parasitoid *Aphidius colemani* (Dalman). The tested products (used in both direct and residual exposure assays) were white thyme essential oil, a commercial product based on sweet orange essential oil (Prev-Am[®]), crude garlic extract and Marseille soap (as an insecticidal soap), with natural pyrethrum as a positive control due to its known low selectivity.

Tested products were scarcely effective against the tested aphid if compared with pyrethrum, and no difference between treatments was observed on parasitoid pupae mortality. However, the other natural insecticides were in general less impactful than pyrethrum on ladybird larvae, meaning that if applied against other pests they have a lower chance of harming this aphid biocontrol agent in real-world scenarios. Some of the products (Marseille soap, Prev-Am[®]) did however show topical toxicity on ladybird larvae, and thyme oil caused extensive phytotoxic effects on the cabbage leaves used as a substrate in the experiment, possibly indirectly leading to higher mortality in ladybird adults via biofumigation. This underlines the necessity for case-by-case evaluations of natural insecticides, considering both effectiveness and side effects, all while avoiding broad generalizations.

KEY WORDS: Pest control, ladybird beetles, parasitoids, natural insecticides, bioinsecticides, selectivity, efficacy.

POSTER



SESSION V

AGRICULTURAL ENTOMOLOGY

Mating disruption of *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae): Five years of field experience

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The intraspecific communication of many insects, including leafhoppers, is mainly mediated by vibrational signals transmitted along the surface of solid substrates, such as plants. Therefore, behavioral manipulation strategies, aimed at controlling these species, can be based on applied biotremology techniques, such as vibrational mating disruption (VMD). The American grapevine leafhopper, *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), is the first species for which a synthetic signal was developed, based on a species-specific signal, capable of interfering with mating communication. In 2017, in San Michele all'Adige (TN), for the first time in the world, a system of transducers was installed to transmit the disturbance signal of *S. titanus* to the plants of a vineyard (1.5 hectares, Cabernet Franc).

In the following five years, the vineyard was subjected to a technical monitoring, to verify the transmission efficiency of the transducers, and a biological monitoring, to evaluate the effect of VMD on the population of *S. titanus* and another leafhopper susceptible to VMD, *Empoasca vitis* (Göthe). For the technical monitoring, the vibrations on the trellis and plants were measured with high sensitivity accelerometers or laser vibrometers, to evaluate the spectral and signal intensity parameters. The populations of the leafhoppers were evaluated in two phases: for the juvenile forms approximately 500 leaves were visually inspected from May to August, while for the adults, yellow sticky traps (Glutor, Biogard) were exposed from July to October and changed weekly. The biological monitoring was repeated in an adjacent vineyard comparable in size, variety and management (control area).

The detection of vibrations along the trellis and on the plants showed that the limit of action of the transducers is 50 m and it is significantly reduced both during the season, due to the increase in the weight of the plants, and over the years, due to the wear of the instruments that were active and exposed to climatic conditions throughout the period of operation. A comparison of the monitoring data between the control area and the treated area revealed a decrease of about 50% of the juvenile forms of both *S. titanus* and *E. vitis* for the first three years; subsequently, in concomitance with the reduction in efficiency of the transducers, the difference between the two areas was dramatically reduced. Adult catches do not appear to reflect the results observed on juvenile forms, with similar and in some cases higher catches in the treated area than in the control. However, this data can be explained by an increase in the flight activity of adults in the presence of the disturbance signal, as it has been shown in laboratory experiments.

In conclusion, VMD is a sustainable and effective control strategy, if a minimum intensity threshold of the disturbance signal is guaranteed. Further area wide studies with different trellis systems will have to be carried out to optimize the installation of the transducers.

KEY WORDS: biotremology, integrated pest management, insect communication, mating behavior.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Invasive mealybugs: a study of the genetic variability of *Phenacoccus peruvianus* in southern Italy

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The growth in international trade and climate change are the main causes of the increase in the number of invasive pests accidentally introduced and acclimatized in Italy and the European Union. *Phenacoccus peruvianus* Granara de Willink (Hemiptera: Pseudococcidae), the bougainvillea mealybug, is an invasive phytophagous causing serious damage to both ornamental and crops, as it produces necrosis, leaf loss, and development of sooty mold. *Phenacoccus peruvianus* is native to neotropical South American areas and was first reported in the Mediterranean basin in Almeria (Spain) in 1999. *P. peruvianus* spread into other Mediterranean regions in the following years and was recorded in Portugal, France, Corsica, the Balearic Islands, and Sicily. In Campania, the first report occurred on bougainvillea in 2021.

Literature reports that *P. peruvianus* can also damage species belonging to the genus *Solanum*. Therefore, in 2022, during the URCoFi project activities (Regional Unit of Phytosanitary Coordination), monitoring was carried out in nurseries and private gardens. Samples from different areas of Naples, Salerno, and Cosenza provinces were collected. The specimens were isolated from the following hosts: Bougainvillea sp. and *Solanum melongena* L. Activities had the main aims to evaluate the possible existence of a specificity host plant - pest population and to estimate the genetic variability of the populations (study of haplotypes) present in Italy. Identification of specimens collected from infested areas was carried out through molecular analysis based on the COI barcoding fragment. The preliminary analysis showed the presence of a single haplotype in all the populations evaluated, except for the sample recovered in Portici on bougainvillea, which showed a silent aminoacidic mutation. The presence of only two haplotypes is probably linked to a bottleneck phenomenon due to the arrival of a few individuals. The presence in Portici of a second haplotype suggests that there were at least two independent introductions of the species.

The comparison with homologous sequences available in gene databases allowed us to evaluate the analogy with specimens collected in different geographical areas. In particular, the same haplotype recorded in Spain and France was found also in Italy. Because the first record of *P. peruvianus* took place in Spain and the found haplotype was the same both in Italy and France, it is possible that the infestation in Spain served as a bridgehead for subsequent settlements in European countries.

Further samplings will be performed to assess the presence of infestations on other host plants and confirm the existence of COI mutations, in association with other molecular markers to better define the invasive populations of *P. peruvianus* in Italy.

KEY WORDS: *Bougainvillea* sp., Campania, haplotypes, invasive species, *Solanum* sp.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Insight into the effects caused by bug feeding on hazelnuts

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In main growing areas, hazelnut is attacked by insects belonging to Coreidae and Pentatomidae (Hemiptera), which cause both quantitative and qualitative production losses, depending on the affected phenological stage. Currently, the most abundant and harmful bug species in North Italy are *Halyomorpha halys* (Hemiptera: Pentatomidae), a highly invasive exotic species, and *Gonocerus acuteangulatus* (Hemiptera: Coreidae), a native species whose populations are increasing due to rising summer temperatures. There are several studies on the presence of bugs in hazelnut orchards and on the effects of their feeding activity on hazelnuts, however, knowledge on the correlation between population levels and damage rate (corked) at harvest, as well as on organoleptic characteristics of corked kernels, is still scarce. Therefore, research was carried out to evaluate: i) the harmfulness of *G. acuteangulatus*, by caging individuals on hazelnut branches and examining the nuts at harvest; ii) the sensory characteristics in kernels undamaged and damaged by *G. acuteangulatus*; iii) the potential of an electronic nose to discriminate corked kernels from healthy ones, as well as kernels damaged by different bug species, such as *G. acuteangulatus*, *H. halys*, *Nezara viridula* and *Palomena prasina*.

Gonocerus acuteangulatus confirmed its harmfulness on hazelnuts, but showing high variability among individuals: in fact, some individuals damaged all available kernels inside the cage, while others damaged none. This variability could be due to behavioural or physiological characteristics of *G. acuteangulatus* (such as feeding on other parts of the plant, or particular characteristics of salivary enzymes), or to plant responses, which need further investigation. Through sensory analysis performing pairing tests, kernels damaged by *G. acuteangulatus* and healthy ones were mostly significantly recognized. In particular, the differences were enhanced both by cold storage and by roasting, therefore the adoption of appropriate post-harvest management protocols could mitigate the effects of the bug damage on hazelnuts. Based on the volatiles emitted from hazelnuts, the electronic nose was able to distinguish healthy kernels from corked ones, and also kernels damaged by different bug species. Therefore, the electronic nose could become a useful post-harvest tool to distinguish healthy kernels from corked ones, or even to determine which bug species the hazelnuts were damaged by in the field.

KEY WORDS: *Gonocerus acuteangulatus*, *Halyomorpha halys*, corked kernels, sensory analysis, electronic nose.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

***Halyomorpha halys*: a model for the use of research dogs in phytosanitary controls**

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The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae), one of the most important worldwide pest organisms, belongs to a group of insects which includes numerous species of phytosanitary interest, whose global diffusion is favored, in particular, by the behavior of the highly mobile adults, which in periods with low temperatures they look for artifacts to take refuge, colonizing not only natural ravines and buildings, but also materials for export as well as containers and large vehicles for the transport of goods, such as ships. The marked polyphagia, the high fecundity and mobility, combined with the difficulties of containing the populations below the economic thresholds of damage with insecticides with low environmental impact, make the BMSB and other Hemiptera "pests" extremely difficult to control. In recent years, in order to face new risks of invasion by other similar species through the development of new and more effective means of early detection, in various countries attention has been placed on the study and verification of the potential of use of specially trained dogs to detect the presence of these insects in ports and airports.

In the present work, a specific training method developed using *H. halys* as a model insect and the results obtained from verification tests of the effectiveness of identifying the "target" by a working dog with previous experience in monitoring programs of other hexapods are proposed. The investigations were carried out in different environments, both in the field, with the search for the target odor on plants, and in closed environments such as storage warehouses and agricultural sheds. The analysis of the data obtained allowed us to confirm the validity of the training method tested, highlighting the possibilities of using research dogs to counter the risks of accidental introduction of invasive harmful organisms with quick checks in the import control sites, also as a complement to other containment methods. It is then discussed how research dogs could represent an important cornerstone of a broader strategy to protect plants from alien species.

KEY WORDS: *Halyomorpha halys*, pest, research dogs, early detection.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Preliminary studies about genetic variability and infestation dynamic of *Bactrocera dorsalis* in Campania

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In 2018 the monitoring activity of the URCoFi project (Regional Phytosanitary Coordination Unit) in Campanian areas, led to the first finding in Italy of some adults of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). The following increase in monitoring activities allowed the interception of some other adults in 2019, while the following two-year period (2020-2021) was without any records. Since the beginning of summer 2022, in several areas of Naples and Salerno, there has been a recovery in the findings of *B. dorsalis* in traps, especially in Naples province. These new captures have brought the intensification of monitoring activities.

Therefore, a study was planned to evaluate a) if the in-progress invasive phenomenon is due to a single species and to single or multiple introductions of several entities belonging to the *B. dorsalis* species complex b) the variability between haplotypes of the invasive population c) the dispersal ability of the species based on the diffusion dynamics in the infested territories. Traps activated with methyl-eugenol or torula were placed and checked weekly. Furthermore, a preliminary genealogy analysis of the mitochondrial haplotypes was performed with the statistical method of parsimony on the COI barcoding region of several collected adults.

The analysis of the captures recorded in the survey periods showed an expansion of the diffusion area in the initial infestation period, coinciding with the summer-fall seasons, which was followed by a progressive reduction in the number of captured adults and in the size of the infested area where the captures have been recorded. No findings have been registered in concomitance with the coldest temperatures. The analysis of the dynamic of the captures has led us to speculate about the most likely starting core of the infestation.

The COI barcoding analysis has highlighted the record in Italy of many new haplotypes to be added to the 7 already identified in previous years. Two of the newly retrieved haplotypes appear to be more frequent and widespread than the others. Furthermore, two of the haplotypes found in 2022 are identical to others found in 2018 and 2019, but the absence of catches for two years, despite many placed traps, leads us to believe that these are new introductions. The analysis of the distribution of the haplotypes, in relation to the time passed since the first records, seems to indicate that new introductions are frequent and repeated over time even if they often do not lead to active infestations. The study of the expansion of the capture area confirms the excellent flight and dispersion ability of *B. dorsalis*. However, despite the moderate variability found in the haplotypes found in 2022, the invasive phenomenon underway is due to a single species.

The analysis of the haplotypes found in Campania will be deepened to identify which haplotype (or group of haplotypes) will be able to settle in our territories and the dispersal and diffusion ability in the long period, to estimate how this invasive species could threaten our agriculture.

KEY WORDS: barcoding, haplotypes, invasive species, oriental fruit fly, population network.

ORAL PRESENTATION

SESSION V
AGRICULTURAL ENTOMOLOGY

***Bactrocera dorsalis* monitoring: comparison of different types of traps**

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Bactrocera dorsalis (Diptera: Tephritidae) (the oriental fruit fly), is an invasive species native to subtropical Asia. This species has been found in Campania since 2018, but only in 2022 there was a copious increase in specimens caught in traps.

The probable *B. dorsalis* establishment causes enormous concern because the species shows a high invasive potential due to the wide host range (about 400 species), high reproductivity, multivoltinism, excellent active spreading ability, and environmental adaptability. *Bactrocera dorsalis* belongs to a group that includes a variable number of species, known as the *Bactrocera dorsalis* complex. Its identification is puzzling because the species of the complex are very close genetically and morphologically. The adults of *B. dorsalis* have high intraspecific variability with overlapping variation ranges of many diagnostic characters; besides, the frequent presence of hybrids further complicates the identification. These issues may affect the phytosanitary investigation strategy, including the choice of attractants to use. It is already known that the species belonging to the *B. dorsalis* complex are attracted by several substances defined as "male-lure", in particular methyl-eugenol (ME), a natural origin compound, and Cue-lure (CL), a synthetic substance. In addition, the type of trap used can also affect trapping efficiency. Two trials were carried out to compare the effectiveness of the two attractants and different types of traps for monitoring the species.

Traps were placed in mixed fruit-tree fields, with a prevalence of plants belonging to the genera *Citrus*, *Diospyros*, *Prunus*, and vegetable species. The two substances (ME and C-L) were compared in McPhail traps at five sites while different types of tephritid traps, usually commercialized in Italy, were compared at six sites. In particular, we compared two types of yellow traps with adhesive panels (Rebell Amarillo and Wing), two Lynfield types with a contact insecticide, and one McPhail type. All traps have been baited with methyl-eugenol.

No catches have been found in traps with C-L, therefore, the comparison between the two attractants showed that the species found in Campania is attracted only by the ME. These results are consistent with claims by several authors that *B. dorsalis* is attracted by ME and no fruit fly species is attracted by both ME and C-L.

The results of the traps comparison showed that those with sticky panels are more effective than the others. In addition, the comparison between Rebell Amarillo and McPhail traps, carried out for a longer period, showed that the former fourfold the catching potential of the latter.

The greater effectiveness of the sticky traps could be due to the exposed surfaces of the sticky panels which makes the captures easier, compared to other traps that require a specific path to enter in the traps.

The results offer interesting opportunities to improve pest monitoring, which is crucial in delimiting the infested area and pest management.

KEY WORDS: Campania, Lynfield, McPhail, methyl-eugenol, oriental fruit fly, sticky traps.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Infection control on Italian Aphrophoridae vectors of *Xylella fastidiosa pauca* ST53

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The *Philaenus spumarius* L. (Hemiptera Aphrophoridae) drives the *Xylella fastidiosa pauca* ST53 invasion, infecting plants in Italy. The consequent OQDS (Olive Quick Decline Syndrome) disease has been decimating olive trees in Apulia. The delayed-to-infection OQDS symptoms lead to invasion mismanagement. Symptoms appear later in the infections, and infected reservoir trees boost the yearly cycles of *Xf* invasion by resident vector acquisition and spread.

Vectors cause damage more than proportional to their number, propagating the borne pathogen in time and space more than one time per vector. Damage occurs in the long term, with the death of the olive trees, annihilating past investments, year cost of cultivation, and future incomes. *Xf* exterminates the susceptible plants, admitting no care exists for the disease.

The action threshold for vector control is unacceptably low in practice, given that each vector can infect by probing a candidate food plant and multiplying the feeding acts in the adult life window. Our first attempt was to model an Infection 1 (the first transmission leading to the infective process to a hitherto non-infected plant) control hypothesis, managing to kill each vector at the time of their first feeding on the olive plant. The attempt suggests lowering to a direct proportionality each vector infection option. Effective infection management involves killing that part of the vector population that causes Infections 2, 3... n by mechanical, chemical, or biological control actions.

The efficacy of infection control depends on the vector death at the first feeding on olive. Killing the same number of vectors after they perform infections 2, 3... n on different plants will result in banal and ineffective vector control, with each insect inflicting multiple infections instead of only one. Transmission control in infected areas can significantly limit the invasion of *Xylella* into disease-free up to stop the pathogen invasion. Mass movements of infectious vectors will diminish as the population decreases during the time of juveniles and olive-feeding adults' extermination. Soon, a scarce vector population would make transmission rare, leading to the isolation of the bacterium in infected plants. The progressive death of diseased plants will reduce the disease to a few active foci, simplifying its management eventually.

Finally, the olive tree has a guild of pests that can compromise crop yield. Modelling a holistic pest management strategy for olive trees will ensure integrated production, limiting insecticides to strictly necessary and targeted interventions. Control actions must consider the TARDIS (Time And Relative Dimension In Space, BBC®) factors of the pests and the crop to make the strategy feasible in all olive-growing contexts.

KEY WORDS: Antifragility, IPM, CoDiRO, Alien, Invasive, Quarantine Pest.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Developing a mass-trapping system for the control of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae) in fruit orchards

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a pest native to Asia that is invasive in Italy, where it is considered a major pest in fruit orchards. *Halyomorpha halys* can develop two generations per year and is characterized by high mobility and a wide range of host plants. These aspects and the low residual activity of insecticides limit the efficacy of current management strategies. In light of increasing the efficacy of these strategies, the use of a mass-trapping system constituted by a sticky panel placed on a tank filled with water and using aggregation pheromones as attracting lures was investigated. In the first experiment, the effect of panel color was tested. The trapping capacity of yellow, black, and transparent panels was compared by considering the number of *H. halys* individuals as well as beneficial insects (i.e., pollinators and natural enemies) trapped during the autumn and spring periods. In a second experiment, the effect of this system was evaluated on the infestation level and associated damage in pome fruit orchards. In a final experiment, the trapping system was combined with *H. halys* repellent products applied in orchards. The black panel captured high number of *H. halys* and low number of beneficial insects. Mass trapping system based on the black panel significantly reduced pest infestation in pome fruit orchards and their use also in association with repellents shows promising results in controlling this pest and reducing the associated damage.

KEY WORDS: brown marmorated stink bug, *Halyomorpha halys*, IPM.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Effect of sulfur on *Halyomorpha halys* (Hemiptera: Pentatomidae) in laboratory, semi-field and field experiments

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Halyomorpha halys (Hemiptera: Pentatomidae) is an insect native to East Asia and invasive to the US and Europe, becoming a severe pest in fruit orchards. Being characterized by high polyphagia and mobility and subjected to low insecticide residual effects, *H. halys* is a challenging pest to manage with the current pest control programs that require frequent synthetic insecticide applications to control economic damage. Behavioral manipulation based on repellents represents a potential tactic that could be used in integrated pest management strategies against this pest. Here we investigated sulfur-based product's repellent and mortality effect in laboratory, semi-field, and field conditions. In the laboratory, we assessed the mortality of *H. halys* induced by sulfur applications at different doses and its response to sulfur in olfactometer and choice experiments. Semi-field experiments were conducted to test repellence towards sulfur-treated apple potted plants. Finally, the effect of sulfur application on *H. halys* infestation and associated damage was studied in pome fruit orchards. No effect on *H. halys* mortality was observed in the laboratory, while in the olfactometer and choice experiments, sulfur treatments were less preferred than untreated ones. A lower infestation level was observed in sulfur-treated plants than in untreated ones, significantly reducing pest damage. These results suggest that sulfur affects *H. halys* population by acting as a repellent, with no effect on insect survival. A significant reduction of *H. halys* infestation with no effect in terms of phytotoxicity was observed in the field, suggesting that sulfur applications can be a valid tactic for reducing losses due to *H. halys* in fruit orchards. The use of sulfur in an integrated program can be a promising tool to minimize the use of synthetic insecticides.

KEY WORDS: Repellent products, invasive pest, IPM.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

The European project "Virtigation": an opportunity to learn more about whitefly pests of vegetable crops and to update on strategies for their control

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The project "Emerging viral diseases in tomatoes and cucurbits: implementation of mitigation strategies for durable disease management (VIRTIGATION)", funded by the European Union under the Horizon 2020 programme, was launched in June 2021 and will end in May 2025. It involves 25 partners, from 12 different countries, and aims to develop solutions for the control of emerging viruses on cucurbits and tomatoes in Europe and the Mediterranean basin, caused by begomoviruses (biologically transmitted by insect vectors) and tobamoviruses (mechanically transmitted). Among the main objectives of the project, particular entomological interest is related to: i) the understanding of plant-virus-vector interactions; ii) the identification of ecological factors that favour outbreaks of infections; iii) the biology of vector insects and their virus transmission efficiency under climate change conditions; iv) the enhancement and optimization of natural resistance, with particular reference to the possible lower attractiveness of plants towards vector insects; v) the development of solutions for the integrated control of the viruses under study and their vectors. As a partner of this project, the research unit of the University of Catania (Department of Agriculture, Food and Environment – Di3A) will overall have to: a) carry out a comparative survey in various partner countries of the project (mainly Belgium, France, Italy and Spain) aimed at the critical analysis of the methods used for the control of *Bemisia* spp. gr. *tabaci* on both protected and open field vegetable crops; b) coordinate field trials to be carried out in Belgium, Italy and Spain, to evaluate the efficacy of new plant extracts with insecticidal action isolated at the Katholieke Universiteit Leuven (Belgian partner of the project), in comparison with both other molecules with reduced environmental impact and with low doses of conventional insecticides commonly used in vegetable production, also analysing the secondary effects on the auxiliary fauna (natural enemies and pollinators); c) carry out field trials in Italy, with 15-20% of the most promising accessions among those identified by the Natural Resources Institute (British partner of the project), to evaluate their performance in terms of resistance to *Bemisia tabaci* MED; d) hypothesize the possibility of combining different approaches studied in the project for the control of virus vector whiteflies, coordinating the formulation of robust and durable strategies to be implemented as a result of the project. Details on the research actions carried out and/or in progress, as well as on the first results achieved, are provided in the poster presented here.

KEY WORDS: Whiteflies, Vectors, Viruses, Tomato, Cucurbits.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Multitrophic interactions induced by water stress on tomato and wheat

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Biotic and abiotic factors can cause bottom-up effects that alter the tritrophic interactions (plant-phytophagous-natural enemy), therefore they can be used to optimize both the use of resources and the control of plant pests. Within this context, the response of *Solanum lycopersicum* and *Triticum aestivum* under the effect of different water regimes (optimal, medium and high drought stress) and different insects (*Phenacoccus solenopsis* and *Sitobion fragariae*) was evaluated in laboratory conditions, observing also the behavioural response of two natural enemies (*Cryptolaemus montrouzieri* and *Aphidius colemani*) to the volatile organic compounds emitted by the plants in a two-way olfactometer. Moreover, the expression of involved plant defense genes was evaluated. The different water regimes and the presence of pests triggered different behavioural responses on the tested natural enemies. The analysis of gene expression showed the activation of some metabolic pathways involved in the defense of plants, such as the jasmonic acid, salicylic acid and abscisic acid pathways. The results could be useful as a basis for the future development of plant protection strategies in the framework of Integrated Pest Management of agricultural crops. However, further studies are needed to assess the volatile compounds emitted by induced plants and field tests to validate the obtained results.

KEY WORDS: blackberry cereal aphid, cotton mealybug, mealybug destroyer, multitrophic interaction, tomato, water stress.

SESSION V

AGRICULTURAL ENTOMOLOGY

***Tuta absoluta* as vector of the emerging Tomato Brown Rugose Fruit Virus**

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Tomato (*Solanum lycopersicum* L., Solanaceae) is one of the most important and widespread crops worldwide and it is constantly threatened by numerous pathogens, including Tomato brown rugose fruit virus (ToBRFV). ToBRFV was characterized in 2014 and currently it is one of the most significant challenges for the tomato production worldwide. It is an extremely infectious tobamovirus whose transmission mainly occurs through plant-to-plant contact or infected sap by adherence to different surfaces and tools. Moreover, it has been recently demonstrated the ToBRFV transmission due to the mechanical action of *Bombus terrestris* L. (Hymenoptera: Apidae), the only arthropod vector known to date. In this context, the potential role of other insects as vectors of this pathogen was investigated testing one of the main tomato pests, the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Laboratory tests were carried out using tomato seedlings and *T. absoluta* reared under laboratory conditions within insect-proof cages. Sap extract of ToBRFV ToB-SIC01/19 isolate was mechanically inoculated into healthy tomato plants through foliar micro-lesions that facilitate the virions' entry. RT-qPCR analysis were carried out to confirm the ToBRFV infection on the inoculated plants; subsequently, uninfected adults of *T. absoluta* were released and left ovipositing on infected plants. The obtained pest progeny resulted positive to ToBRFV presence in the emerging adults. In a second test, *T. absoluta* ToBRFV-infected adults were released on healthy plants, in order to evaluate their ability to transmit the virus. The latter hypothesis was confirmed. Finally, pupae obtained from larvae fed on infected plants have been disinfected and compared with not disinfected pupae. Similarly, adults obtained from disinfected and not disinfected pupae were analysed and compared. The results confirmed the ToBRFV presence in both adults and pupae of disinfected and not disinfected *T. absoluta*, showing that the ToBRFV is located within the insect body. In conclusion, this study demonstrated for the first time that *T. absoluta* is able to acquire and transmit ToBRFV, thus having a key role on the disease epidemiology. Further tests will be needed to verify the ToBRFV virions localization in the insect vector body.

KEY WORDS: South America tomato pinworm, insect vector, ToBRFV, tobamovirus.

SESSION V

AGRICULTURAL ENTOMOLOGY

***Xylosandrus compactus*: a potential danger for the cultivation of Olive (*Olea europea* L.) in Italy**

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Xylosandrus compactus (Eichhoff) is a scolytid native to the tropical and sub-tropical areas of South-east Asia (India, Sri Lanka, Indochina, the Indonesian archipelago, Micronesia, the Philippines and Japan), which is currently widespread also in Africa, Indian Ocean islands and the USA. After being reported for the first time in Europe (in the province of Naples, Italy) in parks, on Holm, Oak and Laurel, it caused strong infestations not only on the latter plant but also on *Ceratonia siliqua*, *Quercus robur*, *Q. pubescens*, *Q. suber*, *Acer pseudoplatanus*, *Cercis siliquastrum*, *Euonymus europaeus*, *Magnolia grandiflora*, *Liquidambar styraciflua* and *Arbutus unedo*. In monitoring programs carried out in central and Southern Italy on Mediterranean maquis attacked by *X. compactus*, a large number of fungal microorganisms were isolated, identified and evaluated for their potential pathogenicity.

More recently, widespread attacks of this Scolytid have been also observed in nurseries in central Italy with massive outbreaks on young potted Olive trees, with extensive branch decay of the affected plants.

From woody tissues of olive trees colonized by *X. compactus*, strains of *Cladosporium perangustum* and *Neofusicoccum parvum*, as well as *Geosmithia pallida* were obtained in the laboratory.

Pathogenicity tests carried out so far, have shown the formation of cancers on olive trees inoculated with *N. parvum*, while isolates *C. perangustum* and *G. pallida* produced necrotic lesions only at the point of inoculation without any formation of cancers.

Following field observations and tests conducted in a controlled environment due to the high polyphagy of *X. compactus*, trials are underway. The aim is to evaluate potential risks not only for olive trees in nursery environments but also in olive groves due to the ability of this pest to colonize the current-year assurgent suckers on the main branches of affected plants.

All activities were funded by the Project MiPAAF “Contributi per il controllo delle emergenze fitosanitarie determinate da *Xylosandrus compactus*, *Xylella fastidiosa*, *Botrytis cinerea*-CO.XI.BO” (DM 0037134 del 31/12/2018)

KEY WORDS: Scolitidae, invasive species, *Olea europea*.

SESSION V

AGRICULTURAL ENTOMOLOGY

**The role of mating systems on reproductive outcomes of *Aromia bungii*
(Coleoptera: Cerambycidae)**

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Insects display a wide range of reproductive behaviors, but two common patterns include female acceptance of copulations with multiple males (polyandry) or repeated copulations with a single male (monogamy). The study of mating systems is crucial for exploring the evolutionary ecology of insect species, as it plays a significant role in determining reproductive success and population dynamics. The Asian wood-boring pest *Aromia bungii*, also known as the red-necked longhorn beetle, has attracted widespread attention in recent years due to its invasive process across Europe, affecting mainly southern Italy in the last decade, and its parallel spread to Japan. *A. bungii* colonizes healthy *Prunus* sp. trees during their early stages, leading to extensive damage to stone-fruit orchards. Given the significant threat, the European Union has classified *A. bungii* as a priority harmful pest. Several studies have recently been conducted to investigate the biological and ecological traits of the invasive beetle, which have provided significant insight into the biotic potential of this species. Nevertheless, the role of mating systems in determining the reproductive success of *A. bungii* remains unclear and needs to be explored. In this work, we investigated the impact of polyandry and monogamy on some key reproductive traits of *A. bungii* females, under controlled laboratory conditions. Our results revealed that polyandry had a positive effect on the beetle's reproductive performance, as females who engaged in polyandrous behavior increased their lifetime fecundity and egg viability compared to those maintained in monogamous relationships. This suggests that the benefits observed could be attributed to the increased genetic diversity resulting from mating with multiple males. These preliminary data emphasize the significance of considering the impact of mating patterns on the reproductive outcomes of *A. bungii*. Furthermore, our study underscores the importance of assessing the reproductive behavior of invasive species to provide valuable insights for formulating effective management strategies.

KEY WORDS: biotic potential, invasive species, longhorn beetle, mating system.

SESSION V

AGRICULTURAL ENTOMOLOGY

Survey on the efficacy of chromotropic sticky traps in *Philaenus spumarius* (L.) adults monitoring

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The meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae) is the main vector of *Xylella fastidiosa* Wells (Xathomonadales: Xanthomonadaceae), a Gram-negative quarantine bacterium causal agent of the "Olive quick decline syndrome" (OQDS). To verify the efficacy of the vector control practices aimed to reduce the spread of the disease, as well as to evaluate the risk of introduction and successive spread of the bacterium, it is of fundamental importance to monitor the spittlebugs both in *X. fastidiosa* infected and free areas.

Adult spittlebugs are mainly monitored by sweeping of ground vegetation and olive canopies by using a sweeping entomological net. This technique requires specialized personnel and is time consuming. To improve the monitoring efficiency, by increasing the investigated areas, it is necessary to validate other sampling strategies. Chromotropic sticky traps are widely used to monitor Auchenorrhyncha species in agriculture, and their positioning and recollection do not require specialized employees.

In the present work, carried out in seven olive groves across Abruzzo region (Italy), the efficacy of different types of chromotropic sticky traps to monitoring *P. spumarius* adults was assessed. Four already commercially available yellow chromotropic traps (the panel traps Biogard Glutor® and Serbios SuperColor®, the star-shaped Isagro Wing® and the roof-shaped Biogard Bactrap®) and an experimental green panel sticky trap (RGB 150, 220, 70) were tested.

The traps were positioned on the trunk and the canopy of olives and replaced every two weeks from May to September. The *P. spumarius* captures were compared to sweepings carried out with an entomological net both on canopy and ground vegetation at trap installation and trap changes.

Isagro Wing® and Biogard Glutor® showed the highest efficacy in *P. spumarius* adult monitoring, with a high statistical correlation with sweepings.

KEY WORDS: Meadow spittlebug, *Xylella fastidiosa*, OQDS.

SESSION V

AGRICULTURAL ENTOMOLOGY

Biology and phenology of the carob moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) an emerging pest of walnut, *Juglans regia* L. in Italy

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The carob moth, *Ectomyelois* (*Apomyelois*) *ceratoniae* (Lepidoptera: Pyralidae) is a highly polyphagous species with a cosmopolitan distribution and can attack crops in field as well as in the post-harvest. In recent years, *E. ceratoniae* started to cause economic damage to the English walnut, *Juglans regia* L. in northern Italy, infesting walnuts mainly in the post-harvest period. Management of this pest requires precise information on its biology and phenology. To study the phenology of this pest in the field and in stored fruits in Veneto region (northern Italy), observations using pheromone traps and fruit sampling in-season and post-harvest were performed in 2021 and 2022. The biology of *E. ceratoniae* was studied with laboratory experiments aimed at evaluating life history parameters on two different diets. Carob moth flights were observed from 2nd May to 24th October 2021, and from 4th May to 17th October 2022. Insect captures in traps fluctuated during the sampling period and peaked during the harvest. Few carob moth larvae were collected from fruits in the field, while high numbers of larvae were found in the walnuts that were stored. In post-harvest, new adults and larvae emerged from nuts, suggesting the development of one complete generation in post-harvest and underlying its role as a food storage pest. The comparison of field and laboratory data suggests the occurrence of three/four generations in the field and one complete generation on stored fruits. Pest activity during harvest time appears to be a critical aspect of its management.

KEY WORDS: English walnut, emerging pest, IPM.

SESSION V

AGRICULTURAL ENTOMOLOGY

Behavioural responses of *Philaenus spumarius* to UV-A and visible light

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The meadow spittlebug, *Philaenus spumarius* (Hemiptera: Aphrophoridae) is the primary vector of *Xylella fastidiosa*, an invasive xylem-limited bacterium causing Olive Quick Decline Syndrome (OQDS), that heavily affects olive groves in Apulia, Italy. Therefore, to limit *X. fastidiosa* spreading, it is of utmost importance to optimize the surveillance and control of *P. spumarius*. Various insects use multimodal cues to orient themselves and find host plants. *Philaenus spumarius* has a well-developed visual system; therefore we investigated in the laboratory its attractiveness towards UV light-emitting diodes (LEDs) combined with different wavelengths of visible light spectrum (blue, red, yellow, and green). We found significant differences between male and female responses to different wavelengths. Males showed higher attraction towards blue and red, while females had higher responses when exposed to red and UV light. Contrary to males, females were not attracted to blue colour. Further investigations are ongoing, aiming to elucidate *P. spumarius* periodicity of attraction and its attraction when exposed to combinations of different colours. Moreover, the most effective wavelength combination will be evaluated in field conditions. The results will provide basic information for understanding behavioural responses of *P. spumarius* to visual cues and for the development of more effective monitoring traps.

KEY WORDS: *Philaenus spumarius*, plant pathogen vector, monitoring.



SESSION V

AGRICULTURAL ENTOMOLOGY

Evaluation of transgenerational phenotypic plasticity of *Tetranychus urticae* Koch (Acari: Tetranychidae) exposed to repeated sublethal acaricide treatments

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Transgenerational epigenetic inheritance is the transmission of epigenetic based information from one organism to the next generations, a process that possibly affects the phenotypic traits of offspring without altering its DNA nucleotide sequence. Because novel epigenetic states can alter gene expression, organisms can show phenotypic plasticity in response to environmental stimuli experienced by previous generations, even in absence of the original conditions that established it (i.e., transgenerational phenotypic plasticity).

Indeed, several studies clearly described the existence of both adaptive and maladaptive transgenerational plasticity able to affect the fitness of succeeding generations. The real implication of this phenomenon in both adaptation to environmental cues and in evolution is still to be exhaustively evaluated and understood. The aim of the present research is to evaluate how and to what extent transgenerational phenotypic plasticity may affect response to acaricide treatments of two-spotted spider mite (*Tetranychus urticae* Koch) a polyphagous agricultural pest able to rapidly develop resistance to different acaricides.

A homozygous *T. urticae* line was developed by crossing full siblings (>20 generations). Thereafter, the homozygous line was divided in two groups (4 genetically identical lines/group). Each line was successively treated for 7 consecutive generations with water (control lines) or with sublethal concentrations of acaricide (5.85 g/l abamectin; abamectin lines), respectively. Then, a common garden experiment (CGE) was performed by treating both groups either with abamectin or with water (factorial design 2 x 2). CGE was repeated for three consecutive generations after the end of abamectin or control treatments. Mites used for CGE were sampled for DNA extraction to perform bisulfited sequencing and to study *T. urticae* epigenetic profile (methylome).

The evidence acquired leads to conclude that transgenerational phenotypic plasticity is involved in *T. urticae* response to the acaricide treatment. During the first CGE, mites previously treated with abamectin showed a maladaptive response, depositing, if currently treated, fewer eggs than mites never treated with abamectin ($p < 0.05$). This difference cannot simply reflect the physiological condition of mites previously treated with abamectin, because not treated CGE control lines showed the same deposition rate as not treated CGE abamectin lines indicating a good physiological condition of all mites. Moreover, during the second CGE, a similar result was obtained utilizing mites born two generations after the last abamectin application, suggesting that transgenerational response is taking place. Differences between previously treated or not treated mites were not evident during the third CGE, suggesting that two consecutive generations might be enough to deactivate the transgenerational response. The adaptive and evolutionary meanings of these observations in the framework of *T. urticae* response to acaricide treatments needs to be further exploited.

KEY WORDS: epigenetic, red spotted spider mite, resistance, abamectin.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

The invasion dynamics of the Japanese beetle (*Popillia japonica*) in northern Italy

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Knowledge of the population ecology of an invasive insect pest is fundamental for elaborating strategies and implementing actions aimed at its eradication or containment. The Japanese beetle (*Popillia japonica* Newman) was first discovered in northern Italy, in the Ticino valley along the border between Lombardy and Piedmont regions in 2014. Since then, it has spread across an area of more than 16,000 square kilometres. Over 100 economically valuable plant species have been identified as potential hosts, including maize, soybean, grapevine, apple, peach, and kiwifruit. In this study, we analysed monitoring data on larvae and adults of *P. japonica* collected between 2016 and 2021 by the Regional Phytosanitary Service of Lombardy Region to investigate the population phenology, growth, and spread of the species during its ongoing invasion in northern Italy.

The results show that both the adult flight period and the population abundance of *P. japonica* increase with the time since the first occupation of a site. The average adult abundance was around 11 and 27 individuals per trap per day during the first two years of presence of the pest, and it steadily increased to more than 200 individuals per trap per day in the fifth year. We simulated the spread of the pest using a reaction-diffusion model. The results of the model showed that the speed of invasion is variable according to the direction of the spread, ranging from 5.5 to over 13 kilometres per year. To better understand the spreading process and the factors affecting the speed of invasion, we analysed 14 different spreading trajectories starting from the area of first detection in Italy. The results show that habitat suitability for *P. japonica* plays an important role in influencing the speed of the travelling fronts. Higher speed has been found in those trajectories characterised by more than 80% of habitats suitable for the pest (arable land, perennial meadows, broadleaf forests, urban green areas, rice fields, or agricultural woodlands). Lower speed was found in those trajectories characterised by over 40% of non-suitable areas (coniferous or mixed forests or heavily urbanised areas).

The present work provides relevant elements of the invasion ecology of *P. japonica* that could be useful for planning and implementing local eradication actions. If eradication is not technically feasible, knowledge of population phenology and growth might support both the timely implementation of control measures, and the prioritisation of the efforts in areas where higher population abundances are expected. The current findings were produced as part of the GESPO project, which aims to develop rational and sustainable solutions to prevent the spread and potential impact of *P. japonica*.

Funding: The project GESPO was funded by "Direzione Generale Agricoltura - Regione Lombardia - D.d.s. 28 marzo 2018 - n. 4403D.g.r. n. X/7353 14 novembre 2017".

KEY WORDS: Japanese beetle, Invasive species, Pest phenology, Pest population growth, Pest management.

POSTER

SESSION V

AGRICULTURAL ENTOMOLOGY

Local management and landscape intensification affect pollinator diversity, pollination services and production in sweet cherry orchards in Mediterranean areas

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Fruit production strongly depends on insect pollination for fruit development. However, intensification of fruit production both at the local and landscape scale can influence pollinator communities, potentially impacting the corresponding pollination services. In this study we explored how local management (organic vs. conventional) and landscape intensification (distance from seminatural habitats) affect pollinator diversity, pollination services and production in sweet cherry orchards (*Prunus avium*) in Mediterranean areas. We found conventional orchard management (compared to organic) and distance from seminatural habitats to negatively affect both abundance and diversity of pollinator communities. Wild pollinator abundance and flower visitation was also negatively correlated to honeybee abundance, indicating potential competition for flower resources. Sweet cherry fruit set was positively influenced by pollinator diversity but not by honeybee abundance. These findings suggest that agricultural intensification both at the local and landscape can impair pollination services in cherry orchards. Interestingly, we found cherry quality (sugar content) to be negatively correlated to fruit set, highlighting a potential trade-off between cherry production and biodiversity conservation. Sustainable management strategies to support sweet cherry production need to consider wild pollinator communities and necessitate both local and landscape interventions.

KEY WORDS: agricultural intensification, biodiversity, honeybee, insect pollinators, sustainable crop production.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

***Epiphyas postvittana* (Walker), *Platynota stultana* Walsingham and *Thaumatotibia leucotreta* (Meyrick) three invasive tortricid pests recently intercepted in Italy
(Lepidoptera)**

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The present paper reports observations on the distribution, host plants, morphology, and biology related to the light apple moth *Epiphyas postvittana* (Walker), the omnivorous leafroller, *Platynota stultana* Walsingham, the false codling moth, *Thaumatotibia leucotreta* (Meyrick), three invasive tortricid pests recently intercepted in Italy. Considering the wide host range of these pests have the potential to damage many cultivated and wild plants in the European and Mediterranean Plant Protection Organization (EPPO) region. Their interception clearly demonstrates that they have the potential to enter and establish itself in the Southern and Mediterranean parts of Europe. Because the eggs, larvae, and pupae can be associated with fruit and plant material, as well as readily transported, vigilance and early detection methods are critical to minimise the probability of these species' introduction and establishment.

KEY WORDS: *Epiphyas postvittana*, *Platynota stultana*, *Thaumatotibia leucotreta*, invasive pests, Italy.

POSTER



SESSION V

AGRICULTURAL ENTOMOLOGY

Carlina acaulis* essential oil is highly effective against *Tetranychus urticae* and harmless to its predatory mite *Neoseiulus californicus

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Tetranychus urticae Koch, a polyphagous cosmopolitan pest, is primarily controlled chemically, resulting in the emergence of several resistant mite populations. Among new and more environmentally friendly control tools, botanical pesticides represent a valuable alternative within Integrated Pest Management strategies. This study investigated the lethal and sub-lethal effects of the essential oil (EO) obtained from roots of *Carlina acaulis* L. (Asteraceae) on *T. urticae* and its predatory mite, *Neoseiulus californicus* (McGregor). Gas chromatography-mass spectrometry (GC-MS) analysis showed the presence of carlina oxide as predominant constituent (98.7%) of the EO. Acaricidal tests were performed on eggs, nymphs, and adult females of *T. urticae* (312.5, 625, 1250, 2500 and 5000 µL/L), as well as on eggs and females of *N. californicus* (1250 and 5000 µL/L on eggs and females, respectively). Two-choice tests were conducted on phytoseiid females. *Carlina acaulis* EO toxicity was higher on *T. urticae* females if compared to the nymphs (LD50 1145 and 1825 µL/L, respectively); egg mortality and mean hatching time were significantly affected by all tested concentrations. Negative effects on the daily oviposition rate of *T. urticae* were recorded testing concentrations ranging from 625 to 5000 µL/L, the population growth rate was negatively affected only when the three higher concentrations (1250, 2500 and 5000 µL/L) were tested. No toxic effects on *N. californicus* females were found, but a strong repellent activity was noted. Repellent activity was reduced or even disappeared after 48 h from applications. Overall, the *C. acaulis* EO reduced longevity and fecundity of *T. urticae* adults, but not that of *N. californicus*, allowing us to propose it as a potential novel active ingredient for developing eco-friendly acaricides against *T. urticae*.

KEY WORDS: Asteraceae, green acaricides, repellence, side effects, Tetranychidae, Phytoseiidae.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

Response of some olive cultivars to *Resseliella oleisuga* (Targioni Tozzetti) attacks

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Although *Resseliella oleisuga* is considered a secondary phytophagous, climate change and cultivation intensification could favor its diffusion and make the species an important phytophagous in olive-growing environments. In general, the species does not represent a problem in olive groves, but it can have a significant impact on young plants and in nursery. The females lay their eggs in the cambial tissues of the host plants and following the trophic activity of the orange-colored apodous larvae, pseudogalleries are formed which lead to tissue necrosis.

The aim of this study was to evaluate the response of different olive cultivars to the attack by this gall midge and to confirm whether the insect was able to wound the plant tissues or needed pre-existing wounds (Bagnoli & Gargani, 2019). The study was conducted for three years (2019-2021) in a private farming in Terranova da Sibari (CS), consisting of a monovarietal olive grove (cv. Tondina), in which there were plants with full-blown *Resseliella* symptoms. Arbosana, Capena, Favarol, Sargano, Pirunara and Sinopolese cultivars were used in 2019; Carolea, Tondina, Nocellara del Belice and Frantoio in 2020 and, finally, Leccino, Coratina, Nocellara etnea, Arbequina, Frantoio, Carolea, Favarol, FS17, Tondina and Sinopolese in 2021. In all three years, 4 plants for each cultivar were used (2 with wounds and 2 for control), placed under the canopy of the infected plants, and the plants were monitored from June to October on a weekly basis.

Statistical data analysis showed that: in 2019 the least infested cultivars were Favarol and Sargano, while Arbosana, Capena, Pirunara and Sinopolese had high and comparable levels of infestation; in 2020 Carolea was the least infested cultivar while the other three (Frantoio, Nocellara del Belice and Tondina) recorded similar infestation values; finally, the least infested cultivars were: Carolea, Coratina, Frantoio and Tondina, while Leccino was the most attacked cultivar. The greater number of larvae found in the wounds of some varieties compared to others can be attributed to the females probably attracted by kairomones associated with bark lesions. In all three years, only the seedlings that showed wounds were attacked by *R. oleisuga*, while no symptoms of attack were ever found in the intact seedlings, confirming the fact that the presence of exposed bark is an essential condition for the oviposition. The results of this survey can provide useful information in the selection of tolerant olive cultivars in the case of new plantings, not only in areas where the pest is already present but also in view of its possible spread. Furthermore, the confirmation of the insect's inability to autonomously damage the tissues of the host plants represents a useful indication of the importance of agronomic management (Vizzarri, 2018) and of natural and/or pruning wounds. Furthermore, an in-depth study of the kairomones emitted by wounds would be useful in order to evaluate their degree of attractiveness towards gall midge.

The research activity was carried out thanks to the funding of the project SALVAOLIVI “Salvaguardia e valorizzazione del patrimonio olivicolo italiano con azioni di ricerca nel settore della difesa fitosanitaria”.

KEY WORDS: *Resseliella oleisuga*, olive tree, Calabria.

ORAL PRESENTATION

SESSION V

AGRICULTURAL ENTOMOLOGY

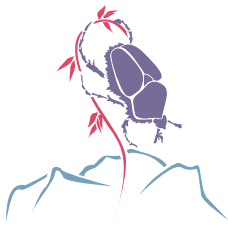
Smart monitoring: leveraging deep learning techniques for effective pest detection

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Early detection of arthropods is crucial for the success of biological control and Integrated Pest Management programs. Recent advancements in technology, such as the development of automatic traps, provide new opportunities for remote monitoring. One of the most promising technologies for agriculture is the use of artificial intelligence (AI) to analyse the data collected by sensors and accurately detect insect pests. AI can be trained to recognize specific shapes, colours, and behaviours of pests using computer vision techniques and distinguish them from other objects or animals in the environment. Integrating automatic traps with AI-based pest detection and identification systems can lead to more efficient and sustainable pest management practices, reducing the use of pesticides while minimizing the impact on the environment and human health. Here, we present a novel AI-based approach aimed at developing a smart trap for monitoring selected key pests of greenhouse tomato, i.e., *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), and *Liriomyza* spp. (Diptera: Agromyzidae). To date, two different networks have been trained: a detection-based approach and a density estimation-based technique. The detection-based method involves training an AI model to detect objects in an image by identifying specific features or patterns. This approach is commonly used in deep learning and consists in training a neural network to classify and localize objects in an image. The model is trained on a dataset containing labelled images of a yellow stick trap with objects of interest (i.e. the insects), along with their corresponding bounding boxes. During training, the model learns to recognize the features that are associated with the target objects and uses this information to detect the objects in new images. The density estimation technique involves training an AI model to estimate the density of objects in an image without explicitly detecting them. This approach is particularly useful when the objects of interest are difficult to distinguish from the background or when the images contain many objects. The model is trained on a dataset that contains images of yellow sticky traps with objects of interest using dot annotation. The target density map is then generated by centring a Gaussian on these points and applying fixed spreads determined empirically based on the average size of the objects. Both detection-based and density estimation techniques have their advantages and disadvantages, and the choice of approach depends on the specific task and dataset. Detection-based methods are generally more accurate but require more labelled data and computational resources. Density estimation techniques are faster and require fewer data but may not be as accurate in certain scenarios. Overall, training AI models for insect detection is a complex and iterative process that involves selecting the right approach, collecting, annotating data, and optimizing the model for performance.

KEY WORDS: artificial intelligence; density estimation; detection; tomato; whiteflies.



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Session VI

SESSION VI

INSECTS AND MICROORGANISMS

Genetic variability of autochthonous isolates of entomopathogenic fungi belonging to the genus *Metarhizium* and implications for the control of *Popillia japonica*

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In 2014, the scarabeid *Popillia japonica* was first reported in Italy. The area of first settlement was identified within the Ticino Valley Natural Park, between the Piedmont and Lombardy Regions. To evaluate the presence of entomopathogenic fungi (EPF) possibly capable of affecting the insect, a collection of soil samples was carried out along the Piedmontese bank of the Ticino River, in areas where treatments with entomopathogenic fungi had not previously been carried out. Entomopathogenic fungi were successfully isolated from 83 samples out of a total of 155, taken in different habitats (perennial meadows, cultivated meadows, uncultivated meadows, wooded areas and river banks).

Isolates were identified to genus and/or species level by sequencing the 5' region of the EF1- α gene and subsequent comparison of the sequences with reference sequences. Fungi belonging to the genus *Metarhizium* were isolated from 94% of the samples, while *Beauveria* and *Paecilomyces* were present in 8% and 7% of the isolates, respectively. Given the low representation of *Beauveria* and *Paecilomyces* species, subsequent studies were focused on *Metarhizium* spp.. Four *Metarhizium* species were identified: *M. robertsii* was the most common (61.5% of isolates), followed by *M. brunneum* (24.4%), *M. lepidiotae* (9%) and *M. guizhouense* (5.1%). Microsatellite marker analysis (multilocus genotyping, MLG), of the *Metarhizium* isolates revealed the presence of 27 different genotypes: 10 for *M. robertsii*, 8 for *M. brunneum*, 5 for *M. lepidiotae* and 4 for *M. guizhouense*. *Metarhizium brunneum* appeared to be associated with woodlands and on average more acidic soils, while the other species did not show any clear associations with particular habitats. Virulence tests carried out in the laboratory against *P. japonica* third-instar larvae allowed the identification of one isolate of *M. robertsii* that showed an efficacy as high as 80.3%, but the tests showed also a high variability of efficacy both between different species and between different genotypes within the same species. The high EPF biodiversity found in this limited territory provided a rich source of isolates to test and select as candidates for microbiological control of *P. japonica* larvae in soil. This confirms that studying the local biodiversity of entomopathogenic fungi, looking for new and efficient isolates, is an important step to find new resources for the microbiological control of harmful insects and increase the number of available strains.

KEY WORDS: Invasive alien species, Microbiological control, Japanese beetle, *Metarhizium* natural occurrence, Habitat, Genotype.

ORAL PRESENTATION



SESSION VI

INSECTS AND MICROORGANISMS

Deformed Wing Virus infection induces gut dysbiosis in honey bees

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Honey bee health decline represents a problem of global importance for the remarkable impact of these pollinators on the environment and human economy. The reduced bee survival is the result of a multifactorial syndrome triggered by several stress factors that synergistically interact. A common element to all collapsing colonies is the high loads of parasites and associated pathogens, such as Deformed Wing Virus (DWV). DWV is an endemic immunosuppressive virus that generates asymptomatic covert infections, kept in check by the bees' immune system when not exposed to stress agents which weaken antiviral barriers. Here we focused on the effects of DWV infection on the modulation of honey bee gut microbiota, which plays a key-role in gut physiology and immunity. We compared the microbiota composition of field-collected bees with low and high DWV levels, pointing out the occurrence of a gut dysbiosis in highly infected bees, characterized by a reduced level of *Lactobacillus* species and an increased level of Rhizobiaceae (Proteobacteria). The same kind of dysbiosis is observed in adult bees injected with viral lysate under lab conditions, suggesting that this community shift is likely the result of an active DWV infection during microbiota colonization and establishment in the gut. The comprehension of DWV effect on microbiota will deepen our knowledge about immunosuppressive strategies used by viruses for host exploitation and will allow us to define blends of probiotic microorganisms which may help to rescue the decay of honey bee immune competence.

KEY WORDS: DWV, immune response, bee microbiota, *Lactobacillus*, *Bartonella*.

ORAL PRESENTATION



SESSION VI

INSECTS AND MICROORGANISMS

Does the SIT technique have a future for the olive fruit fly control? Evaluation of the effects of irradiation on adults of *Bactrocera oleae*: survival, behaviour and presence of the endosymbiont *Candidatus Erwinia dacicola*

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In most of the Mediterranean olive-growing areas, *Bactrocera oleae* is the species responsible for the most serious damage to the olives, affecting both quantity and quality of drupes and olive oil productions. In Italy, until a few years ago, traditional control methods were based on the use of organophosphates with larvicidal action. The development of integrated pest management programs and the increased awareness of low-impact strategies have led to the evaluation and application of more sustainable methods, which have spread even more widely after the banning of dimethoate by the European Commission. Since the knowledge on the olive fruit fly symbiont, *Candidatus Erwinia dacicola*, has been deepened, the research has begun to reevaluate the sterile insect technique (SIT) as it represents the most effective control method against fruit flies, suitable for large-scale application and with low environmental impact.

Unfortunately, the mass production of sterile olive fruit fly insects still presents several difficulties and technical-biological problems that need to be overcome. In fact, adults reared on artificial cellulose-based diets show aberrant behaviors and physiological deviations, while released sterile males should be competitive with wild populations. This research was conducted to assess whether the symbiont *Ca. E. dacicola*, which is essential for the survival of *B. oleae* adults in nature, may be responsible for the fitness of irradiated flies. Olive fruit fly puparia obtained from infested olives were irradiated with Co60 as source of γ -rays applying irradiation doses of 100 and 150 Gy. Adults emerged from irradiated puparia were used in lab experiments to evaluate their survival, content of the endosymbiont and behavioral parameters in comparison to non-irradiated flies. As a result, flies irradiated at 150 Gy survived significantly less than the other two treatments. Also mating competitiveness bioassays showed a less performing behavior of the adults irradiated at 150 Gy respect to the other two treatments. Irradiation induced a different response also in olfactometer bioassays, when males were exposed to the pheromone. Adults irradiated at 150 Gy showed less walking activity and moved more slowly. Concerning molecular analyses, Real Time PCR was performed to assess *Ca. E. dacicola* content at four ages. One-day-old-irradiated flies at 150 Gy showed a significantly lower bacterial content in comparison to the other treatments. Overall, *Ca. E. dacicola* content increased as flies aged, with a more evident trend in 100 Gy rather than in other treatments. The unexpected recovery in the endosymbiont content in flies irradiated at the lower irradiation dose, together with the behavioral data and survival, highlight the possibility of a concrete hope. Thus, further research should be done in order to clarify the viability of *B. oleae* irradiated males to start improvements on the possible application of SIT for the olive fly control.

KEY WORDS: SIT, mating competitiveness, bacterial symbiosis, Real-time PCR.

POSTER

SESSION VI

INSECTS AND MICROORGANISMS

Investigations on the facultative bacterial community hosted by *Philaenus spumarius* and *Lepyronia coleoptrata* (Hemiptera: Aphrophoridae): a study on hindgut, Malpighian tubules, and nymphs' foam

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Spittlebugs (Hemiptera: Aphrophoridae) are xylem-sap feeding insects that can exploit a nutrient-poor diet, thanks to mutualistic endosymbionts residing in various organs of their body. Although primary symbionts in some spittlebug species have been quite well studied, little is known about their facultative endosymbionts, especially those inhabiting the gut. Recently, the role played by spittlebugs as vectors of the phytopathogenic bacterium *Xylella fastidiosa* (Wells et al., 1987) aroused attention to this insect group, boosting investigations aimed at developing effective yet sustainable control strategies. Since spittlebug nymphs are currently the main target of applied control, the composition of gut bacterial community of the juveniles of *Philaenus spumarius* (Linnaeus, 1758) was investigated using molecular techniques. In addition, the gut microbial community of *Lepyronia coleoptrata* (Linnaeus, 1758) nymphs was also studied to compare Aphrophoridae species belonging to different tribes.

The bacterial biomass in each gut sample was quantified performing quantitative real-time PCR, while Denaturing Gradient Gel Electrophoresis (DGGE) analysis was carried out to explore the composition of the bacterial community associated with the gut and the Malpighian tubules of both species. Moreover, bacteria associated with their froth, sampled from different host plants, were studied. Results revealed that the bacterial genera *Sodalis* and *Rickettsia* are the predominant taxa in the gut of *P. spumarius* and *L. coleoptrata* nymphs, respectively, while a species belonging to the genus *Rhodococcus* was found in both spittlebugs. Our investigations also highlighted the presence of recurring bacteria in the froth. Furthermore, the foam hosted several bacterial species depending on the host plant, the insect species, or on soil contaminants. Overall, our first findings showed that nymphs harbor a large and diverse bacterial community in their gut, Malpighian tubules and froth, providing new accounts to the knowledge on facultative symbionts of spittlebugs. Thus, the complete sequencing of the extracted DNA via next-generation sequencing (NGS) is ongoing in order to provide a detailed description of these insects' facultative endosymbionts, clarifying their roles in the relationship with their hosts.

KEY WORDS: facultative symbionts, juvenile stages, *Lepyronia coleoptrata*, *Philaenus spumarius*, *Xylella fastidiosa* vectors.

POSTER

SESSION VI

INSECTS AND MICROORGANISMS

***Acheta domesticus* densovirus (AdDV) detection in an experimental cricket
(*Acheta domesticus*) colony by real time PCR**

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The European house cricket, *Acheta domesticus*, thanks to its high protein content and easy farming conditions is currently among the most reared insects for edible purposes for animals and has recently been authorized for human consumption (Regulation (EU) 2022/188). Among its pathogens, the parvovirus *Acheta domesticus* densovirus (AdDV) is responsible for massive and sudden outbreaks in industrial farms, causing the death of most of the insects and therefore constituting simultaneously a serious health problem and a considerable economic loss for the farmers. During summer of 2022, a sudden death outbreak of crickets in a single box in the colony of the experimental rearing facility in the Istituto Zooprofilattico Sperimentale delle Venezie has been recorded. The possible causes of this mortality have been investigated. After the compliance of some possible stress factors common to all boxes, such as temperature, humidity and diet, was verified and since no new introduction had been carried out in the facility, we focused on the possible presence of pathogens, in particular AdDV. Both crickets at different stages of development (eggs, nymphs, adults) and their frass were analysed, given that AdDV transmission occurs through the fecal-oral route. In addition, environmental swabs were carried out in some critical points of the room (benches, warehouse, ventilation system) in which the colony was reared to evaluate the spread of viral particles in the environment. After viral DNA extraction from the different matrices, two genes of the AdDV genome have been amplified by real time PCR: the viral capsid protein (VP) and a non-structural region (NS) (PCR fragment sizes of 357 and 304 bp, respectively) (Semberg et al. 2019). The analysis revealed the presence of AdDV both in cricket specimens (all the development stages) and in their frass as well as in environmental swabs. AdDV detected by real time PCR was also confirmed by sequencing. The application of this molecular protocol was useful for detecting the presence of AdDV in the analysed samples and therefore it could be considered a promising diagnostic approach to identify the presence of AdDV in farms. The frass, as already reported by Semberg et al. (2019), and environmental swabs proved to be suitable substrates for AdDV detection without unnecessarily sacrifice insect specimens. Its detection in environmental swabs underlines the importance of applying stringent biosecurity measures and good husbandry practices. However, since it was not possible to identify the real trigger of the mortality event in the facility, despite the application of good farming and hygienic practices, it is necessary to investigate the role and mechanisms of stress factors in influencing latency and reactivation of AdDV, with particular reference to the industrial rearing of *A. domesticus*.

KEY WORDS: Insect pathogens, Edible insect, good farming practices.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Interaction between bacterial associates and ambrosia beetles

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Ambrosia beetles (Curculionidae: Scolytinae) are fungus growing insects that mainly develop in wood tissue. These beetles are distributed worldwide, and some of them are recognized as successful invaders. Most ambrosia beetles preferentially attack dying or stressed and ethanol-emitting trees, but if substances beside ethanol can affect host selection and colonization is still unclear. In addition, galleries of ambrosia beetles are inhabited by multiple associates including filamentous fungi, yeasts, and bacteria. Hardly any of these microbes, besides nutritional symbionts, have been described or associated to functional capabilities. In a study performed at a forest site in Veneto, Italy, we investigated whether host selection and colonization success changed in eight tree species under two different treatments, a real stress (flooding) vs. a simulated stress (ethanol-injection). Additionally, microbial isolation was conducted to obtain fungal and bacterial cultures of microbes colonizing successful insect galleries. Isolated fungi were confronted and the interactions between the insect associated microbes, endophytes, and pathogens were described. We observed plant colonization by five species of ambrosia beetles: *Xylosandrus crassiusculus*, *Xylosandrus germanus*, *Xyleborinus saxesenii*, *Xyleborus monographus*, and *Anisandrus dispar*. Interaction between microbes suggest the presence of protective symbioses within the gallery associates that may benefit the insects, of mycopathogens cohabiting the insect galleries, and endophytes that successfully compete against the insect associated fungi. We also found closely related bacteria species in the galleries of the different insects. These bacteria might be functionally relevant for insect colonization success. Our findings help aid in understanding the multikingdom symbiotic web associated to ambrosia beetles.

KEY WORDS: Ambrosia beetles, flooding, symbiosis, microbial interaction.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Inhibiting the uncultivated insect-endosymbiont '*Candidatus Erwinia dacicola*': insights from genome data and antimicrobial resistance profile of closely-related free-living *Erwinia* species

Ilaria Checchia, Elisa Salvetti, Gioele Lazzari, Nicola Vitulo, Nicola Mori, Giovanna E. Felis

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Bactrocera oleae (Diptera: Tephritidae) is the main parasite of the olive tree, which causes important productive and economic losses, mainly in the countries of the Mediterranean basin.

A promising strategy of sustainable control of the parasite is based on the interruption of host-bacterial symbiosis that plays a key role in insect fitness. The obligated symbiotic bacterium '*Candidatus Erwinia dacicola*' is a vertically transmitted microorganism, which cultivation in vitro has never been obtained to date, probably due to the adaptation to the host and a significant reduction in genome size. Therefore, the species could not be described in sufficient detail, but phylogenetic and phylogenomic analysis indicate that it has a high 16S rRNA gene similarity (> 98.36%) with the free-living and cultivated plant pathogens *Erwinia aphidicola*, *Erwinia persicina* and *Erwinia rhapontici*. As for genome sequences, data are available for several strains of the different species as well as for '*Ca. E. dacicola*': six sequences of genomes have been assembled from metagenomes (MAGs) of esophageal bulbs belonging to specimens from different geographical areas, whose average size is between 2.13 and 3.02 Mb.

On this basis, this study aims to i) determine the antimicrobial efficacy of six substances currently allowed in olive cultivation in some strains of *E. aphidicola*, *E. persicina* and *E. rhapontici* through an in vitro inhibition test; ii) investigate the presence of potential antimicrobial resistance genes (ARG) in the genomes of the same strains and iii) assess the level of possible extrapolation to '*Ca. E. dacicola*' of the phenotypic and in silico data obtained for cultivable species.

Antimicrobial activity was tested on eight strains (3-*E. aphidicola*, 4-*E. persicina*, 1-*E. rhapontici*) through the evaluation of the Minimum Inhibitory Concentration (MIC) towards fungicides (copper oxychloride-Neoram WG[®], dodine-Syllit 544 SC[®], pyraclostrobin-Cabrio Olivo[®], difeconazole-Score 250 EC), elicitors (propolis-Serbios[®]) and fertilizer (copper and citric acid biocomplex -Dentamet[®]).

As regards the available genomic sequences, their quality was assessed by BUSCO (Assessing Genomic Data Quality and Beyond), while databases such as CARD (The Comprehensive Antibiotic Resistance Database), KEGG (Kyoto Encyclopedia of Genes and Genomes) and ResFinder were employed to study possible ARGs.

Phenotypic tests showed that copper oxychloride, dodine and the biocomplex of zinc, copper and citric acid are the most effective compounds on *Erwinia* free-living species, while in silico analysis revealed the presence of 11 ARGs in both the genomes of reference strains *E. aphidicola* and *E. persicina*.

The high-quality genomes of '*Ca. E. dacicola*' strains IL and Oroville displayed 6 and 7 putative ARG, respectively, involved in general resistance mechanisms, such as non-specific efflux pumps or mutations of receptors that bind the antibiotic. The presence/absence patterns and similarities between the genetic determinants of the different species revealed a complex framework, which could limit the extrapolability of the results. However, the analysis of '*Ca. E. dacicola*'- related species could serve in testing compounds with low environmental impact possibly able to interfere the symbiosis with *B. oleae*.

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KEY WORDS: *Candidatus Erwinia dacicola*', antimicrobial resistance genes, antimicrobial activity, genome analysis, free-living *Erwinia* species, MIC, phenotypic analysis.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Endophytic development of the entomopathogen *Beauveria bassiana* reduces the immunocompetence of *Spodoptera littoralis* larvae

Ilaria Di Lelio¹, Andrea Becchimanzi¹, Eleonora Barra¹, Corentin Clavé¹, Maria Giovanna De Luca¹, Giulia Magoga¹, Giobbe Forni¹, Elfie Perdereau², Géraldine Dubreuil², Matteo Montagna¹, David David², Francesco Pennacchio¹

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Beneficial microorganisms can promote plant growth and defence barriers, offering a valuable alternative to synthetic agrochemicals. *Beauveria bassiana* is one of the most important entomopathogenic fungi that can colonize a wide variety of plant species as an endophyte, limiting the growth and survival of plant pests and pathogens. Here we contribute to this research topic by studying the effect of tomato plants colonization by *B. bassiana* on the survival, development, and immunity of *Spodoptera littoralis* (Lepidoptera, Noctuidae). Endophytic colonization of plants did not affect the survival of larvae feeding on them, which showed a weight increase associated with a higher pupal mortality. Interestingly, encapsulation and nodulation responses of larvae fed with *B. bassiana* colonized plants were reduced, while, in contrast, phagocytosis slightly increased. These larvae proved to be more sensitive to *B. bassiana* or *Bacillus thuringiensis* infection, given their reduced immune competence. "Omic" studies are currently being performed to unravel the molecular mechanism underlying these changes. Our results shed new light on the complex network of multitrophic interactions which underlie the evolution of entomopathogenic lifestyle in soil fungi.

KEY WORDS: soil microbiota, insect immunity, pest management, biocontrol.

SESSION VI

INSECTS AND MICROORGANISMS

Beneficial fungi in the genus *Trichoderma* are effective control agents of noctuid moth larvae

Ilaria Di Lelio, Andrea Becchimanzi, Eleonora Barra, Maria Giovanna De Luca, Corentin Clavé, Sheridan Lois Woo, Matteo Lorito, Maria Cristina Digilio, Francesco Pennacchio

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Beneficial fungi belonging to the genus *Trichoderma* have well-known positive effects on plant biology, such as growth promotion, nutrients uptake efficiency, seed germination rate, influencing plant interactions with the environment and promoting plant defense against biotic and abiotic stress agents, including phytophagous insects. The fungal biocontrol agent *T. afroharzianum* strain T22 (T22) is capable to colonize the roots of tomato plants, eliciting an active response against insects. Here we obtained colonized tomato plants (var San Marzano nano) by coating the seeds with spores of *Trichoderma afroharzianum* strain T22 (T22-plant), and scored the plant-mediated effects of T22 on the moth *Spodoptera littoralis* (Lepidoptera: Noctuidae). Larvae fed with T22 plant leaves (T22-larvae) showed developmental delay and weight reduction associated with lower survival. Furthermore, T22-larvae showed an immunosuppressed phenotype, more susceptible, at both IV and VI instars, to sublethal doses of a biopesticide containing the entomopathogen *Bacillus thuringiensis*. The results obtained show a clear plant-mediated effect of *T. afroharzianum* on *S. littoralis* larvae, which could contribute to the reduction of pesticide use in agriculture by suppressing this pest or enhancing its susceptibility to biocontrol agents.

KEY WORDS: *Spodoptera littoralis*, *Bacillus thuringiensis*, Fungal endophyte, Induced defence, Tritrophic interaction, Immune suppression.

SESSION VI

INSECTS AND MICROORGANISMS

Investigating the role of cholinergic signaling in *Drosophila melanogaster* defense against bacterial pathogens

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Cholinergic signaling takes place at synapses to mediate neurotransmission in animals, but is also implicated in different cell-to-cell communication mechanisms that operate in non-neuronal tissues and play important roles for the maintenance of physiological homeostasis. In humans, acetylcholine is involved in the regulation of immune functions, but information about its immunomodulatory potential in invertebrates is still limited. Since neonicotinoids, neurotoxic insecticides that are able to target insect nicotinic acetylcholine receptors (nAChRs), impair the immune response in both *Apis mellifera* and *Drosophila melanogaster*, we decided to investigate the role of acetylcholine signaling in insect defense against pathogens. By using a Gal4-UAS expression system, we altered nAChR function in *Drosophila* immune tissues and tested if and how these manipulations affected fly survival to systemic infection. The obtained results support the occurrence of acetylcholine-based immune regulation in *Drosophila*, whose impact may differ depending on the species of invading bacteria.

KEY WORDS: Innate immunity, Acetylcholine, Nicotinic acetylcholine receptors, *Drosophila melanogaster*.

SESSION VI

INSECTS AND MICROORGANISMS

***Trichoderma* confers resistance to tomato plants against *Spodoptera littoralis* larvae by disrupting the symbiotic role of their gut microbiota**

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Beneficial fungi belonging to the genus *Trichoderma* are important biocontrol agents of fungal pathogens and promotes plant growth. Here we investigate how the interaction between tomato plants and a phytophagous insect, *Spodoptera littoralis* (Lepidoptera, Noctuidae), can be driven by hidden interactions involving their respective microbiotas. We describe the mechanism underlying this insect-plant interaction as modulated by the colonization of tomato roots by strain T22 of *Trichoderma afroharzianum* (T22-plants). Larvae fed with T-22-plant leaves show a marked and significant reduction of their growth, delayed development and a much higher mortality, compared to controls.

Since the observed negative effects on survival and development could be mediated by the fungal induction of plant defense barriers targeting midgut functionality and thus its physiology, the midgut structure, its enzymatic profile and transcriptional activity were investigated as well as the composition and functional properties of gut microbiota. The changes induced in tomato plant by the fungus do not cause structural damage to the gut and/or alterations of its digestive capacity, but mediate gut dysbiosis in *S. littoralis* larvae. This dysbiosis is largely responsible for the reduced nutritional support by the gut microbiota to the host and the consequent negative impact on insect development and survival. Indeed, this phenotype can be fully rescued by oral administration of the functional *S. littoralis* gut microbiota or of the bacterial species *Enterococcus casseliflavus*, the symbiont that accounts for most of the changes in the metatranscriptomic analysis of the microbiota. Our results shed new light on the role played by a soil microorganism in the modulation of an interkingdom competition influencing nutritional resource allocation, laying the foundation for the development of sound strategies for the sustainable management of agroecosystems and for an in-depth assessment of the ecological impact by biocontrol agents.

KEY WORDS: Holobionts, insect-plant interactions, insect gut microbiota, soil microbiota.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Filamentous fungi isolated from buckwheat nectar affect the olfactory responses of stink bug egg parasitoids

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Flowering plants, like buckwheat, are integrated into the agricultural landscape to support and enhance parasitoid fitness. These plants are expected to provide essential resources such as nectar and must be relatively enticing for the parasitoids to guarantee constant visitations. Commonly, flowers exhibit inherent scents that the parasitoids used to associate with the presence of nectar reward and thus elicit favorable attraction. However, recent studies have shown that nectar is colonized by microbes that are thought to influence the nectar's overall traits, such as scent and chemical composition. Moreover, it has been demonstrated that these changes in nectar traits have cascading impacts on the foraging behavior of parasitoids. However, to date, most studies only focused on nectar bacteria and yeasts, and the possibility of filamentous fungi altering nectar traits and how these alterations impact the parasitoids has received much less attention. Here, we isolated filamentous fungi from buckwheat (*Fagopyrum esculentum*) flowers to examine the hypothesis that nectar-colonizing filamentous fungi affect the foraging behavior of parasitoids through metabolic modification of nectar volatile composition. *Nezara viridula* egg parasitoids *Trissolcus basalis* and *Ooencyrtus telenomicida* were used as study insects. These two egg parasitoids are known to coexist in areas where their primary host is present. Culturable nectar filamentous fungi were used to ferment synthetic nectars, and their impacts on parasitoid olfactory behaviors were systematically evaluated. Out of six nectar filamentous fungi (3 *Cladosporium* spp., 2 *Aspergillus* spp., and 1 *Stachybotrys* sp.), only nectar fermentation by *Cladosporium* sp. 22.2.11 attracted *T. basalis*. However, nectar fermentation by both *Cladosporium* spp. 22.2.11 and 22.3.29 elicited a positive olfactory response in *O. telenomicida*. Headspace analyses disclosed variability in the volatile compositions of nectars fermented by filamentous fungi that supported the contrasting behaviors of the two egg parasitoids. Overall, our results are highly relevant from an ecological point of view as nectar-rich flowering plants such as buckwheat are widely integrated into crop habitats to enhance the conservation biological control of insect pests.

KEY WORDS: Nectar-inhabiting microbes, Conservation biological control, Parasitoid olfaction, *Trissolcus basalis*, *Ooencyrtus telenomicida*, *Nezara viridula*.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Studying the function of $\alpha 7$ nicotinic acetylcholine receptor in *Drosophila* humoral immunity

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Acetylcholine (ACh) is one the major neurotransmitters, whose role in mediating synaptic interactions between neurons in the central nervous system in vertebrates and invertebrates is well characterized. It also plays largely unexplored regulatory functions in non-neuronal tissues. Here we demonstrate that ACh signaling is involved in the modulation of the innate immune response of *Drosophila melanogaster*. Knockdown of ACh synthesis or ACh vesicular transport in neurons reduced the activation of drosomyacin (drs), a gene encoding an antimicrobial peptide, in adult flies infected with a Gram-positive bacterium. Our results indicate that the nicotinic acetylcholine receptor $\alpha 7$ (D $\alpha 7$) is involved in the cholinergic pathway that modulates drs gene expression in both neuronal and non-neuronal tissues, demonstrating a cholinergic activity in hemocytes.

In conclusion, our results allowed us to establish that the innate humoral immune response is modulated by neuronal and non-neuronal cholinergic signals.

KEY WORDS: Innate immunity, acetylcholine, nicotinic acetylcholine receptors, neuroimmunity.



SESSION VI

INSECTS AND MICROORGANISMS

Olfactive preference of *Drosophila suzukii* toward different strains of viticultural-enological yeasts, to develop new control strategies

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Drosophila suzukii (Diptera, Drosophilidae) is native to Asia, and was introduced in Europe in 2008. It is a generalist species, which preferentially attacks soft red or black-skinned fruits. Unlike native species in the *Drosophila* genus, female flies have a serrated ovipositor, which allows the deposition in berries before ripening. Since 2011, qualitative and quantitative problems have been encountered on grapevine berries as well. The damage is both direct, due to larval feeding on the grape, and indirect, following exposure of the grapes to *Botrytis* bunch rot or sour rot. To avoid chemical residues in wine, the available management strategies are very limited and mostly rely on physical barriers, with poor results in terms of effectiveness. The aim of this work was to test the potential of several yeasts to be used as attractants or repellents of *D. suzukii* females by means of treatments on the berries before harvest. Yeast strains were selected among non-*Saccharomyces* strains that are commonly used in enology during alcoholic fermentations, and among isolates recognized as biocontrol agents.

Multi-choice trials were performed in arena by exposing *D. suzukii* females to the selected strains grown in solid medium, along with a control with no yeast cultures. The most selected yeast strain was subsequently used for direct comparisons by means of two-way olfactometer bioassays. In this experiment the comparison was directly performed between a sample of berries (cv. Nebbiolo) sprayed with a yeast suspension and an equal sample containing untreated berries.

Multi-choice trials showed that the most attractive strain was *Starmerella bacillaris* FC54; the olfactometer bioassays confirmed the significant attraction capability of this strains when sprayed on the grape berries. Therefore, the selected strain may be exploited to implement attract and kill strategies, or to develop new traps for monitoring purposes and for mass trapping. Moreover, the recognized enological of *S. bacillaris* suggest that its possible persistence after harvesting (which may be a critical issue for several biocontrol agents) could provide a further benefit in the wine-making process.

KEY WORDS: *Drosophila suzukii*, food baits, enological yeasts.

SESSION VI

INSECTS AND MICROORGANISMS

Volatiles of fungal mutualists can act as aggregation cues during host-selection by an ambrosia beetle

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The use of aggregation pheromones during mass colonization of host trees is a well-known strategy for several insect species belonging to different orders and families. However, the role of semiochemicals during host selection by ambrosia beetles, an ecological clade within the bark beetles, has been poorly investigated. Ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) are wood-boring insects sharing a common ecological strategy, i.e., they use fungal mutualists as the sole source of nutrition. In some cases, mass colonization of trees growing in nurseries and orchards by non-native ambrosia beetle species has been reported. However, mechanisms involved in this aggregation behavior have remained enigmatic. Here, we evaluated the role of microbial volatile organic compounds (MVOCs) produced or elicited by fungal species associated with *Xylosandrus germanus* (Scolytinae) on its host-selection behavior. Fungal associates from dispersing individuals of this non-native ambrosia beetle were first isolated and characterized. Then, dual choice olfactometer bioassays revealed a significant attraction of *X. germanus* to its nutritional mutualist *Ambrosiella grosmanii*. Adult females were also attracted by MVOCs of another common fungal isolate (*Acremonium* sp.) but not from those of a selected non-symbiont contaminant (*Cladosporium* sp.). Further dual choice bioassays conducted with beech branch sections (i.e., bolts) also revealed a strong attraction of beetle females for bolts either pre-colonized by conspecifics or pre-inoculated with *A. grosmanii*. Analyses of VOCs showed a group of eight compounds that were commonly released from beech bolts infested by the beetle, beech bolts infected by *A. grosmanii*, and *A. grosmanii* growing *in vitro*. Our results show that identified MVOCs act as attractive cues for dispersing *X. germanus* individuals, thus providing the first evidence for volatiles released by mutualistic fungi as aggregation pheromones of a *Xylosandrus* ambrosia beetle. Overall, this study provides new knowledge on the chemical ecology of this beetle and useful insights toward the development of semiochemical-based control methods based on the potential use of MVOCs as specific and effective beetle attractants.

KEY WORDS: *Ambrosiella grosmanii*, Invasive pest, MVOCs, Symbiosis, *Xylosandrus germanus*.

SESSION VI

INSECTS AND MICROORGANISMS

Characterization of Smoke Waters and their effects on *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) microbiome

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From the pyrolysis of organic feedstock, aside from Biochar and Bio-oils, it is possible to obtain volatile fractions that can be bubbled in distilled water to obtain the "smoke waters" (SWs), rich in biological compounds and usable for energy purposes or technological applications. Overall, 10 different SWs types were generated and investigated from 5 organic feedstocks treated at 300 and 500°C, with which 2 mixtures were produced. The SWs investigated were obtained from solid olive mill waste (OLI mix) and from dried and finely crushed corn (*Zea mays* L.) leaf and stem; (3) alfalfa (*Medicago sativa* L.) hay; (4) sawdust of beech (*Fagus sylvatica* L.) wood; and (5) cellulose from filter paper (MIX). The SWs and the two mixtures obtained from them were characterized with GC-MS. The effect of oral administrations of OLI and MIX on the *Bactrocera oleae* fitness was evaluated throughout bioassays carried out with groups of wild new-emerged adults of both sexes, collected from clearly attacked olives harvested in Parco Gussone in Portici (NA). The compounds were administered through a diet of water and sugar added with known amounts of the two mixtures. The alteration effects on the microbiome of the fly were evaluated at the end of each replication. Real Time qPCRs were carried out on pools of 4 females to quantify the presence of the primary endosymbiont, "*Candidatus Erwinia dacicola*". The analysis were completed on DNA extracted from the head, esophageal bulb's seat, and the intestine's abdomen. The results highlight how the MIX administration limits the bacterial titer and even halves it in the gut, with strong phonological effects also in the subsequent generation. The OLI mixture promotes, instead, the symbiont proliferation both in the bulb and in the gut.

KEY WORDS: Olive fruit fly, "*Candidatus Erwinia dacicola*", induced alteration.

POSTER

SESSION VI

INSECTS AND MICROORGANISMS

Seasonal variations in the *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) microbiome

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Bactrocera oleae is well-known for the wide symbiotic relationships linked with the adult and larval stages phenology. In the whole microbiome of the fly stands out the primary bacterium "*Candidatus Erwinia dacicola*", usually vertically transmitted and hosted in an oesophageal diverticulum, the "Bulb," and in the gut of larvae and adults. Quantitatively, the titer is not so much high in the new-emerged adults. Still, it increases quickly during the first days until it is impossible to remove the bacterium through oral antibiotic administrations after the fifth day. Due to the peculiar characteristics of "*Ca. Erwinia dacicola*" and its symbiotic relation with the fly, it is commonly used as an indicator to evaluate the actual symbiotic titer. The absolute quantization of "*Ca. Erwinia dacicola*" was obtained with Real-Time qPCRs after an extraction of both symbionts and host DNAs from the heads and the abdomens separately, on pools of 4 females. The wild adults were obtained from olives attacked by *B. oleae* and collected in Portici (NA) all along the fly's activity period, from June to the end of December, for two subsequent years. The results highlighted a cyclical and seasonal variability in the symbiont presence that follows and adapts to the seasonal modifications acting on the phenology of the host and modifying its physiology due to the environmental and climatic conditions. Such results underline the need to accurately define the timing and control techniques of the fly populations based on the alteration of the fly's microbiome.

KEY WORDS: Olive fruit fly, "*Candidatus Erwinia dacicola*", endosymbiosis.

SESSION VI

INSECTS AND MICROORGANISMS

Biotic and abiotic factors affecting the microbiota of leaf beetles (Coleoptera, Chrysomelidae)

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Little is known about the factors shaping the phytophagous insects' microbiota composition and diversity and the role of microorganisms in supporting the metabolization of the host plants compounds. In this project, we focus our attention on the bacteria associated with the phytophagous insects of the families Chrysomelidae and Orsodacnidae. By performing a large-scale screening of the bacteria present in the abdomen of these non-model organisms, we aim at testing which biotic and abiotic factors can shape the bacterial diversity and composition, including among others the host plants spectrum, the sex and the living environment. The microbiota of different leaf beetle species is characterized by a DNA metabarcoding approach targeting the bacterial 16S rRNA. Up to now, the microbiota of 35 species, representative of the different Chrysomelidae subfamilies, was characterized. From this screening, we observed that the species with a wide host plant spectrum (polyphagous) harbour a more diversified microbiota than the more specialized ones. Moreover, males were found to be likely more prone to acquire bacteria from the surrounding environment, while females' microbiota of most of the species resulted dominated by reproductive manipulators (e.g. *Wolbachia* and *Rickettsia*) or symbionts with functional roles. Two putative primary symbionts belonging to the Enterobacterales and previously isolated only from *Bromius obscurus*, have been detected in two species of the same subfamily, suggesting a widespread symbiosis in Eumolpinae. Surprisingly, a taxon closely related to the well-known aphid symbiont *Buchnera* was found well represented in the microbiota of *Orsodacne humeralis* (Coleoptera, Orsodacnidae). At present, we are processing more than 100 other Chrysomelidae species (from at least three populations per species, three individuals per population) in order to better understand the influence of the species' host plant spectrum and geography on the microbiota diversity. The host plants of these individuals will be characterized by analyzing the plants DNA present in their gut through a DNA metabarcoding approach targeting the plant ITS gene. The achieved results will also help to identify the bacteria with a possible role in conferring to the insect the ability to exploit different host plants, and thus possibly involved in the expansion of the host plant spectrum.

KEY WORDS: Insects microbiota, phytophagous insects, 16S rRNA metagenomics.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Comparison of the immune response of *Galleria mellonella* and *Rhynchophorus ferrugineus* after infection with the entomopathogenic nematode *Steinernema carpocapsae*

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier 1790) (Coleoptera: Curculionidae), a pest of palm trees, and *Galleria mellonella* L. (Lepidoptera: Pyralidae), a pest of beehives, were used as models to deepen our understanding of the host-entomopathogenic nematode relationship.

The effects of the entomopathogenic nematode *Steinernema carpocapsae* (Nematoda: Steinernematidae) on the immune activity of *R. ferrugineus* and *G. mellonella* larvae were compared. The ability to complete the life cycle of *S. carpocapsae* in the two insects was also evaluated.

Although *S. carpocapsae* is effective in the biological control of the red weevil, it is not able to complete its cycle within the host, whereas the same nematode successfully reproduces in *G. mellonella*.

On the basis of previous laboratory data and a systematic literature review, the nematode-induced effects on the immune response of the Coleoptera and the Lepidoptera were compared. In particular, the immunocompetent haemocyte population (number and cell types), the activity of the prophenoloxidase-phenoloxidase system (proPO) and finally the inhibition and proliferation of the Gram-negative symbiont bacterium *Xenorhabdus nematophila* (Enterobacteraceae) were analysed.

KEY WORDS: Red Palm Weevil; Greater Wax Moth, palm; nematode; bacteria, symbionts; immune re-sponse.

SESSION VI

INSECTS AND MICROORGANISMS

Revision on the effects of an entomopathogen bacteria *Bacillus thuringiensis* on the im-mune response of Red Palm Weevil (*Rhynchophorus ferrugineus*), is it a question of gender?

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Since their discovery, bioinsecticides based on *Bacillus thuringiensis* (Bt) have demonstrated targeted insect control with minimal environmental impact. Understanding the biology of insect pests, as well as interactions with pathogens and immune systems, can help to enhance their effect within these interactions.

On the basis of previous laboratory data and a systematic literature review we analyzed the effects induced by the entomopathogen bacteria Bt, on red palm weevil (RPW), an highly invasive pest of palms that had a significant economic, environmental and social impact when introduced into Italy. The bacteria-induced effects on physiology, immune response and stress answers in the RPW. *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) were considered. Previously a Bt strain, commercialized against another family of Coleoptera, was found to be a potential pathogen of RPW. Data showed that Bt interacts negatively with the growth and the vitality of RPW moreover with hemocytes of *R. ferrugineus* whose numbers decreased drastically in the hemolymph both in total number and in type. In particular, we focused on the Bt stress-induced infections considering the genders and the instars. The interaction between Bt and RPW hemocytes was analyzed by evaluating the expression heat shock proteins (HSPs) particularly HSP70 in the supernatant of the hemocyte lysate obtained from larvae and adults. HSPs are rapidly synthesized in the cells after stress exposition including infection by pathogens. The western blot analysis, showed that the HSP70 expression was modulated in the time (3h, 6h, 12h, 19, 24h) in the response to Bt treatment, highlighting that Bt is a stress factor for the larvae but also for adults. The protein expression was increased approximately seven times after 3 hours from treatment and after 6 hours it returning to control value. Data show difference in how males and females invest in Bt infection answer. Our data on immunity support the theory that males choose investment in reproduction over investment in immunity. In fact, *B. thuringiensis*-induced mortality data show the effect that females mount a stronger immune response and they also suffer lower mortality.

KEY WORDS: Red Palm Weevil; Hemocytes, HSP70, immune response.

POSTER



SESSION VI

INSECTS AND MICROORGANISMS

Possibility of impairing the vertical transmission of the olive fly symbiont

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The olive fly, considered the most destructive pest of olives, is strictly associated with the bacterial symbiont *Candidatus Erwinia dadicola*. The symbiont is vertically transmitted from the mother to the offspring, by smearing the egg surface with bacteria, during oviposition. Upon hatching, the larva ingests the symbiont through nibbling the egg surface and the bacteria acquired during this period become associated with the alimentary tract and proliferate in the larval caeca.

The association between the olive fly and its hereditary symbiont is of paramount importance for all stages of the insect. Larvae deprived of symbiont are unable to develop on unripe olives because of their high polyphenolic content. In adults, the symbiont plays a key role in the degradation of nitrogen compounds contributing to the fly nutrition.

Given the key role of *Ca. E. dadicola* in host fitness, the present study aimed at evaluating the possibility of disrupting the vertical symbiont transmission with a focus on the egg stage. This stage offers a good opportunity for disrupting symbiont acquisition, since in this moment symbionts are exposed to the surrounding environment. Commercial products with putative antimicrobial activity were used for treating olives infested with eggs. Symbiotic bacterial load was quantified by Real-Time PCR in larvae derived from treated and untreated olives. Our results showed a significant reduction in the symbiont titre in larvae obtained from olives treated with dodine and Dentamet®, a zinc-copper-citric acid biocomplex. In addition, a reduction in the pupation rate of the insect pest was observed after treatments with both products.

This study highlights, for the first time, the possibility of disrupting symbiont acquisition in the olive fly through the application of antimicrobial compounds in olives infested with newly laid eggs.

Overall, these results open future perspectives on the possibility of compromising the olive fly fitness through symbiont control strategies, by targeting the eggs inside the fruits.

KEY WORDS: *Bactrocera oleae*, antimicrobial, real-time PCR, symbiotic control.

SESSION VI

INSECTS AND MICROORGANISMS

Virome of Hemiptera: the case of three insect vectors of plant pathogens

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The use of viruses as sustainable alternatives to conventional chemical-based insecticides for controlling agricultural pests is a hot topic due to EU quest for pesticide reduction. Insect viruses are well known biocontrol agents of lepidopteran species but so far largely unexplored for hemipterans. In this work we aimed at providing new opportunities to develop virus-based solutions for vector-born plant disease, by unveiling the virosphere of three Hemipteran vectors. As a first step, RNAseq was applied to characterize the virosphere of *Scaphoideus titanus*, vector of Flavescence dorée phytoplasma, *Euscelidius variegatus*, laboratory vector of Flavescence dorée and 'Candidatus Phytoplasma asteris', and *Philaenus spumarius*, vector of *Xylella fastidiosa*. Samples of *S. titanus* were collected from Italy, France, Switzerland, Hungary, and the USA. *Euscelidius variegatus* adults were sampled from two laboratory rearings originally established with insects collected in France and Italy. *Philaenus spumarius* samples were collected from eight localities across Italy and France. Eight RNA viruses were newly identified in *S. titanus* populations, two new I flaviviruses were identified in *E. variegatus*, and others are expected from ongoing analyses of *P. spumarius* virome. Prevalence within the population, and potential pathogenicity to the respective hosts is under evaluation for the newly identified viruses. Moreover, some viruses have been selected to measure their effects on insect fitness parameters and transmission efficiency. For each pathosystem, the non-pathogenic viruses (covert infections) will be tentatively exploited to alter the expression of insect genes involved in phytoplasma transmission through virus-induced gene silencing (VIGS).

KEY WORDS: *Euscelidius variegatus*, *Philaenus spumarius*, *Scaphoideus titanus*.

SESSION VI

INSECTS AND MICROORGANISMS

**Shared evolutionary signatures of the shifts to insect endosymbiosis across
Enterobacterales evolutionary history.**

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The tight endosymbiotic associations evolved between bacteria and insects underlie several hosts adaptations and dramatically altered endosymbionts' genome evolution. The more striking consequence faced by insect endosymbionts is genome reduction and it has been characterized in several instances, as in the case of endosymbionts of the genus *Buchnera*, *Carsonella* and *Stammera*. Yet, a broad-scale perspective of the evolutionary processes which are responsible for this phenomenon is lacking. Here, we unravel the main evolutionary mechanisms associated with this shift in lifestyle using the Enterobacterales order of Gammaproteobacteria as a representative model. This clade can in fact provide unique insight, as shifts to endosymbiosis are considered to have happened independently numerous times. Leveraging multiple phylogenetic approaches - that should accommodate the idiosyncratic sequence evolution of endosymbionts - novel phylogenetic hypotheses were generated, encompassing over two hundred bacterial species, including insect symbionts and pathogens of plants and animals, alongside free-living species. Ancestral state reconstruction retrieved between nine and eleven independent events of endosymbiosis establishment, with insect endosymbionts' clades closely related to pathogenic ones. Inferences on gene families evolution support extensive gene losses concurrently with the shift to endosymbiotic lifestyle, but subsequent gene loss rates do not differ between free-living species and insects endosymbiont. Our analyses found instead a reduction of horizontal gene transfer in endosymbionts, suggesting that their genome erosion results from the reduced acquisition of genes, which is not able to compensate the continuous genome contraction which characterize bacteria evolution. Furthermore, we found a correlation between gene selection regime and their loss rate in endosymbionts and free-living species, highlighting gene essentiality as a driver of gene loss, independently from lifestyle.

KEY WORDS: insects endosymbionts, comparative genomics, molecular evolution, genome reduction.

POSTER

SESSION VI

INSECTS AND MICROORGANISMS

New viral sequences identified in the brown marmorated stink bug *Halyomorpha halys*

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New viral sequences identified in the brown marmorated stink bug *Halyomorpha halys*

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The brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae) is a serious agricultural pest causing damage to fruit crops across Europe upon its introduction from Eastern countries. Although the extensive use of insecticides, *H. halys* is still a major pest against which alternative non-chemical solutions are often not completely effective. The urgent need for more targeted and sustainable pest management approaches includes the exploration for alternative biocontrol agents such as insect viruses. Here, we characterized the virome of ten *H. halys* populations collected during summer/autumn 2020-21 in Lombardy region. The ten collection sites represent different agroecosystems and pedoclimatic conditions. Total RNA was extracted from over 100 single insects and then checked for purity and integrity. Ten RNA pools (one for each collection site) were then generated for library construction: each pool was obtained using the same amount of RNA extracted from four males and four females. Stranded libraries were prepared and sequenced from both ends (150 bp) using the Illumina NovaSeq System. RNA sequencing resulted in 41 to 42 million reads per library, which were independently assembled into contigs and compared to NCBI protein non redundant (nr) database for virus discovery. These transcriptome data allowed us to identify six viral RNA genomes associated to *H. halys*: three of the viruses described are expected to have a double-stranded RNA (dsRNA) genome, two a positive-sense single-stranded RNA (+ssRNA) genome, and one a negative-sense single-stranded RNA (-ssRNA) genome. Only complete or nearly complete viral genomes were considered in this computation. Despite these strict criteria, we could identify sequences matching to viruses in all the libraries except one. Six specific primer pairs were then designed to estimate virus prevalence in single insects included in the same pooled samples previously analysed by RNAseq. DNase-treated samples were retro-transcribed with specific reverse primers and analysed by qPCR. Insect actin transcript was also analysed in all samples as endogenous control. The presence/absence of the six viruses in the ten pooled samples was confirmed by qPCR. The possibility of exploiting these naturally occurring viruses as specific and targeted biocontrol agents of *H. halys* is a possible answer to the increasing demand for a more sustainable agriculture.

This work is part of the project “DEBUG – New knowledge for the control of the alien pest *Halyomorpha halys* in vineyards of Lombardy Region” funded by Lombardy Region.

KEY WORDS: Hemipteran pest, virome, viral biocontrol agents.

POSTER

SESSION VI

INSECTS AND MICROORGANISMS

Detection of honey bee viruses in adults and larvae of *Vespa orientalis*

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The Oriental hornet (*Vespa orientalis*) is native to the southeastern Mediterranean, north-eastern and eastern Africa, the Middle East, Central Asia and it is well established in southern Italy. However, recent reports in Liguria, Trieste and Tuscany show great expansion of its areal probably due to climate change or involuntary anthropic actions. Adults feed on carbohydrates collected from fruit and nectar while brood is fed by workers with animal proteins (grasshoppers, flies, yellowjackets and bees). The interaction between the two species could lead to possible spillover of pathogens in both directions. Previous studies have already reported the presence of honey bee pathogens (virus, fungi and bacteria), in *Vespa* and have underlined their role in disseminating pathogens which could represent a threat for honey bees.

Aim of this study was to detect the presence in *V.orientalis* of six honey bee viruses, more precisely Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Kashmir Bee Virus (KBV), Sac Brood Virus (SBV), and to investigate possible transmission route. 30 adults and 29 larvae of *V.orientalis* and 2 pools of 10 honey bees (*Apis mellifera ligustica*) each were collected from the managed nest and apiary located both at the Department of Agricultural, Food and Forest Sciences-University of Palermo, and sent to the Department of Veterinary Medicine and Animal Productions-University of Naples "Federico II". Samples were observed by stereomicroscope to assess possible alterations which could be indicative of the action of viruses and then subjected to multiplex PCR to detect viruses.

No morphological alterations were identified despite the biomolecular results showed 25/30 adults and 24/29 larvae were infected with at least one virus (DWV). Adult samples presented also ABPV (19/30), BQCV (13/30), SBV (1/30); while larvae presented SBV (10/29), ABPV (5/29), BQCV (5/29). No sample resulted positive for CBPV and only 1/30 adult resulted positive for KBV. Honey bees' positivities reflected those of the hornets: 2/2 DWV, 2/2 SBV, 2/2 BQCV and 1/2 ABPV. The viruses detected in our study are the most prevalent in apiaries across Italy and the overlapping of positivities between hornets and honey bees collected in the same site suggests possible transmission of honey bee viruses through ingestion of infected honey bees.

KEY WORDS: Oriental hornet, virus, pathology, *Apis mellifera*.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Variable effects of gut dysbiosis in the southern green stink bug *Nezara viridula* and its implications for symbiotic control

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Symbiotic interactions with microorganisms play a key part in insect ecology and evolution. Insects belonging to the family Pentatomidae harbour an obligate symbiont in a specialized ventricle of the gut, vertically transmitted via egg surface contamination by the females. Symbiotic control is a pest management strategy based on the disruption of symbiont acquisition. The mechanisms explaining the insect response to the imbalance in the gut microbial community (dysbiosis) are essential to understand the potential success of symbiotic control. The aim of this study was to assess dysbiosis induction by egg masses treatment in the southern green stink bug *Nezara viridula* (Hemiptera, Pentatomidae), evaluating the effect of symbiont elimination in nymphal survival and in the gut microbiome, by means of third generation sequencing techniques. Symbiont presence declined in nymphs deriving from treated egg masses; moreover, they underwent increased mortality. 16S rRNA gene sequencing allowed for detection of six different strains of the symbiont, assigned to the genus *Pantoea*. Only one of these strains was detected in all samples, while the others were present in non-treated samples exclusively. The microbiome of non-treated nymphs showing low mortality was characterized by the dominance of the primary symbiont, whereas the community internal diversity in the other samples increased accounting for a dysbiosis. Nonetheless, dysbiosis was not in all cases associated with high nymphal mortality. Moreover, symbiont absence was detected in some non-treated samples as well. A possible inefficient symbiont transmission happening in nature could explain the variable response of *N. viridula* to symbiont elimination stated in previous studies. Further research addressing the gut microbiome dynamic along *N. viridula*'s complete biological cycle will allow for a deeper comprehension of the interaction between *N. viridula* and its gut microbial community.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

Surface proteins of entomopathogenic bacteria interacts with insects during pathogenesis. The case of *Brevibacillus* and Diptera.

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The surface of a living organism represents the first physical contact with the surrounding environment, which has led over time to the development of specific adaptations, resulting in the differentiation of specific virulence factors of pathogenic microorganisms.

The insect immune system in turn has evolved by developing specific cellular and humoral response mechanisms to surface microbial proteins. Spore-forming insect pathogenic bacteria are characterized by both spore-surface and vegetative cell-surface that may be respectively involved in interaction with the host either in the early stages of infection or in the later haemocelic proliferation.

The present study investigated the involvement of cell S-layer and spore-surface proteins of *Brevibacillus* entomopathogenic bacteria. Cell wall and spore surface proteins were identified, their multilayer structures characterized by electron microscopy, and their encoding genes sequenced. Laboratory bioassays with different fractions of these proteins were conducted on house fly adults as model insects, which highlighted their role as virulence factors documenting their contribution to the pathogenic process. Their action is concentration dependent with an LC50 on *Musca domestica* of 12 µg/ml and 3 µg/ml for the cell wall and major spore proteins, respectively.

Taken together, *Brevibacillus* S-layer and spore-surface proteins appear to be part of a complex mode of action involving several toxins and virulence factors produced by these invertebrate pathogens. The study model employed, which can be replicated on other entomopathogenic species, may help to clarify the physical and molecular pathogen-host interaction.

KEY WORDS: plant-host interaction, entomopathogens, flies, mosquitoes, biological control.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

The influence of foodborne pathogens in the rearing of *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae

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Edible insects have attracted the attention of the European Union as they represent one of the most promising alternative protein and energy sources for food and feed. Among them, *Tenebrio molitor* (Coleoptera: Tenebrionidae) represents one of the most popular species used for the conversion of several food by-products into protein. Such by-products can contain foodborne pathogens. Consequently, a proper risk assessment during rearing should be carried out to evaluate potential risks. The contamination that might occur in insect rearing may influence the health of consumers, as well as the insect welfare. Among biological hazards harbored by edible insects, microorganisms are the most noteworthy. Moreover, the presence of microbial pathogens might lead to an alteration of the intestinal microbiome that plays an important role in *T. molitor* health, thus potentially affecting the development of the intestinal epithelium, the growth performance of the organism, and the survival rate. Therefore, the aim of this study was to investigate the effects of *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Campylobacter* spp. on *T. molitor* larvae growth performance. The analysis of the intestinal epithelium and the growth performance were carried out on insects reared on organic wheat middlings inoculated with three different contamination levels (1, 5, and 7 log cfu/g) of each pathogen. The results obtained from the present study highlighted that *T. molitor* growth performance was not influenced by the presence of contaminants, so much so that even the intestinal epithelium didn't show any difference among treatments. Moreover, under the optimal rearing conditions, *T. molitor* seemed to represent an inhospitable or even hostile environment for the multiplication of the pathogens, irrespective of the initial contamination level. While this is promising, it is still recommended to monitor and control these pathogens in *T. molitor* rearing and further research is needed to better clarify the interactions established among the target microorganisms and those naturally harbored by the insect gut.

This study was funded by Ricerca e Sviluppo 2020 Fondazione Cariverona, Italy, Project N° 2020.0071 "Insetti edibili e sicurezza alimentare: vecchie sfide per nuovi alimenti - INNOVA". The authors wish to thank Nutrinsect srl, via Enrico Mattei, 65, 62010, Montecassiano, Italy, and via Primo Maggio, 156, Ancona, 60131, Italy, for having co-funded this research.

KEY WORDS: *Tenebrio molitor*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter* spp., growth performance, intestinal epithelium.

POSTER

SESSION VI

INSECTS AND MICROORGANISMS

Fermentation by nectar-inhabiting bacteria affects parasitoid performance

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Parasitoids are frequent flower visitors, relying on floral nectar to cover their energetic and nutritional needs. An extensive body of literature documents the benefits of nectar provisioning on life history traits of parasitoids, such as longevity and fecundity. Although it is well-known that nectar is inhabited by microbial communities which can alter nectar traits, the effect of nectar fermentation by bacteria on parasitoids has not been sufficiently explored. In this study the longevity of the adult parasitoids, relying on nectar provisioning, was studied using synthetic nectar fermented by different bacterial strains belonging to the phyla Firmicutes, Proteobacteria, and Actinobacteria. The bacteria had been previously isolated from flower nectar of *Fagopyrum esculentum* (Polygonales: Polygonaceae), which has proved beneficial for parasitoid longevity. Synthetic nectar was prepared by filter-sterilizing sucrose solution mixed with casamino acid. Then the nectar was inoculated with different bacterial strains, representing the different treatments, while the control was the non-inoculated synthetic nectar. Female adult parasitoids were individually kept in vials and provided ad libitum with synthetic nectar fermented by the different bacterial strains. Statistical analysis was based on the null hypothesis that the parasitoids' longevity is not affected by the bacterial strains in the synthetic nectar. Parasitoid longevity was either positively, neutrally or negatively affected indicating a species-specific effect of the different bacteria on parasitoid performance. The results of this work will be discussed in the context of conservation biological control programmes.

KEY WORDS: nectar microbiome, *Fagopyrum esculentum*, Firmicutes, Proteobacteria, Actinobacteria, parasitic wasps.

ORAL PRESENTATION

SESSION VI

INSECTS AND MICROORGANISMS

**Interactions between entomopathogenic fungi and entomopathogenic nematodes
in microbial control**

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Entomopathogenic fungi and entomopathogenic nematodes are globally distributed soil organisms capable of infecting and killing a wide variety of insects. Therefore, these organisms are frequently used as biocontrol agents in insect pest management. Both entomopathogenic fungi and nematodes share the soil environment and therefore can infest and compete for the same insect host. However, natural co-infections are found rarely due to the cryptic soil environment. Our current knowledge on their interaction within the host comes mainly from laboratory experiments. Due to a recent trend of combining biocontrol agents to increase their efficacy, many studies focused on the co-application of different species of EPF and EPNs against various insect pests with variable outcomes for instance, synergistic effect, additive effect, and antagonism. Also, the effect on the development and reproduction of each pathogen varied from normal reproduction to exclusion, and generally, the outcomes of the interactions were dependent on the pathogen and host species, pathogen doses, and timing of infection. The present review aims to summarize current knowledge on the interactions of entomopathogenic fungi and nematodes within an insect host and estimate the possible effects of the interactions on natural pathogen populations and their use in microbial control.

KEY WORDS: microbial control agents, synergy, antagonism, biocontrol; effectiveness.



SESSION VI

INSECTS AND MICROORGANISMS

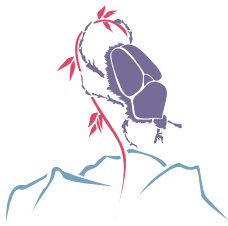
Scotch broom's psyllid fauna and '*Ca. Liberibacter europaeus*' infection in northwestern Italy

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The Scotch broom *Cytisus scoparius* (L.) Link is a deciduous leguminous shrub belonging to the Fabaceae family. It is native to western and central Europe, it typically colonises heaths and mountain grasslands. It has been widely commercialized as an ornamental plant in temperate and subtropical regions of the world but has also become an invasive species and a serious weed in several temperate areas. The bacterium '*Candidatus Liberibacter europaeus*' (CLEu) was firstly described in Italy in 2011 from pear trees, where it seemed to behave as an endophyte, and pear psyllids. Subsequently, CLeu was recorded in New Zealand in symptomatic Scotch brooms (stunting of shoots, shortened internodes, leaf dwarfing and leaf tip chlorosis) and in the psyllid *Arytainilla spartiophila* (Förster) that was introduced as a biocontrol agent in the early 1990s in order to slow down the uncontrolled spread of the plant. More recently, CLeu was reported in Scotch broom and broom psyllids in the UK. The similarity of UK and New Zealand sequences fits with a scenario where CLeu arrived accidentally in New Zealand following the introduction of *A. spartiophila* from the UK. Consequently, a need for investigating the CLeu infection status of Scotch broom plants and its associated psyllids have raised in Italy. In particular, a wide screening was done in different ecological niches in the Piedmont region (North-western Italy), focusing on significant *C. scoparius* stands. Insects were sampled by means of the beat-tray method and subjected to DNA extraction. Molecular analyses were performed by PCR with specific primers for CLeu. *Arytainilla spartiophila* and *Arytaina genistae* (Latreille) were the most abundant psyllid species and both resulted positive to CLeu. This is the first report of CLeu in *A. spartiophila* in Italy and of *A. genistae* as host of the bacterium. Further analyses on the presence and incidence of symptoms in plants are currently underway. In addition, more in-depth investigations are in progress in order to assess the population dynamics of *A. spartiophila* and *A. genistae*, their competence in transmitting the bacterium as well as CLeu epidemiology.

KEY WORDS: *Arytainilla spartiophila*, *Arytaina genistae*, *Cytisus scoparius*, epidemiology.



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P A L E R M O

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Session VII

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

The use of RNA interference for the management of arthropod pests in livestock farms

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Pest management in farm animals is an important action to prevent transmission of severe diseases to the stock and contain economic damage to livestock production. Use of chemical insecticides is still the most common approach followed by farmers, however avoiding possible toxic effects on animals is a fundamental task for pest control measures compatible with animal well-being. Moreover, legal constraints aimed at taking off the market harmful insecticides and insurgence of resistance by target species to the available active ingredients are increasingly complicating farmers' operations. The application of RNA interference techniques has enabled the production of new means of pest control in agriculture and it is opening a promising avenue for controlling arthropod pests in livestock. The interference is based on the action of double strand RNAs (dsRNA) capable of specifically silencing genes of the recipient organisms impairing the production of fundamental proteins. Their mechanism guarantees selectivity against non-target organisms potentially exposed, in addition there are physical and chemical barriers to dsRNA uptake by mammalian cells that render these products practically innocuous for higher animals. The presentation will furnish an overview of the recorded cases of resistance to active compounds commonly used by livestock farmers. Then, summarising the existing literature on gene silencing for the main taxa of arthropod pests of livestock (Acarina, Diptera, Blattoidea), the perspectives of practical applications of dsRNA-based pesticides against the main pests of farm animals will be explored. Existing lack of knowledge are indicated to stimulate additional research in this area.

KEY WORDS: dsRNA, biopesticides, animal health, biotechnology.

POSTER

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

First evidence of natural breeding sites and a noteworthy record of adults of *Anopheles superpictus* (Diptera:Culicidae) in Tuscany region

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Anopheles superpictus (Grassi, 1899) is a malaria mosquito vector found along the South and Eastern Mediterranean basin (including northern Africa and the Middle East), in Eastern Europe, and into the Caucasian area of the Western Palaearctic Region (Vincent et al., 2019). In Italy, the presence of this species, considered a secondary malaria vector in the past (Romi et al., 2001), is now strongly restricted to limited areas and protected wetlands of Southern regions (Benelli et al., 2018) due to extensive land reclamation and vector control activities. In these territories, larval breeding sites are mainly represented by gravelly beds of hill streams with scattered vegetation. Adults show a typical phenology of late summer mosquitoes with an increasing density throughout the summer, usually reaching a peak in August and September. Females are hematophagous and need to take several blood meals on animals or humans to complete the development of eggs (Jetten et al., 1994). This opportunistic behaviour is exploited by Plasmodium genus to complete its biological cycle. This parasite is the etiological agent of malaria causing hundreds of thousands of deaths every year, in particular in tropical regions. Therefore, surveillance activities represent an important tool to prevent potential epidemic outbreaks and to increase the knowledge about vector species. In this context the aim of this study is to deepen the knowledge about presence and distribution of *An. superpictus* in Tuscany, an endemic malaria area before eradication, where also several malaria episodes were registered until last decades (Panin F. et al., 2019).

Within a CCM 2019 project, samplings were performed from June to October 2022 in Magliano in Toscana (Grosseto municipality, Tuscany region). Mosquito collections were carried out as follows: traditional larval catching methods were used along the Albegna river during the last week of September; adult mosquitoes were collected by using CDC-light and BG-sentinel traps, placed in five different sites in two farmhouses and activated from sunset to dawn, supported also by manual collections (electric aspirators). Environmental parameters (temperature and relative humidity) were also recorded. Both larvae and adults were identified by morphological keys (Severini et al. 2009) and confirmed by molecular analysis using ITS-2 of ribosomal RNA as a marker. Up to now, analysis reveals presence of 164 specimens (20 larvae and 144 adults), belonging to the *An. superpictus* species, collected during the season, with a peak of abundance between the end of August and early September. The rest of the sample is still being analysed, as some specimens still need to be sorted and identified. These findings have allowed the rediscovery of *An. superpictus* in Grosseto municipality after hundred years from the last record (Galimini A., 1923), but above all the detection for the first time in this area of larvae of this mosquito in natural breeding sites. To confirm a local stable presence or a recent reintroduction of this species, further investigations, also in neighbouring areas, are required. Although preliminary, these results reinforce the importance of entomological surveillance activities, especially in a context of residual anophelism.

This research was supported by CCM 2019 project "Definition of the integrated vector management strategy and its application in the municipal arbovirus plans within the Rete Città Sane" and by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

KEY WORDS: *Anopheles superpictus*, malaria, breeding sites, mosquitoes.

ORAL PRESENTATION



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Entomological investigations carried out in seven Italian regions highlighted hot spot areas for phlebotomine-borne diseases circulation

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Italian Phlebotominae sand flies are distributed mainly close to Tyrrhenian, Ionian and middle-south Adriatic coastline, including islands. Currently, their range is expanding towards regions of northern Italy, especially in Piedmont, Veneto and on the eastern side of the central-northern Apennine ridge.

This expansion due to of climate change and environmental conditions leads this ectothermic insects to length their activity period increasing larval survival during winter period and also adults biting activity during summer season.

This scenario linked to sand flies vector role in the transmission of visceral and cutaneous leishmaniasis caused by *Leishmania infantum* as well as Toscana Virus and other phleboviruses affects the expansion of Italian Phlebotominae-borne diseases.

In this study, we report the results of longitudinal study carried out in the frame of a CCM 2019 project: "Definition of the integrated vector management strategy and its application in the municipal arbovirus plans within the Rete Città Sane" funded by Italian Health Ministry. Samplings were performed twice a month, from 2019 to 2022 in seven Italian regions namely: Emilia-Romagna, Friuli Venezia Giulia, Latium, Piemonte, Sardinia, Tuscany and Veneto, using CDC and BG-sentinel traps taking advantage of West Nile virus surveillance program.

Collected specimens were morphologically identified pooled for site, date species and sex up to 30 specimens and tested by RFLP-PCR and RT-PCR for pathogen detection, *Leishmania* spp. and Toscana Virus respectively. To obtain live specimens and proceed to dissection for parasite isolation and culture, hand capture was conducted in Magliano in Toscana (GR), Este (PD) and one Sardinia site (Olmedo, SS).

Overall, 1210 capture sessions were carried out in 138 Italian municipalities with a total collection of 192859 sand flies, 49.0% (N = 94450) of which were morphologically identified as follows: *Phlebotomus perfiliewi* (91.81%), *Ph. perniciosus* (6.40%), *Sergentomyia minuta* (1.61%), *Ph. mascittii* (0.17%), *Ph. papatasi* (0.01%) and *Ph. neglectus* (0.004%); with strongly unbalanced sex ratio in favor of females (76.6%).

Altogether 45746 specimens (pool=746) were molecularly analyzed, of which 66 (0.4%) tested positive for *Leishmania* natural infection, with higher vector species prevalence for *Ph. perniciosus* (1.3%). The highest value was registered in Veneto (6.5%), followed by Sardinia (1.9%), Emilia Romagna and Tuscany (0.2% respectively). Focusing on dissection analysis, only in Sardinia isolation and culture highlighted presence of 3 *L. tarentolae* (10.0%) and 1 *Tripanosoma platyductyli* (3.3%) strain obtained only from *Se. minuta*.

A total of 441 pool (N=29194) were analyzed for Toscana Virus, of which 3 (0.1%) *Ph. perfiliewi* pool tested positive, in Latium (25.0%) and Emilia Romagna (0.04%) and for the first time in Piedmont (1.8%).

These results demonstrate *L. infantum* circulation by *Ph. perfiliewi* in Emilia-Romagna and Tuscany, where the species results locally extremely abundant, instead in Sardinia pathogen circulation is related to *Se. minuta* and for the first time to *Ph. perniciosus* positive for *L. tarentolae*.

Concerning Toscana virus detection confirms its circulation in Northern, Central and Sardinia island regions. In conclusion this study improve knowledge of presence and distribution of phlebotomine-borne diseases in the Italian territory highlighting the importance of surveillance activities.

KEY WORDS: sand fly, species, phlebotomine-borne diseases, molecular detection.

ORAL PRESENTATION

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Entomological mosquito surveillance for West Nile, Usutu virus and *Dirofilaria* spp. in Basilicata region in 2021-2022 years

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INTRODUCTION - The entomological survey is a fundamental action of the national surveillance plan of arboviruses (PNA, 2020-2025) at a level region to prevent risk of pathogen transmission by arthropods, including mosquito species. Mosquito monitoring was conducted in the Basilicata region as part of an integrated surveillance plan for Usutu and West Nile (WN) virus and *Dirofilaria* infection. This work reports the results obtained in 2021-2022 years.

MATERIALS AND METHODS - In the period April-November, mosquito traps (CDC and BG sentinel®) were positioned in selected livestock farms from each province in Basilicata region. After identification using morphological keys according to Severini et al., 2009, the culicidae were counted and pooled according to species, sex, date, trap. Females were molecularly tested for WN and Usutu RNA using real-time RT-PCR and for *Dirofilaria repens* and *D. immitis* using both qPCR and LAMP. A leg from each *Anopheles maculipennis* s.l. complex mosquito was used for molecular identification at species level. The sequences thus obtained were compared with the NCBI database through BLAST runs.

RESULTS - A total of 1971 (1941 F, 30 M) adult mosquitoes from 7 genera and 16 species *Aedes* (*Ae*) *albopictus* (6/1971,0.30%), *Ae. vexans* (10/1971,0.51%), *Anopheles* (*An*) *algeriensis* (121/1971,6.14%), *An. claviger* (4/1971,0.20%), *An. maculipennis* s.l. (= *An. labranchiae*) (82/1971,4.16%), *An. plumbeus* (1/1971,0.05%), *An. superpictus* (22/1971,1.12%), *Coquillettidia richardii* (85/1971,4.32%), *Culex* (*Cx*) *pipiens* s.l. (505/1971,25.63%), *Cx. theileri* (6/1971,0.30%), *Culiseta* (*Cul*) *annulate* (147/1971,7.45%), *Cul. longiareolata* (12/1971,0.61%), *Ochlerotatus* (*Ochl*) *caspius* (880/1971,44.65%), *Ochl. communis* (11/1971,0.56%), *Ochl. detritus* (62/1971,3.15%), *Uranotaenia unguiculata* (2/1971,0.10%) were collected at the 7 selected sites (6 in MT province and 1 in PZ province) for a total of 158 catches. Out of 310 examined pools, all of them were negative for both WNV and USUV RNA and 9 pools positive for *Dirofilaria*, with 8 being *D. repens* and 1 *D. immitis*. Specifically, *D. repens* was found in *Ochl. caspius*, *Cx. pipiens* s.l., *Coquillettidia richardii*, *An. maculipennis* s.l. (= *An. labranchiae*), e and *D. immitis* was only recorded in *Ochl. caspius*.

CONCLUSIONS - The results obtained show the presence of a wide culicidic entomofauna in the monitored farms, confirming the variety of biotopes present in the indagated area. The presence of mosquito species (*Cx. pipiens* s.l., *Ochl. caspius*, *An. labranchiae*, *An. superpictus*) vectors or suspected vectors of a variety of human and animal pathogens suggests the importance to continue the monitoring activities. The positivity to *D. repens* is recorded for the first time in *An. labranchiae*.

KEY WORDS: entomological survey, West Nile, Usutu, *Dirofilaria*.

POSTER

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

**Comparison of 4 different field trapping methods for surveillance of adult
Anopheles mosquitoes**

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Mosquitoes of the genus *Anopheles* have been subjected to intense research in Italy during the period of the malaria eradication campaign. Although Anopheline mosquitoes can transmit several disease-causing pathogens to humans and animals, they are not intensively studied anymore and to date the knowledge on the presence and diffusion of the various species in Italy is largely incomplete.

The aim of this study, conducted from July to October of 2019 in 3 different sites in Lombardy, was to compare and evaluate different trapping techniques for the active sampling of *Anopheles* spp. for a subsequent development and standardization of a sampling protocol usable on a large scale. Four different trapping systems including i) modified CDC CO₂ baited trap, ii) CDC light trap, iii) BG Sentinel with CO₂, iv) direct manual aspiration were used and compared. The traps worked overnight (from 05:00PM until 10:00AM of the next day). Morphological methods were used for species identification.

A total of 9956 mosquitoes were collected with the 4 trapping methods of which 2786 were anopheline mosquitoes including both males and females. Regarding species identification *Anopheles maculipennis* s.l. was the most abundant species. Most of the *Anopheles* were collected using BG Sentinel with CO₂ and modified CDC CO₂ trap (1559 and 916 specimens respectively), while the CDC light trap and manual aspiration captured significantly less individuals (61 and 250 specimens respectively).

Our results support the use of BG Sentinel with CO₂ traps as a successful tool for the surveillance of *Anopheles* population which represent a crucial for the risk assessment and management of possible cases of autochthonous transmission of malaria.

KEY WORDS: *Anopheles*, Entomological Surveillance, Trapping Methods.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Sterile Insect Technique pilot application targeting *Aedes albopictus* (Diptera; Culicidae) in Bologna

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A Sterile Insect Technique (SIT) pilot project for the suppression of *Aedes albopictus* was started in the 2019, involving an urban area of about 100 hectares in Bologna city. The project has been supported by the Municipality of Bologna with the collaboration of the Emilia-Romagna Region (through the CCM project “Definition of an integrated strategy for the management of vectors and its application within the Municipal Plans Arboviruses in collaboration with the WHO Healthy Cities network”).

The SIT is a biocontrol strategy aiming to reduce the reproductive rate of the target species thus causing a progressive decline of the population density. The SIT application requires the establishment of a mass rearing facility, the sorting of the males, their sterilization by radiation and release in the environment. The sterile males by mating with the wild virgin females will make them sterile for the entire life (the *Ae. albopictus* female is basically monogamous and the first mate will shape its reproductive status).

In the period 2019-2021 the irradiation of the male pupae in the water has been conducted by the gamma ray machine available through a collaboration with the S. Anna hospital in Ferrara, while in the 2022 an X-ray irradiator has been acquired and made operational.

Laboratory tests on the males’ sterility levels and field studies on the survival and dispersal of sterile males (through marking-release-recaptures experiments) didn’t show significant difference between the two radiations source.

In the pilot area sterile males were released twice a week during the reproductive season: 4.5 million were released in the 2021 and 6.5 million in the 2022. The induced sterility rate has been monitored regularly by collecting and hatching the eggs in the SIT treated area in comparison with a control non-treated area. The seasonal mean induced egg sterility resulted 45.5% (SD 20.1%) with maximum values about 70 %. The reduction in the number of eggs, in comparison to the density observed in the non-SIT treated urban territory of Bologna, resulted 52.2% (SD 15.3%) in the 2021 and 54.4 % (SD 34.1%) in the 2022.

A survey conducted locally showed that the opinion declared by residents is positive on the project without reporting any critical issue.

The project is ongoing by focusing on improving the methodology of male irradiation to obtain sterile males of higher performances and aerial release by drone to achieve a more homogeneous field distribution.

KEY WORDS: SIT, biocontrol, mosquitoes, *Aedes albopictus*.

ORAL PRESENTATION

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

**Description of the entomofauna associated with the remains of the Cistercian nun
Angela Veronica Bava (1591-1637)**

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Barbara Napoli¹, Aurora Nigro¹, Emy Tonini¹, Omar Larentis², Marta Licata², Stefano Vanin¹

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In an archaeological context, the knowledge of the ecological and biological peculiarities of the arthropods' species associated with the human or animal remains can be useful to reconstruct the funerary practices, to describe the cadaver taphonomy and to understand the hygienic and social conditions of the investigated human populations. This research aims to describe the entomological community associated with the body of the Cistercian nun Angela Veronica Bava (1591-1637), stored in the Fossano Monastery (Northern Italy) in order to provide information about her funerary ceremony, the taphonomic process affecting her body and eventually her sanitary and hygienic status when alive and so doing verifying the historical data.

Veronica Bava died on April the 14th 1637 at the age of 46. Historical notes report that, after the death, her body was exposed in the cloister and then preserved in a wooden coffin and initially placed in a common grave. Subsequently the body was transferred several times and, for a while, the remains were split in two jars, one for the bones and the other one for the clothes. With the last transfer the remains were reunited in a single coffin.

Entomological samples were manually collected with sterile tweezers and paintbrushes from the bones and the coffin and stored in sterile plastic vials. Samples identifications were performed using specific keys and by comparison with already identified specimens.

The arthropod fragments collected from the Veronica Bava remains belong mainly to the insects (Insecta) while only two fragments belong to a spider (Arachnida, Aranea). Among the insects, puparia of *Hydrotaea capensis* (Diptera: Muscidae) count more than the 84% of the analysed specimens. Fragments of five families of Coleoptera (Dermestidae, Zosteridae, Staphylinidae, Endomychidae and Carabidae) and one family of Hymenoptera were also associated with the bones. No arthropod ectoparasites as lice or fleas were found among the analysed material.

The entomological evidence highlighted that the body of Veronica Bava, when exposed to the devotees was in an environment limiting the arrival of Calliphoridae (Frankincense smoke, cloister, chapel, etc), not found during the remain inspection. The body was preserved in a sheltered, underground environment where decomposition took place as documented by the presence of *H. capensis* puparia. The conservation condition of the bodies changed during the time with periods of high humidity with the formation of mold as indicated by the presence of Zopheridae and Staphylinidae species and periods of low humidity (dry) as documented by the presence of Dermestidae beetles and spiders. This reconstruction is in agreement with the historical records about the body transfer at different times after death. The lack of ectoparasites reveals a good hygienic condition of the body but it cannot be excluded the usage of funerary practices composed by the cleaning of the body after the death typical of the catholic tradition. This work shows how the analysis of the traces of the insects associated with a body can provide evidence to confirm or to amend historical information also in a religious context.

KEY WORDS: Taphonomy, human remains, crypt.

POSTER

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Ecological evaluation of the entomofauna in a dry hypogeous funerary context in northern Italy (Azzio, Varese)

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Funerary archaeoentomology is the application of the study of insects and other arthropods in archaeology and in particular in burial contexts. The study of these organisms provide useful information for the reconstruction of past events and to understand changes in environmental and climatic conditions.

In 2022, the tomb of the Della Porta family (18th century) in the church of Saints Antonio and Eusebio in Azzio, located in northern Italy in the province of Varese, was explored and a multidisciplinary study (anthropology, paleopathology, entomology, etc) took place. The tomb is located under the floor of the central nave of the church. The tomb contains three partially preserved wooden coffins. The environment was dry and there was no trace of recent animal activity.

The entomological study began with the documentation and the on-site collection of the entomological samples carried out with brushes and scoops. All the collected material was subsequently analysed in laboratory, where the samples of entomological interest were isolated, identified and subsequently photographed.

The analyses revealed the presence of several taxa belonging to the orders Diptera, Coleoptera and Lepidoptera. Among the Diptera, the dominant group was the Muscidae family, with the species *Hydrotaea capensis*. This species is known as one of the most important colonizers of bodies in confined environments such as crypts, hypogean tombs and coffins. Puparia of Phoridae and Sphaeroceridae were also collected. Among Coleoptera, specimens of Cryptophagidae, Histeridae, Staphilinidae and Mycetidae were identified. *Mycetatea subterranean* in the family of Mycetidae is worth of be mentioned because found in several archaeological burials. No other arthropods have been found, except for few specimens of mites.

The absence of insects specialised in the colonisation of exposed bodies during the early stages of the decomposition such as the Diptera Calliphoridae and/or Sarcophagidae, suggests that the corpses were buried shortly after death.

The results of this study highlight the very low entomological diversity of the site, which may suggest that excessively dry and unfavorable environmental conditions combined with difficult accessibility to the burial environment hind the colonisation of corpses even in favorable climatic contexts. In fact, entomological analyses carried out in other burials in the same church provided very different results in terms of number of species and abundance of specimens.

KEY WORDS: Archaeoentomology, taphonomy, crypts.

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Entomological study of the III order Franciscan friars' tomb in the Saints Antonio and Eusebio church (Azzio, Varese, Northern Italy)

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The application of entomology in the archaeological field can contribute to the understanding and reconstruction of past events and habits. Funerary contexts can promote the development of an entomofauna which, when properly analysed, can provide information about the decomposition of the bodies and about the climatic and environmental conditions.

Under the floor of the church of Saints Antonio and Eusebio in Azzio, in the province of Varese (Northern Italy), there is a crypt that was reserved to the burial of the Franciscan friars of the III order. The crypt was explored in spring 2022. Samples were collected in order to describe the entomological fauna of the site and consequently to derive the information about events related with it.

Sampling was performed by mean of tools not affecting the fragile material. Subsequently, laboratory analyses were carried out starting with the sieving of the sediments which allows for an easier detection of the entomological fragments. All the isolated species were identified and photographed in order to create a pictorial archive.

During the inspections and samplings, it was noted that the environment had a high level of humidity, also linked to water infiltration. A clear brownish line with some Diptera puparia was also noted on the walls. It suggests a flooding of the crypt after the decomposition of the bodies.

The dominant specimens were puparia of Diptera, belonging to the Muscidae and Phoridae families, typical of colonization in hypogeal contexts and present in massive quantities. Puparia belonging to Sphaeroceridae were also present.

Among Coleoptera, both species associated with body decomposition (Cleridae, Histeridae) and xylophagous species (Ptinidae) were identified, but also species associated with the environment (*Blaps* sp., Tenebrionidae). The finding of mycetophagous species belonging to the families of Mycetidae (*Mycetaea subterranea*), Cryptophagidae (*Cryptophagus* sp.) and Zopheridae (*Langelandia* sp.) is also particularly interesting. Predatory species linked to the external environment such as Carabidae and Staphilinidae were also collected. Other arthropods found, such as pseudoscorpions, spiders and, in small numbers, mites are more related to the environment.

The results allow to hypothesize 1) a flooding of the tomb, after the decomposition of the bodies, as suggested by the presence of puparia on the walls, 2) a high level of humidity allowing the development of fungal species pabulum of the mycetophagous species and 3) a connection of the tomb with the external landscape allowing the access to ground - and rove - beetles.

POSTER



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Observations on Heteroptera Lygaeidae infestations in urban environments

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In recent years we have followed numerous infestations by Hemiptera Lygaeidae in Umbria and Emilia-Romagna regions, with a large spread of bedbugs from the cultivated fields of rapeseed (*Nysius cymoides*) and chamomile (*Metopoplax ditomoides*) towards the surrounding towns. The invasion mainly involved the nymph stages in the case of *N. cymoides* and the adult stage in the case of *M. ditomoides*. In the first case there was the invasion of houses through the ground with millions of individuals who carpeted the streets and covered the walls of the houses; in the second case, on the other hand, the invasion occurred by adults, who were attracted by the thousands by the lights of the houses and flew inside. However, unlike *N. cymoides*, which invaded inhabited areas, but did not actively interact with humans, *M. ditomoides* attacked people, stinging them repeatedly in every part of the body, causing micro-papulopustular and vesiculobullous lesions, almost always excoriated by scratching due to the intense itching caused. In both cases the invasion occurred after the crops were cut. While it was possible to intervene with mechanical means to deal with the infestation by *N. cymoides*, to eliminate the infestation by *M. ditomoides* inside the houses it was necessary to use chemical treatments based on pyrethroids.

KEY WORDS: *Nysius cymoides*, *Metopoplax ditomoides*, puncture.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Problems related to the massive presence of *Apis mellifera* (Hymenoptera: Apidae) in anthropic context: analysis of some cases recorded in South-Western Piedmont (Northern Italy)

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During the period between 2010 and 2020, the reports for massive presence of *Apis mellifera ligustica* in highly anthropized contexts were collected and analysed. These reports occurred because this hymenoptera causes a source of annoyance for the risk of stings, particularly feared by sensitive sections of the population including subjects with a full-blown allergy to bee venom. The reports refer to cases that occurred in north-western Italy, in south-western Piedmont.

All the cases found have all been traced back to incorrect management practices of hives bordering urban areas, or to incorrect planning or management of green areas for public or private use. As regards the damages found by pests, it was possible to record a temporary state of alarm, linked to the disturbance induced by the insect and the phobia of stings, the death of two heads of cattle, feeding disturbances in stabled cattle and seven cases of humans stung.

The management of these events is not simple and in all cases requires a careful analysis of the problem; in any case the management must go through the modification of the environment such as for example the mowing of the attractive vegetation. Based on the cases analysed, if the cause has been correctly identified, the infestation resolves itself in 4-5 days.

KEY WORDS: *Apis mellifera*, risk for sensitive population groups, management of apiaries.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Risk of dengue outbreaks in Italy: evaluation of the vector competence of Italian *Aedes albopictus* populations for DENV-1

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Dengue is a mosquito-borne viral disease widely spread in tropical and subtropical areas, caused by four serotypes of dengue virus (DENV) and transmitted to humans by *Aedes* spp. *Aedes aegypti* is considered as the main vector. Although considered a secondary vector of DENV, *Ae. albopictus* is associated to the virus transmission in different areas of the world. Its actual role as dengue vector needs to be better investigated given its widespread presence also in non-tropical regions. Indeed, in August 2020, the first outbreak of autochthonous dengue was reported in Italy with 11 locally-acquired cases detected in Montecchio Maggiore (Vicenza province, Veneto Region).

The aim of our study was to evaluate the vector competence of two *Ae. albopictus* Italian populations for a DENV-1 strain circulating in Singapore, phylogenetically similar to the Indonesian strain responsible for the 2020 Italian epidemic.

Experimental infections by a membrane feeding system were performed, providing an artificial infectious blood meal to mosquitoes. *Ae. albopictus* females (field populations collected in Montecchio Maggiore and Rome) and *Ae. aegypti* (as control lab-colony) were fed and monitored after the infectious blood meal for 28 days and reared under controlled conditions (T=26±1°C, 70% RH, 14/10h light/dark cycle). Infection, dissemination and transmission rates (IR, DR, TR) were assessed by detection of the virus in abdomen, legs plus wings and saliva of fed females, respectively. DENV titre of infected mosquitoes was evaluated by quantitative Real Time PCR.

Preliminary results highlighted an increasing IR in *Ae. albopictus* populations with a maximum value at 14 days post infection (p.i.); the IR value decreased progressively at 21 and 28 days p.i. Conversely, a progressive increase of IR value in *Ae. aegypti* population was observed over the study period. Our data demonstrated a disseminated infection starting at 7 and 14 days p.i. in *Ae. albopictus* and *Ae. aegypti*, respectively, and a maintenance of high viral titres until 28 days p.i. Analyses of the saliva and vertical transmission are still in progress.

Our preliminary results highlight the possible vector competence of both Italian *Ae. albopictus* populations for DENV-1. The TR analyses will be able to confirm the actual vector role of this species in the transmission of DENV and the potential risk of further outbreaks in Italy and Europe.

This research was supported by ISS funding within the Bando Ricerca Indipendente ISS 2020-2022: Evaluation of the vector competence of Italian *Aedes albopictus* populations for dengue virus type 1 and 2 in order to contribute to the risk assessment of dengue in Italy (Project cod: Iss20-80089926288b) and by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

POSTER



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Field trials of mosquito control with Mosquitorex

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In recent years in the Mediterranean area, reports of mosquito-borne diseases have increased significantly and this has brought back to the forefront the need to manage the risk of infection by trying to limit mosquito populations. However, recent studies have highlighted the emergence of resistance to some insecticides commonly used to control mosquito adults and larvae.

This study aimed to evaluate the larvicidal effect of the commercial product Mosquitorex (Newpharm). This product, based on oils of vegetable origin, exerts a physical-mechanical action by creating a thin film on the surface of the water that prevents the larvae from breathing and favors the drowning of the females that lay eggs.

Mosquito larvae control tests were carried out in 2021 in Cagliari (Italy) in a little pond of around 2000 m² surrounded by thick reeds and closed to the public, within the 'Terramaini' municipal park. The remaining part of the park was used as an untreated control area. Treatment with Mosquitorex was carried out on 23 July 2021 by distributing the product over the surface of the identified wet area at a dose of 2 ml/m². To evaluate the efficacy of the treatment, weekly sampling was carried out with dippers to determine the abundance of larval stages and assess the potential impact of the larvicide product on non-target organisms in the treated and untreated areas. To verify a real decrease in the adult mosquito population, 3 CDC Light Trap and 3 BG-Sentinel traps, activated with a specific chemical lure, were placed in the treated area and the same number in the untreated area.

In the untreated area, the number of Culicidae larvae remained almost constant at about 15-16 larvae during the trial, while in the treated area, the larval populations significantly decreased to 3 and 2 larvae per 500 ml of water in the two weeks following the treatment.

Morphological analysis of mosquito adults caught in the traps revealed the presence of three species: *Aedes albopictus*, *Aedes caspius*, and *Culex pipiens*. Following treatment, *Ae. caspius* and *Cx. pipiens* populations were significantly reduced for 2 weeks while no effect was observed on the *Ae. albopictus* density.

No effect was observed on populations of non-target organisms. However, the low densities of these arthropods do not allow us to draw any definitive conclusions.

KEY WORDS: *Aedes albopictus*, *Culex pipiens*, Field trials, Mosquitorex.

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Identification of *Culicoides* species transmitting Schmallenberg virus in Sardinia (Italy)

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Schmallenberg virus (SBV) is an arbovirus belonging to the genus Orthobunyaviruses and the family Bunyaviridae. SBV affects domestic and wild ruminants. The vectors of SBV are small Diptera belonging to *Culicoides* genus. In Sardinia, SBV infection was first observed in 2012 when deformed lambs were born. From 2012 to 2021, when the virus was again isolated from foetuses, there were only sporadic reports of typical malformations of SBV infection. However, in early 2021, several cases of SBV infection in sheep were reported, resulting in the need for continued entomological surveillance to better assess the spread of the virus and to determine which *Culicoides* species are involved in host transmission.

From May to December 2021, all *Culicoides* captured under the Bluetongue entomological surveillance plan were used for this study. The plan foresees weekly captures in eight farms in Sardinia. *Culicoides* caught in the field with Onderstepoort Black Light Traps were then sent to the laboratory for counting and morphological identification, after being separated by species and sex. Females were also differentiated into nulliparous (that had no blood meal), parous (that had at least one blood meal), gravid (that had well-formed eggs in their abdomen), and engorged (that had blood in their abdomen). Only parous, gravid, and engorged females were subjected to Real Time RT-PCR analysis to detect the virus, as only they had consumed at least one blood meal and could have ingested the virus.

A total of 226 captures were made, yielding a total of 133,711 *Culicoides* belonging to 19 species. The two most abundant species were *C. imicola* (56%) and *C. newsteadi* (35%). A total of 1946 pools were analysed with a variable number of individuals per pool ranging from 1 to 25. Real Time RT-PCR analysis revealed 35 positive pools: 28 of *C. imicola*, 3 of *C. newsteadi* and 1 of *C. cataneii*, *C. sahariensis*, *C. paolae* and *C. obsoletus*. Positive pools were found from July to October at all capture sites.

This study confirmed the role of *C. imicola*, *C. newsteadi*, and *C. obsoletus* as vectors of the SBV virus and allowed for the first time detection of positivity in *C. cataneii*, *C. sahariensis*, and *C. paolae*, whose role in virus transmission should be better defined.

KEY WORDS: Schmallenberg virus, *Culicoides*, Vectors, Sardinia.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Laboratory infection of *Culicoides* species with Bluetongue virus

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Bluetongue (BT) is an infectious disease that occurs in a severe form mainly in sheep. The disease is transmitted by biting midges belonging to the *Culicoides* genus. Currently, the species considered vectors are: *C. imicola*, *C. obsoletus*, *C. scoticus*, *C. montanus*, *C. dewulfi*, *C. pulicaris*, *C. punctatus*, and *C. newsteadi*. *Culicoides* from stable colonies are generally used in experimental infections, while only for a few species wild-caught adult females are directly used.

This study aimed to assess the potential vector competence of other *Culicoides* species that are often found in abundant numbers on livestock farms.

Culicoides captured with ultraviolet light traps were brought into the laboratory, anaesthetized with CO₂ for 30 seconds, and separated by species and sex. Laboratory infection were carried out with nulliparous females (females that have not yet had a blood meal and are free from possible infection) of the following species: *C. newsteadi*, *C. circumscriptus*, *C. jumineri*, and *C. paolae*.

Females of each species were kept separately in cylindrical cardbox with both ends closed with a fine-mesh net for 3 days and fed with a cotton ball soaked with a sugar solution. Blood meal infected with BTV-1 was supplied to the *Culicoides* by placing a Hemotek feeder on the cardbox. Each meal lasted 60 minutes. After blood meal, *Culicoides* were anaesthetized with CO₂ for 30 seconds and, under the stereomicroscope, females with a red abdomen due to the presence of freshly ingested blood were separated. These were placed in a cardbox and kept for 7 days in the insectarium until REAL-TIME RT-PCR analysis for virus detection. About 1000 *Culicoides* were used in the experimental infection tests. Of these, only 153 females took the blood meal and survived up to 7 days p.i.. At the end of this period, females belonging to 4 species were analyzed: 112 *C. newsteadi*, 15 *C. jumineri*, 10 *C. circumscriptus*, and 6 *C. paolae*. A total of 92 pools were analyzed with a number of individuals per pool ranging from 1 to 10. REAL-TIME RT-PCR analysis revealed 5 positive pools: 3 of *C. newsteadi* (Ct values ranging from 32.47 to 34.21), 1 of *C. jumineri* (Ct value 40.2), and 1 of *C. circumscriptus* (Ct value 39.7).

Experimental infection tests confirmed the vectorial capacity of *C. newsteadi* and contributed to *C. circumscriptus* being considered even more of a potential vector species, although the small number of individuals used does not allow definitive conclusions to be drawn.

KEY WORDS: Laboratory infection, Bluetongue, *Culicoides*.

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Laboratory evaluation of the insecticidal effect of Mosquitorex on two mosquito species

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Traditional strategies for containing mosquito populations based on the use of chemical insecticides now have limitations mainly related to the development of resistance, risks to human health, and effects on non-target organisms. These issues make it necessary to promote the development of alternative control methods.

The objective of this study was to evaluate the insecticidal effect of the commercial product Mosquitorex (Newpharm). Based on oils of plant origin, this product exerts a physical-mechanical action by creating a thin film on the surface of the water that prevents larvae from breathing and promotes the drowning of the females that settle for egg-laying. Laboratory tests were conducted in 2022 to verify the effect of Mosquitorex on the viability of larvae, drowning of adult mosquitoes resting on the water surface for egg-laying, and simultaneously also on the number of eggs laid.

Culex pipiens and *Aedes albopictus* mosquito species from breeding in the IZS Sardinia insectarium were used in the laboratory bioassays. Different doses of the product (1, 2, 3, 4, 5 ml/m²) were assayed on early-life larvae and mortality was checked at 24 and 48 hours.

To evaluate the effect of Mosquitorex on mosquito drowning and egg laying, two tests were conducted: trials with choice and trials without choice. In the trials with choice, two small egg-laying containers were placed inside the mosquito breeding cages, one with the insecticide and the other without the product. In trials without choice, only the container with the insecticidal product was placed inside the cages of the two species. Two doses of the product were tested: 1 and 2 ml/m², and after 24 hours, eggs and dead adults were counted.

Mortality tests on the larvae of *Cx. pipiens* and *Ae. albopictus* revealed excellent efficacy of the insecticidal product. In fact, at 48 h, doses 1 and 2 ml/m² resulted in more than 95% mortality, while doses 3, 4, and 5 ml/m² resulted in the death of all tested larvae.

In the trials with choice and those without choice, high mortality of mosquito adults was recorded in the containers with the insecticidal product. A significant reduction in the number of eggs laid by *Cx. pipiens* and *Ae. albopictus* was also detected in these containers.

KEY WORDS: Laboratory evaluation, larvae, *Aedes albopictus*, *Culex pipiens*.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Does the South spreading of *Aedes koreicus* (Diptera: Culicidae) increase the risk of Mosquito Borne Diseases transmission?

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Aedes koreicus is an invasive mosquito species native to East Asia, which recently invaded several European countries. This species was detected in the Northeast of Italy (Belluno province) in 2011. After the first detection, this species has been able to colonize all the north Italian Regions in a relatively short time period (approx. 10 years), contrary to what has been observed in Europe (limited spreading). Here, we discuss the findings of *Aedes koreicus* in mosquito collections sampled in the framework of entomological surveillance for West Nile virus (WNV) and Usutu virus (USUV).

According to the National Plan for Arboviruses, the regional health Authorities annually fund entomological surveillance for WNV and USUV; the surveillance is ongoing since 2010. We placed 65 CDC-CO2 traps in 2015 and 75 CDC-CO2 traps in 2016-2022 per year, in the flat area of Friuli Venezia Giulia (FVG) and Veneto regions, at an altitude below 200 m a.s.l. Traps worked from sunset to sunrise every two weeks from May to October. Collected mosquitoes were counted, morphologically identified and then analyzed by molecular methods for WNV and USUV.

From 2015 to 2022, we collected a mean of 143,657 mosquitoes/year (range 103,634-217,581) belonging up to 18 species. Among them, *Culex pipiens* was the most abundant species (about 74%), which is the target species of the surveillance plan. Although a collection method designed for *Cx. pipiens* was used, we were able to collect 231 *Ae. koreicus* (0.02%). Despite the number of *Ae. koreicus* was very low, it is increasing ranging from 0 to 27 and to 41, in 2015, 2017 and 2022, respectively. Moreover, we found this species in flat areas with an elevation ranging between 6 m a.s.l. (Mirano, VE) and 112 m a.s.l. (Premariacco, UD).

In FVG and Veneto regions, we observed an altitude and latitude shift in distribution of *Ae. koreicus* from the initial stage of colonization. It occurred in mountainous and hilly areas, but after several years, we have found an increasing number of specimens in flat areas, characterized by warmer temperatures.

The southwards *Ae. koreicus* spreading in warmer areas represents a risk factor for arboviruses transmission. This species has been proved being a competent vector for Chikungunya and Zika viruses in laboratory conditions (Jansen et al., 2021; Ciocchetta et al., 2018), but the involvement of *Ae. koreicus* in arboviruses autochthonous outbreaks has so far never been reported.

In conclusion, *Ae. koreicus* should be included in ordinary entomological surveillance programs, due to its presence in warm climate areas, where mosquito-borne diseases, such as Chikungunya or Dengue viruses may circulate among human population.

This program was funded by Public Health Department of Veneto and Friuli Venezia Giulia regions.

KEY WORDS: *Aedes koreicus*, entomological surveillance, mosquito-borne diseases.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Phlebotominae sand flies surveillance reveals Toscana virus and *Leishmania infantum* circulation in Northeastern Italy

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Phlebotominae sand flies are vectors of pathogens for humans and animals. In 2021, Toscana virus (TOSV) circulated for the first time in the Veneto region (Northeastern Italy), causing symptomatic infections in humans. Following this TOSV detection, regional health authorities established a surveillance plan to assess the occurrence and abundance of sandflies and the presence of pathogens in the vectors.

We conducted the surveillance placing a CDC-CO2 light trap (white light) and 10 sticky trap sheets in eight sites located in hilly places across Veneto region. We activated the traps every two weeks from May to September 2022.

After morphological identification, sand flies were pooled up to 30 specimens by sex and collection date. Pathogens screening were performed following Sanchez-Seco et al. (2003) and Francino et al. (2006) for Phleboviruses and *Leishmania infantum* detections respectively.

A total of 4343 sand flies were collected (N=4065 by CDC-CO2 light traps and N=178 by sticky traps), of which 79.8% attributed to *Phlebotomus perniciosus* followed by *P. neglectus*, *P. perfiliewi*, *P. mascittii*, and *Sergentomyia minuta*. *Phlebotomus perniciosus* had different abundance peaks during the season among sites.

We analyzed 194 pools, one of which resulted positive for TOSV and eight for *L. infantum*. These results confirm TOSV circulation in the same area where human cases occurred in 2021 and 2022. Of note, it is the finding of a positive pool of TOSV of 35 *P. perniciosus* males to confirm vertical or sexual virus transmission, as reported in literature.

More research are necessary in order to deeply investigate the epidemiological cycle of these pathogens. Our study highlights the importance of integrated surveillance in monitoring and control programmes of vector-borne diseases.

This program was funded by Public Health Department of Veneto region.

KEY WORDS: Phlebotominae sand flies, Virus Toscana, *Leishmania infantum*.

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

**First record for Italy of the beaver beetle *Platypsyllus castoris* Ritsema, 1869
(Coleoptera: Leiodidae: Platypsyllinae)**

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The Eurasian beaver *Castor fiber* L. 1758 is present in Central Italy since 2020, probably due to an unauthorized release. Beavers are potential hosts for a wide range of endo- and ecto-parasites species, some also dangerous for wildlife and humans. The 'Rivers with Beavers, Italy' project, in addition to behavioral studies and surveys on the impact of these animals on the environment, has also focused its attention on the possible implications that beavers would have on spillover and spillback risks. The collection of a road-killed adult female of Eurasian beaver in the Province of Perugia was the occasion for a more accurate analysis of these animals.

Necropsy occurred on 26th October 2022. After defrosting, the beaver was assessed for ectoparasites by hair brushing throughout its body, and by dividing it into four parts: head, back, belly, and legs. Moreover, cotton swabs were used to search for parasites in each nostril and ear. Swabs and brushed hairs were then stored in Falcon tubes with 70% ethanol before lab analyses.

Within the sample containing hair from the ventral part of the dead beaver, an ectoparasitic larva of the beetle *Platypsyllus castoris* Ritsema, 1869 (Coleoptera: Leiodidae: Platypsyllinae) (0.9 mm in length) was found. The animal is preserved inside a slide with Hoyer's medium. A careful morphological analysis was also carried out for this insect, using tables and photographs found in the literature. The identification was confirmed by the experts Stewart B. Peck and Sándor Szekeres.

The subfamily Platypsyllinae comprehends only 4 genera worldwide. This discovery is of entomological interest concern because *P. castoris* is one of the most modified and well-adapted ectoparasites of all beetles. All this beetle subfamily is characterized by wingless and eyeless or with reduced eyes, and with a striking dorsoventral flattening. The larva has even more specializations than the adult, such as hooked tarsi and mandibles.

The first record of this beetle from a wild population of Eurasian beavers seems to be from the Camargue region of the Petit Rhone River near the Mediterranean coast of southern France. Since then, European collection records of this beetle are from France, Germany, Norway, Russia, and Sweden. More recent reports are for Belgium, Belarus, the Czech Republic, the Netherlands, Poland, Slovakia, Switzerland, and Hungary. This is the first record of this insect in Italy. Subsequent research on this beetle could give us more information on the possible area of origin and the ecology of its host.

KEY WORDS: *Castor fiber*, Eurasian beaver, ectoparasitic beetle..

POSTER

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Diptera of medical-veterinary and forensic interest in Calabria

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Diptera have been extraordinarily successful in their evolution, colonizing many habitat types. The Diptera include species living in close contact with humans that can be used to solve forensic cases, but also species of great interest in the medical and veterinary fields, because they can be parasites or vectors of pathogens. A study on Diptera Brachycera of forensic and medical-veterinary interest was conducted in the Aspromonte National Park, in the Sila National Park and in the Serre Regional Natural Park (Calabria, Southern Italy) and in an extra-urban area near the Botanical Garden of the University of Calabria, using “bottle bait” activated with bovine liver. Among the species identified, some have never been reported for Southern Italy, including the Muscidae of forensic interest *Phaonia angelicae* (Scopoli, 1763) and *Thricops simplex* (Wiedemann, 1817), and *Phaonia errans* (Meigen, 1826), of forensic and medical-veterinary interest. Other species of medical-veterinary interest that were found were *Protocalliphora azurea* (Fallen, 1817) (Calliphoridae), identified for the first time in Calabria and parasite of passerine chicks, *Musca autumnalis* De Geer, 1776 (Muscidae), intermediate host of the nematode *Thelazia* sp. Bosc, 1819 which causes conjunctivitis in cattle, *Haematobosca stimulans* (Meigen, 1824) and *Hybomitra montana* (Meigen, 1820) (Tabanidae), hematophagous, and *Eristalis tenax* (Linnaeus, 1758) (Syrphidae), agent of accidental enteric myiasis. The following genera of medical-veterinary and forensic interest were found: *Calliphora* Robineau-Desvoidy, 1830 and *Lucilia* Robineau-Desvoidy, 1830 (Calliphoridae), *Fannia* Robineau-Desvoidy, 1830 (Fanniidae), *Hydrotaea* Robineau-Desvoidy, 1830, *Phaonia* Robineau-Desvoidy, 1830 (Muscidae), *Piophilina* Fallen, 1810 (Piophilidae) and *Sarcophaga* Meigen, 1826 (Sarcophagidae).

KEY WORDS: Diptera, agent of myiasis, taxa of forensic interest, taxa of medical-veterinary interest.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Is the evolution of fly control moving forward?

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The control of synanthropic flies is still a complex problem to manage but particularly in animal husbandry. Despite many years of training in using an approach that starts from the management of livestock, waste and the environment with the related food sources, the general approach is still too often activated only in the face of an emergency. Even in modern farms, IPM for flies is not considered and programmed only when is too late to prevent. The inspection experience of recent years, addressed to IPM operators, has mainly pointed a series of gaps concerning the lack of knowledge of the life cycle of flies or the relationship between temperature and speed of the biological cycle. The role of flies as vectors is not perceived. There is a general failure to recognize the different larval stages and pupae and related critical role in larvicidal treatments. The operators do not consider the usefulness of physical-mechanical actions, preferring biocides whose active chemicals are often little known and the biological control is considered only as a convenience cover and not an actual management strategy. Often the lack of efficacy in control derives from poor training on flies IPM for both clients and technicians, as the flies control are still too often perceived as a simple disturbance to operators and animals rather than a factor that can cause production losses. The control of flies in many Italian realities is relegated to the spring-summer season only. There is still no real basic programming that starts from an integrated approach and too often we focus on a flying approach to the mere containing intervention of adults or larvae. The new provisions on biosecurity and animal welfare focus above all on the aspects of prevention, monitoring and defense of the environments in which animals are stationed. The integrated and guided approach becomes more and more the only winning perspective against these pests and their important role in transmission of pathogens.

KEY WORDS: Fly, control.

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Historical collection of Culicidae (Diptera: Culicidae) of the Istituto Superiore di Sanità

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At the Section of Diseases Transmitted by Vectors of the Department of Infectious Diseases of the Istituto Superiore di Sanità there is a collection of Culicidae collected over almost one hundred years of history. In fact, the Public Health Institute, which only took the name of Istituto Superiore di Sanità (ISS) in 1941, was officially born in 1934 under the Ministry of Home Affairs, which for some time had housed the General Directorate of Public Health which coordinated the laboratories that had control and research functions. Among these activities that formed the basis of what would have been the ISS, there was applied research to the fight against malaria, largely supported by funding from the US Rockefeller Foundation. In this context, entomological collections were carried on aimed at both the study of species of health interest and the retrieval of teaching materials for the young doctors and entomologists of the time. Part of these collections are still preserved today in the Culicidae historical collection of the ISS Medical Entomology laboratory which has almost 3000 specimens between pre-imaginal and adult stages, the former fixed on a slide and the latter stored dry in entomological boxes, collected since 1925 to date. The collection consists of 53 species belonging to 7 genera, mostly collected in Italy and in some cases from campaigns conducted abroad. The collection includes species considered uncommon or rare today such as *Aedes cataphylla*, *Aedes refiki*, *Aedes annulipes*, *Aedes cinereus*, *Culiseta morsitans*, *Culex martinii* and *Anopheles sacharovi*, the latter no longer found in Italy since 1960.

This collection represents a testimony of research activity in the field of medical entomology and a useful study tool that is continuously expanded and updated.

This research was supported by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

KEY WORDS: collection, Culicidae, ISS, mosquitoes.

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Saints, aristocrats, and common people for science: where, when and how the entomological fauna associated with mummified bodies provides information to forensic entomology

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Insects from archaeological contexts provide useful information to reconstruct the events occurred in the past. In funerary contexts, they may help to reconstruct the related practices or rituals as well as to understand the taphonomic processes. Of note, they provide key information that can be applied in forensic entomology in a communicating vessel system. Indeed, carrying out experiments in a long-term perspective, such as when the mummification process is studied, is a hard challenge. For this reason, all the evidence collected from archeo-funerary contexts is vital to understand and interpretate nowadays forensic cases. The Italian cultural heritage counts a large amount of mummified or skeletonized bodies that can be considered, in addition to their historical, cultural, and religious value a good “test bench” for forensic entomology. This contribution is a sort of journey through the “Bel Paese”; it deals with the entomological analysis of some Saints, blessed people, members of the clergy, aristocrats, and common people buried in crypts and Italian churches to describe the entomofauna associated with those bodies, and provide additional data – at national and regional scale – about the mummies-related arthropods. The entomofauna associated with the bodies from different Italian regions (e.g., Piedmont, Lombardia, Veneto, Tuscany, Umbria, Puglia, Sardinia, Sicily, among others), were manually collected by entomologists and/or archaeologists. Insect species belonging to the orders Diptera, Coleoptera, Lepidoptera, Hymenoptera, Siphonaptera, and Anoplura were sampled, in addition to mites, pseudoscorpions, spiders, and terrestrial isopods. Among Diptera the most common taxon was *Hydrotaea capensis* (Muscidae) followed by Phoridae and Fanniidae species, instead, among Coleoptera the Cleridae in the genus *Necrobia*. The beetle fauna, in addition to the already well known Cleridae, Histeridae, Staphylinidae, Dermestidae, Trogidae, and Ptinidae, shows a high abundance of species belonging to the families Mycetophilidae, Cryptophagidae, Zopheridae, and Latridiidae, revealing an important mold-feeders community. Among Lepidoptera, Tineidae represent the most important taxon, while the retrieved Hymenoptera mainly belong to Formicidae and parasitoids of various families. The fleas collected from the bodies are usually mainly associated with mice and rats that use the mummified bodies in the crypts for nesting, whereas lice (nits) were collected only in a site. Overall, this work allowed us to draw some useful insights also on the effect of the bodies’ accessibility on the insect colonization, as well as to show significant variations in the Italian body-related fauna during the last centuries.

KEY WORDS: Forensic Entomology, archaeoentomology, Funerary Archaeoentomology, mummies, decomposition.

ORAL PRESENTATION

SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

The mummies of Cerreto di Spoleto: description and interpretation of the entomofauna of two groups of mummies of Valnerina (Umbria, Italy)

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The study of arthropods from archaeological contexts is fundamental to gain useful information to reconstruct the events occurred in the past, as well as to define effective conservation strategies of our cultural heritage. This research deals with the entomological investigation of two groups of mummies (18th-19th century) from the church of Santa Maria Annunziata (Cerreto di Spoleto) and from the church of SS. Gesù and Maria (Borgo Cerreto). The study aimed to verify and eventually support the archaeological hypotheses about the burial of the bodies, their taphonomic processes and to plan an effective conservation strategy. Statistical analyses were carried out to verify the association of the fauna records with geographical and taphonomical data. Diptera puparia and Coleoptera adults represented most of the sampled specimens. In addition to the common species typical of the hypogean burial, such as *Hydrotaea capensis* (Muscidae), several mycetophilous beetle species (mold feeders) were collected. Pseudoscorpions and mites were also sampled. The presence of Calliphoridae species suggests that some bodies were exposed before their burial. The importance of this work is also related with the search of museophilous species (e.g., *Anobium punctatum*) to plan an effective conservation strategy of a so precious source of historical and cultural data such as the mummified bodies of Valnerina.

KEY WORDS: Archaeoentomology, Funerary Archaeoentomology; museophilous species, mummies.



SESSION VII

FORENSIC, MEDICAL AND VETERINARY ENTOMOLOGY

Entomological mosquito surveillance for West Nile and Usutu virus in Apulia region (Southern Italy) in 2021-2022 years

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INTRODUCTION - The entomological survey is a fundamental action of the national surveillance plan of arboviruses (PNA, 2020-2025) at a level region to prevent risk of pathogen transmission by arthropods, including mosquito species. Mosquito monitoring was conducted in the Apulia region (Southern Italy) as part of an integrated surveillance plan for Usutu and West Nile (WNV) infection and funded by Regional Grants. This work reports the results obtained in 2021-2022 years.

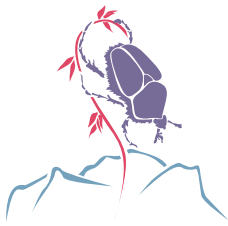
MATERIALS AND METHODS - In the 2021-2022 years (April-November), mosquito traps (mostly CDC, unatantum BG sentinel[®], Gravid traps) were positioned by Veterinary Services in selected livestock farms in each province of the Apulia region. The obtained caughts were delivered to the Medical Entomology Laboratory of the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata for the analyses. After identification according to Severini et al., 2009 morphological keys, the culicidae were counted and pooled according to species, sex, date, trap. Females were molecularly tested for WNV and USUV RNA by real-time RT-PCR in use. A leg from each *An. maculipennis* s.l. complex mosquito was used for molecular identification at species level. The sequences thus obtained were compared with the NCBI database through BLAST runs.

RESULTS - A total of 5971 (5589 F, 382 M) adult mosquitoes from 8 genera and 18 species *Aedes* (*Ae. albopictus* (3.40%), *Ae. vexans* (1.56%), *Anopheles* (*An. algeriensis* (0.82%), *An. claviger* (0.02%), *An. maculipennis* s.l. (= *An. labranchiae*) (0.20%), *An. petragrani* (0.05%), *Coquillettidia richardii* (8.81%), *Culex* (*Cx. laticinctus* (0.03%), *Cx. modestus* (0.05%), *Cx. pipiens* s.l. (20.30%), *Cx. theileri* (0.28%), *Culiseta* (*Cul. annulata* (0.75%), *Cul. longiareolata* (0.17%), *Ochlerotatus* (*Ochl. caspius* (53.60%), *Ochl. communis* (0.24%), *Ochl. detritus* (6.75%), *Orthopodomyia pulcripalpis* (0.05%), *Uranotaenia unguiculata* (0.05%) were collected at the 36 selected sites (6 in FG province, 2 in BA province, 5 in BAT province, 5 in BR province, 10 in LE province and 8 in TA province) for a total of 773 caughts. The prevalent species resulted *Ochl. caspius* (3202/5971, 53.60%) and *Cx. pipiens* (1212/5971, 20.30%), respectively. Out of 682 examined pools, all of them were negative for both WNV and USUV RNA.

CONCLUSIONS – The rich culicidic entomofauna in the monitored farms is related to the variety of biotopes in the Apulia region. Together with *Cx. pipiens* s.l., the most important vector of WNV and Usutu virus in Europe and Italy, *Ochl. caspius* resulted the most spread and abundant species. The types of traps and their location could also explain the scarce number of *Ae. albopictus* (202/5971, 3.40%), very abundant in urban and periurban areas. With regard to the collected anophelinae, *An. maculipennis* s.l. specimens result belonging to the single species *An. labranchiae*, historically the primary human malaria vector in Italy; *An. algeriensis* species, suspected malaria vector in Nord Africa result in seven Apulian sites, including the Promontory of Gargano where it is numerically predominant and where the species was detected in 2020 in the ambit of the RC IZSPB 01.2018 project, forty years after the last survey.

KEY WORDS: entomological survey, West Nile, Usutu.

POSTER



CNI E²⁰₂₃

P A L E R M O

XXVII Congresso Nazionale
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Session VIII



SESSION VIII

FORESTRY ENTOMOLOGY

Evaluation of endotherapeutic treatment against the alien pest *Toumeyella parvicornis* Cockerell (Hemiptera: Coccidae) on *Pinus pinea* L.

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The pine tortoise scale (PTS) *Toumeyella parvicornis* (Cockerell) is native to North America and was found for the first time in 2014, in Campania (Italy). This alien species gradually colonized woods and urban areas in Latium and moved northward to Tuscany and Abruzzo, southward to Apulia. The scale was responsible for severe damages on stone pine *Pinus pinea* L., causing progressive defoliation and lack of vegetative renewal. The quick decline and dieback of several *P. pinea* trees occurring in Naples and Rome prompted studies on control strategies with low environmental impact and feasible in urban areas. Endotherapy can be considered as a suitable approach, avoiding the environmental risks associated with spraying.

An experimental trial was performed in April 2021 in an urban park area of Rome, which was heavily and homogeneously infested by PTS, using the “Nuovo Metodo Corradi®” endotherapeutic technology. An abamectin-based insecticide was trunk-injected in 61 *P. pinea* trees distributed in three plots whereas a plot of 11 untreated plants served as control. Visual inspections of the trees, branch sampling and microscope observations of sampled biological material were carried out to assess the treatment effect on *T. parvicornis* density until November 2022. At each sampling, the presence and abundance of PTS females, males, nymphs and eggs were recorded as well as the presence of possible parasitoids and predators. The surveys carried out in 2021 showed that the trunk-injected plants appeared to be recovering, and the scale colonies sharply decreased or disappeared on these trees. Indeed, the number of mature females on the one-year-old twigs was significantly lower in treated than untreated pines at both samplings performed in July (2 vs 40 females/twig on average) and October (1.8 vs 14 females/twig on average). Also, no injury and bark lesions ascribable to the trunk drilling were observed. In 2022, the level of PTS infestation was still lower in treated than in control plots, providing evidence that this endotherapeutic method and abamectin-based insecticides can efficiently reduce the high populations density of *T. parvicornis* until 18 months.

Comparative studies on the injection of abamectin-based insecticides by means of different endotherapeutic techniques are currently ongoing, to provide the stakeholders with an overview on their optimal application. Meanwhile, the present study suggests that the “Nuovo Metodo Corradi®” technique can be usefully employed to provisionally control the high populations of *T. parvicornis* that are threatening the forest heritage of some of the most important urban areas in Italy.

The study was carried out in the frame of the projects PROTEGGO 1.3 and PROTEGGO 1.4 funded by the Ministry of Agriculture, Food Sovereignty and Forests (MASAF), DISR-V.

KEY WORDS: pine tortoise scale, trunk injection, urban area.

ORAL PRESENTATION

SESSION VIII
FORESTRY ENTOMOLOGY

Consequences of a wildfire on beetles (Staphylinidae) in an ancient wood of the Aspromonte National Park (South-Italy)

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The ancient forest of *Pinus nigra* Arnold subsp. *laricio*, located in Serro di Acatti, in the Aspromonte National Park (municipality of San Luca in the province of Reggio Calabria) and falling within an integral reserve, due to the peculiarity of the origin and its geographical position, it represents an interesting natural forest and biodiversity hotspot. The important role played by natural forests in the conservation of biodiversity is also recognized by various international regulations and conventions intended for their protection. In August 2021, a large wildfire involved this area, almost destroying the ancient forest. A study was conducted to understand the effects of the forest fire on entomological biodiversity, following the occurred environmental alteration. One of the groups on which we have focused is the Coleoptera family Staphylinidae, about which this one is the first study conducted to check the effects of a fire. These insects, for the high number of species known for the Italian fauna and the high capability to colonize different kinds of macro and micro-environments, even subjected to stress conditions, are very suitable to be used as bioindicators. The study on the composition of the communities and the relative species abundance was conducted in two Pinus stand by pitfall traps, comparing the data with another neighboring P. nigra stand, with comparable characteristics. In the samplings from June to November 2022, 1741 Staphylinids specimens were collected, representing 42 species, 19 of which present in the burned stand with crown fire, 28 in the unburned stand and 21 in the transition stand with grazing fire (between the burnt and unburnt stands). In addition to the direct effects on the specific composition of the taxon group, there was a population reduction of 78,050% at the burned stand, with 272 specimens collected. The transition stand also experienced a clear reduction (81.43%) compared with the unburnt stand. In addition to the reduction in the number of species and abundances, there was the almost disappearance of *Quedius* sp. (group of Staphylinidae typical of woods), such as *Quedius aspromontanus* Bernhauer, 1908, *Quedius abietum* Kiesenwetter, 1858 and *Ocypus italicus*, with many saproxylic species, indicators of a high degree of maturity and good conservation of forest habitats. The diversity specific comparison and the species affinities present was carried out with the NMDS analysis and result has highlighted that Staphylinids species present in the burnt and in transition stands were similar and superimposable, while in the unburned stand the species specific composition differs from the other two stands. Likewise, both in the burnt and in the transitional stand, a greater number of Staphylinids fliers species was found, as probably they represent the first colonizers of the area that suffered the effect of the wildfire.

KEY WORDS: Wildfire, Coleoptera, Staphylinidae.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

Effects of a wildfire on Arthropod biodiversity in an ancient wood of *Pinus nigra* Arnold subsp. *laricio* ("Acatti Giants") in Aspromonte

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Wildfires can constitute an ecological agent capable of continuously regulating and shaping ecosystems. Concern about these phenomena arises when these events are intensified (especially in terms of duration and extent) due to man.

Invertebrates succumb more easily to fire, due to their reduced mobility. This study has the objective of evaluating the impact of the fire on the Arthropod community through a multi-taxa approach, in a centuries-old *Pinus nigra* Arnold subsp. *laricio* forest. It is located in Serro di Acatti, in the Aspromonte National Park (municipality of San Luca in the province of Reggio Calabria) falling in integral reserve, which was destroyed during a fire in August 2021. The research compared the effects on the specific richness and abundance of Arthropods one year after the forest fire. Moreover, the seasonal variation of these communities in three *Pinus* stands: burned stand by a crown fire (A), transition stand burned by a grazing fire (B) both in the "old forest", and a not burned area (C) adjacent to the first two investigated. Five samplings were carried out in 2022 using pitfalls and aerial traps for the Arthropod fauna collection and three soil collections for chemical and chemical-biological analyses. The results obtained were significant and, although they exclude the results of aerial traps, for which processing is still in progress, showing that the fire profoundly affected the entire forest ecosystem. The number of Arthropods collected in the two-burned stands proved to be around 50% of that obtained in the unburnt area. Furthermore, in the burned area the soil organic matter and the enzymatic activity of the microorganisms in the soil was significantly reduced. The sampled Arthropods in the three stands (Tot N=64,450) are mostly represented in succession by the orders Diptera, Hymenoptera, Coleoptera, Araneae, Collembola and Lepidoptera. The ratio among the different groups differed in relation to the stand and the typology of the fire. The samples obtained, in each monitoring, showed overall an altered seasonal dynamics which became more evident in the stand with crown fire. The results have highlighted the clear reduction in the abundance and richness of Arthropods and evident environmental imbalances. The extensive damage caused by forest fires in the Mediterranean area, has become an ever-increasing problem, with severe ecological, economic and social consequences. They add to other unknowns that represent the fundamental environmental emergencies of recent years: desertification, climate change, decrease in water resources, disappearance of habitats and, as highlighted in this study, loss of biodiversity. The study has set further goals to be investigated in the future to obtain a holistic approach in natural environments characterized by valuable habitats and species of community interest, including the analysis of the temporal variability of the communities as a function of the temporal distance from the fire and by seasonality.

KEY WORDS: Wildfire, Arthropod, Biodiversity.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

Remote sensing for the early-detection of the Eurasian spruce bark beetle (*Ips typographus*) outbreaks

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Increasing frequency and intensity of extreme weather events due to climate change determine greater stress to European conifer forests and create conditions suitable for pests' infestations. Since 2019, the south-eastern Alpine populations of the Eurasian spruce bark beetle (*Ips typographus* L.) shifted from an endemic to an epidemic phase, elicited by the dramatic windthrow event occurred at the end of 2018 (Vaia). The successful management of outbreaks and vulnerable areas faces difficulty in detecting the infested trees at their early stages, before the beetle expands to larger areas becoming unmanageable. Remote sensing techniques (such as time-series analysis of multispectral imagery acquired by satellites, airplanes, or drones) have a great potential to obtain useful information about damage due to bark beetles' infestations. The study explored the possibility to develop a method to characterize and detect the symptoms of the infestations at their early stages, employing multispectral images acquired by a drone to identify a spectral signature specific for *Ips typographus*. An attack was induced on individual spruce trees in ten experimental sites by the deployment of an aggregation pheromone. The precise position of the infested allowed to monitor the development of the bark beetle in the bark while collecting multispectral images by drone once every two weeks for three months. The use of two vegetation indices NDRE (Normalized Difference Red Edge index) and SAVI (Soil Adjust Vegetation Index, with a correction factor of 0.44) allowed to recognise the infested trees at least one month before the appearance of visible symptoms, i.e., the colour change of the tree crowns from green to red. This remote sensing early-detection tool could allow the automatic diagnosis of the bark beetles' disturbances and provide a better guidance for operational decisions and management of areas more at risk of pest outbreaks.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

**Further range expansion of *Thaumetopoea pityocampa* (Denis et Schiffermüller),
and its originary distribution range in Sicily**

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Additional monitoring of the pine processionary moth, *Thaumetopoea pityocampa* (Denis et Schiffermüller) in Sicily was carried out in the years 2017, 2018, and 2023, in order to assess its expansion, compared to the 2008-2010 monitoring (Lo Duca et al. 2011), and some data from 2012 and 2013. The presence of winter nests, in pinewood and residential areas, was monitored inside UTM quadrants (10 km side) located along the western Sicilian limit of known presence of the moth until 2013. For the other UTM quadrants at the limit of the known range, we used carefully analysed and validated reports from local operators and mayors' ordinances. A bibliographic survey and surveys in some entomological collections were also conducted in order to ascertain the reliability of the reporting of *T. pityocampa* in the Madonie territory by Minà Palumbo & Failla Tedaldi (1888), which was taken up and considered valid in the last century by other entomologists; this report, based on the analysis of adults, was immediately criticized by E. Ragusa (1889) who, having seen winter nests on Pantelleria, and knowing that instead no winter nests had ever been found on the Madonie, considered it only as an erroneous identification of *Thaumetopoea processionea* (L.); specimens of the latter species were found in the Mariani collection as the only adults of *Thaumetopoea* Hübner from Madonie.

The original range of distribution of the pine processionary moth in Sicily is thus represented by the native ranges to *Pinus nigra* J.F. Arnold subsp. *laricio* Palib. ex Maire on Mount Etna. Another ancient range of a pine processionary moth in the borders of the Sicilian Region is that of the recently described *Thaumetopoea mediterranea* Trematerra et Scalercio on Pantelleria.

Thaumetopoea pityocampa was first reported with certainty in Madonie mountains only in 2005 in Gangi and San Mauro Castelverde (Lo Duca et al. 2011), and then in 2012 it was found for the first time within the boundaries of the Madonie Regional Park.

Our monitoring shows that *T. pityocampa* is now widespread in the central and higher areas of the Madonie Park, having also reached the settlements of Isnello and Castelbuono; the latter location although it was only 8 km away from the nearest infested sites of San Mauro Castelverde in 2005, was reached by the pine processionary moth in 2017, through a route that started from Gangi about 20 km long, and with greater continuity and proximity between the pines.

Compared to the 2008-2010 monitoring (Lo Duca et al. 2011), the UTM quadrants of Sicily in which the presence of *T. pityocampa* nests has been recorded have increased by 73%. The pine processionary moth in Sicily is currently known to us to be present in 35% of the UTM quadrants of the island, up from a presence in 5% of the quadrants until the middle of the last century.

Having already reached the highest and coolest areas of the island, the future spread of *T. pityocampa* in the warmer hilly and lowland areas is likely to be slowed by global warming.

KEY WORDS: Processionary pine moth, winter nests, original distribution.

ORAL PRESENTATION



SESSION VIII

FORESTRY ENTOMOLOGY

Improving trapping strategies for *Agrilus* beetles at international scale

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Jewel beetles (Coleoptera: Buprestidae) of the genus *Agrilus* includes over 3000 species at the global scale. Larvae feed on the living subcortical tissues of trees and shrubs, and feeding activity can kill the host, especially when it has already been weakened by other abiotic or biotic factors. Many *Agrilus* species have a proven invasive potential, being easily transported within nursery plants and wood products. As a consequence, some species have become important invasive pests into new geographic areas, leading to wide environmental, economic and social impacts. Hence, trapping strategies to be used at entry points are of utmost importance to intercept incoming individuals. Reliable trapping protocols exist so far only for the emerald ash borer *Agrilus planipennis*, one of the most destructive species existing nowadays, while only a few and scattered studies targeted other *Agrilus* spp. can be found. The Euphresco project “Developing and assessing surveillance methodologies for *Agrilus* beetles” aimed to assess the effectiveness of the trapping protocols commonly used for *A. planipennis* also for other *Agrilus* spp., testing 1) green sticky prism traps vs. green multifunnel traps, 2) green sticky and multifunnel traps with and without the leaf alcohol 3Z-hexenol, 3) multitrap vs. multitrap on which adult beetles of a target species were attached. These results will contribute to aid in surveillance and monitoring approaches for the early detection of these wood-boring insects.

KEY WORDS: *Agrilus*, Euphresco project, invasive species, trapping protocols, early detection.

SESSION VIII
FORESTRY ENTOMOLOGY

Monitoring study of ambrosia beetles species (Coleoptera: Curculionidae, Scolytinae and Platypodinae) using different types of traps in a rewilded hazelnut orchard in Latium region

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Italian hazelnut production is among the most important worldwide, with the 2nd largest global production. However, biological factors such as insect and mite pest attacks seem to be a major limitation for a more efficient production. Hazelnut orchards are frequently infested by several insect species belonging to the subfamilies of Scolytinae and Platypodinae (Coleoptera: Curculionidae), which can cause relevant economic damages and threaten the survival of infested plants. Particularly concerning is the “ambrosia beetles” group, which includes some invasive alien species in Italy (e.g. *Xylosandrus germanus* Hoffman, *Xylosandrus crassiusculus* Motschulsky e *Xylosandrus compactus* Eichhoff). These species are responsible for substantial negative impacts on forests and agro-ecosystems. Due to the increasing international trade and climate change, the introduction and establishment rate of these species is globally growing, resulting as one of the major threats to biodiversity.

In the present study, carried out in the province of Viterbo (Latium Region) from March to October 2022, we aimed to estimate the qualitative and quantitative species composition of the “ambrosia beetles” group in a hazelnut orchard that is returning to its pre-management condition and evaluate the efficiency of three different kinds of traps. The site, now part of the Valle del Treja Regional Natural Park, was cultivated until the early 1990s and then abandoned due to the difficulty of mechanisation practices. The monitored area was divided into 8 randomized blocks, 75 m apart, in each of which 3 different traps were placed at the vertices of a 5 m side triangle, positioned on the plants at 2 m above the ground. Two commercial traps (Rebell Rosso and Multifunnel) and a handmade bottle-trap called Tornado Tube were used in the field testing. Ethanol (95%) was used as a lure in the traps, which were inspected weekly for counting and identifying the specimens. The lure, instead, was replaced every 2 weeks.

Preliminary results show that *X. germanus*, recently reported in Latium, was the most captured species, thus confirming its importance also in ecosystems dominated by hazelnut trees. Even though specific studies should be conducted in the future, the commercial traps recorded significantly higher numbers of catches than those obtained with the handmade trap Tornado Tube. Further monitoring studies for these organisms are essential in order to better understand the impact of alien species on native beetle communities in the ecosystems of interest.

KEY WORDS: ambrosia beetles, *Xylosandrus germanus*, invasive alien species, hazelnut orchard.

ORAL PRESENTATION

SESSION VIII
FORESTRY ENTOMOLOGY

The push-and-pull technique reduces bark beetle damage in Norway spruce forests affected by Vaia storm.

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The European spruce bark beetle, *Ips typographus* (Coleoptera: Curculionidae, Scolytinae), is one of the most destructive pests of Norway spruce forests in Europe. Severe windstorms could favor population growth and lead to epidemic outbreaks affecting as well healthy forest stands. In a two-year field study carried out in South-Eastern Alps, we tested a large scale use of a semiochemical push-and-pull strategy to protect susceptible forest edge trees from *I. typographus* colonization in a context of post-windthrow disturbance. Slot traps baited with aggregation pheromones and three repellent semiochemicals (verbenone, a blend of Green Leaf Volatiles (GLV) and trans-conophthorin) were used in both years. In 2020, 26 windthrown areas were classified into three levels of infestation risk (low, medium, and high) and each randomly assigned to three protocols: 8 push-and-pull sites protected with repellents and pheromone traps; 9 only-trap sites with just pheromone traps; 9 control sites with no protection. In 2021, the trials were repeated in 22 clear-cuts of infested spruce forests, comparing only push-and-pull (16 sites) to control (6 sites). At the end of each summer, the effectiveness of the push-and-pull technique was evaluated on the infestation status of the forest edge trees. Results show that mitigation of spruce bark beetle infestation along forest edges can be achieved. In particular, the used push-and-pull technique performed well in post windthrow conditions (2020 trials), especially at high infestation risk, with significantly lower damage to forest edges when compared to untreated sites. Minor infestations were found in sites treated with the push-and-pull protocol, with in average only 0.05% of the margin affected by new infestations. In sites protected only by pheromone traps the mean infestation level was 7.24% of the edge, and the control sites showed a mean infestation of 6.39%. The use of pheromone baited traps alone was not enough to avoid colonization of forest edges, especially in high risk sites, with no significant differences in catch rate. Differently, when the push-and-pull protocol was applied in clear-cut areas (2021 trials) and higher beetle densities, this technique was less effective, resulting in a not significant damage reduction compared to untreated sites. In 2021, 3.69% of the margins protected by the push-and-pull were colonized by bark beetles compared to 5.47% in the control sites. The push-and-pull technique works better in post windthrow conditions rather than clear cuts probably thanks to the presence of natural enemies still occurring in the wind fallen trees. The choice of the repellent lures is determinant in the success of this technique, being trans-conophthorin the most effective, always used in combination with pheromone traps. Extremely high bark beetle population densities make however this control technique less effective in forest edges protection.

KEY WORDS: Push-and-pull, spruce bark beetle, Vaia storm.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

Current distribution and host plants of alien species belonging to *Xylosandrus* genus (Coleoptera, Curculionidae, Scolytinae) in Italy

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Alien species are considered one of the most important threats to biodiversity. Globalisation and thus the continued intensification of trade in goods around the world, makes such invasions constantly facilitated. On this regard, invasive insect species can easily reach new territories, usually through the transportation of wood products of various kinds. Moreover, small dimensions of the body, cryptic life cycle and a very efficient reproductive system may increase their capacity to invade new territories. Insects belonging to the Xyleborini tribe (Coleoptera, Curculionidae, Scolytinae) exhibit all these characteristics. Species of this tribe are defined as ambrosia beetles, for their capacity to bring on the body different fungi species, potentially dangerous for the plants health, making them a concerning group of insects. Xyleborini tribe contains the *Xylosandrus* genus of whom three well established alien species are present in Italy. *Xylosandrus germanus* (Blandford, 1894), *X. crassiusculus* (Motschulsky, 1866), and *X. compactus* (Eichhoff, 1876). The aim of this work is to map the current distribution of these four alien species and their potential host plants in the Italian Peninsula. In order to revise all the information available in the literature, we used Google Scholar and the SAMFIX life project (LIFE17 NAT/IT/000609) database to obtain the necessary datasets. Doing so, we elaborated host plants list and distribution maps, for each of the four species. Considering the wide range of potential host plants, ranging from nursery plants, cultivated plants or forest species, the monitoring of this species is crucial for their correct management and to promptly contain their dispersion.

KEY WORDS: Scolitinae, Xyleborini, *Xylosandrus*, Plant health.

SESSION VIII
FORESTRY ENTOMOLOGY

Bark and ambrosia beetles associated with chestnut trees in Piedmont region

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Chestnut trees (*Castanea sativa* Miller) represent a potential host for several species of insect pests. Although most of them do not represent a severe threat to plant production and survival, some of them are capable of causing serious damage, compromising the plant health and production.

Among the main pests affecting chestnut trees, some species of bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) have recently posed particular concern, especially in orchards with young plants and subject to stress from biotic and abiotic factors such as rising temperatures and prolonged drought conditions.

These small xylophagous insects can penetrate the bark and dig tunnels within which mating, oviposition and larval development occur. These activities can cause irreparable damage to the vascular system of the plant, compromising its survival within a short time.

In order to identify the species present, the entity of the populations, and the flight period of the adults, in 2021 a monitoring started in two chestnut groves in the province of Cuneo, using traps with bioethanol. These traps were placed in each chestnut stand from April to the end of September. The collection of caught insects was carried out every 10 days. Captured individuals were collected, placed in plastic test tubes and morphologically identified at the DISAFA - Entomology laboratories.

In total, 5 different species have been identified. The native species collected included *Anisandrus dispar* F. and *Xyleborinus saxesenii* Ratzeburg, which represented the highest number of individuals found. Among the exotic species, on the other hand, in addition to *Xylosandrus germanus* Blandford and *X. crassiusculus* Motschulsky already reported for chestnut, a few specimens of *A. maiche* Kurentzov were captured for the first time in Piedmont. Flight peaks were found to vary by species, but in general they were recorded between late April and mid-June.

Mild winters, however, have created optimal conditions for an increasingly early swarming. These conditions make the management of these insects more and more delicate and complex. These xylophagous insects may be able to perform more generations than usual, especially at altitudes where the chestnut tree finds its ecological optimum. For this reason, it is getting harder for professional technicians to forecast flight peaks and apply effective control strategies to cope with these species.

Thus, it is necessary to keep the monitoring of these beetles active, to assess the population trends, the damage caused in chestnut groves and to identify the most effective control strategies in prediction of ever higher temperatures and more intense and frequent abiotic disturbances.

KEY WORDS: bark and ambrosia beetles, *Castanea sativa*, climate change.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

Distribution of *Toumeyella parvicornis* (Cockerell) nine year after its introduction in Campania Region, Italy

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The pine tortoise scale *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae) is considered a major threat for pine species in Italy. Strong phytosanitary measures have been adopted by the Ministerial Decree of 3 June 2021 with the aim to contain the spread of this harmful pest in the country. Native from the Nearctic region, *T. parvicornis* has been recorded in Campania during 2014 infesting mainly Italian stone pine (*Pinus pinea*) in the province of Naples. In following years, the pine tortoise scale has been reported in different Italian regions (Abruzzo, Lazio, Puglia and Tuscany) and in 2021 a first outbreak has been recorded for France. The Plant Protection Organization of Campania region suggested the application of the first containment measures in two executive decrees in 2015 that were updated in 2021. Among different pine species *P. pinea* resulted to be the most susceptible host with huge infestations and high mortality rate interesting mainly pines already weakened by environmental and anthropic factors. In less than a decade *T. parvicornis* spread over a great part of the region, from urban areas to forest plantations by means of wind dispersal of crawlers or favored by their passive transport with vehicles. The phytosanitary surveys carried out in Campania since 2015 to 28.02.2023, reported in the database SIMFito, list as monitored 328 of 550 municipalities. 28% of the 2700 surveyed sites resulted infested. To date *T. parvicornis* is infesting pines in 203 municipalities of the region located along the coastal areas from northern regional limit to the gulf of Policastro to the south. The species is largely present also in the internal zones of the Avellino, Benevento and Caserta provinces. The elevation of infested areas ranges from 2 m of coastal plains to 840 m a.s.l. of hilly zones. The main infestations have been observed on Italian stone pine and Maritime pines (*P. pinaster*), with clear prevalence of the first species (95%). Aleppo pine (*P. halepensis*) infestations resulted usually inconsistent. The first serious attacks on Austrian pine (*Pinus nigra*) have been observed in November 2022 at Avellino. The spatial analysis on the progress of the infestations by *T. parvicornis* showed a spread rate of 7 km/year heading north, 8 km/years eastwards and 15 km/year southwards with new territorial records located along the main regional road network.

KEY WORDS: invasive species, pine the tortoise scale, distribution.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

The successful eradication of the pine processionary moth from Sardinia (Italy)

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The pine processionary moth, *Thaumetopoea pityocampa*, was reported for the first time in Sardinia in March 2006. After this first report, the Department of Agricultural Sciences of the University of Sassari together with the Regione Autonoma della Sardegna started a monitoring program aimed to evaluate the distribution of the pest and the spread of infestation in the surrounding area of the first report. Since the area of infestation resulted to be limited to approximately 20000 ha in the south-western part of the island (i.e. Medio Campidano), a three-year eradication program was funded by Regione Autonoma della Sardegna in 2011 and involved the following actions: 1) defining the spread of the pest through a network of pheromone traps; 2) detecting and mapping pine trees and pine forests within the infested area; 3) detecting infested trees by visual inspection; 4) applying ground insecticides and removing nests from isolated trees; and 5) spraying *Bacillus thuringiensis kurstaki* on pine forests by aerial application.

A significant reduction in the territory occupied by *T. pityocampa* was observed immediately after the beginning of the eradication program, and the infested areas in Medio Campidano decreased from approximately 18000 ha in 2010 to 280 ha in 2013. Unfortunately, pine trees infested by *T. pityocampa* were observed approximately 200 km away from the first outbreak in a limited area in the northern part of Sardinia in 2013 (i.e., Alta Gallura). After this record, a pheromone trap network covering the whole Sardinian territory was established in 2013 and 2014, and data from monitoring at regional spatial scale indicated that the outbreaks of *T. pityocampa* were limited to Medio Campidano and Alta Gallura territories. Consequently, the eradication program was extended to Alta Gallura in 2014 taking into consideration all the control actions successfully adopted in Medio Campidano in the previous years. After that, the area infested by pine processionary moth decreased in both historically infested territories, and no adults in baited traps have been captured since 2017 and 2020 in Medio Campidano and Gallura, respectively.

According to the International Standards for Phytosanitary Measures of Food and Agricultural Organization (FAO) of the United Nations, as well as the suggestion provided by different authors, the pine processionary moth can be considered as eradicated from Sardinia as suitable surveillance activity has resulted in no subsequent detections for at least 3 times the normal generational time. Nevertheless, also considering the possibility of a multi-year diapause in pine processionary moth pupae, the monitoring of adults through a network of baited traps will be carried out in the next years in order to confirm the species eradication.

KEY WORDS: *Thaumetopoea pityocampa*, control methods, *Bacillus thuringiensis*.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

***Coraebus undatus* (Coleoptera Buprestidae): a new threat to cork production in Sardinia (Italy)**

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The flathead oak borer, *Coraebus undatus* (Fabricius), is widely spread throughout the Mediterranean area and is considered one of the main emerging pests for cork production as larvae damage phellogen layer through their feeding activity. In the years following the infestation, feeding galleries are gradually incorporated into the cork layer, causing a change in physical and mechanical characteristics of cork and a significant reduction in product quality. Moreover, the extraction of the cork from infested and damaged trees can be difficult, thus increasing the possibility of irreparably damaging portions of phellogen and compromising the future productivity of the plants. In Sardinia (Italy), where more than 80% of the Italian cork industry is present, infestations of *C. undatus* have occurred sporadically until 2015 and have dramatically increased since 2016, especially in the northeastern part of the island.

This work aimed to evaluate the distribution of *C. undatus* in Sardinia, the pest season phenology and the dynamics of attacks between two debarking seasons (period 2011-2021). The distribution area of the infestations was estimated by visual inspecting cork oak growing areas debarked in 2021 and 2022, as well as by obtaining records from people involved in debarking. The dynamics of *C. undatus* attacks was estimated by observing feeding galleries on cork strips and dating them based on their positions between annual cork-rings. Moreover, the seasonal dynamics of *C. undatus* adult flight was monitored in an infested area using purple traps baited with a mixture of green leaf volatiles (GLVs).

The distribution area of *C. undatus* infestations was mainly restricted in the north-eastern part of the island, with attacks of the pest mainly concentrated in the cork oak forests closest to the industrial cork storage areas. Everything considered, the area where *C. undatus* infestations occur extend for approximately 250 km². Observations made on cork strips allow us to highlight that the frequency of *C. undatus* attacks were relatively low in 2011-2015, increased exponentially from 2016 until 2019, and decreased dramatically afterwards. Field trapping revealed that adults flight from the first week of July to the end of August, showing a peak at the second half of July.

The increase of *C. undatus* infestations in the last years might be related to ecological unbalance between this insect and its natural regulating factors, as well as to the spread of the pest from cork storage areas through cork planks imported from other cork-growing regions. However, a multi-year study is needed to confirm our hypothesis. In particular, it would be important to study the population dynamics of the insect over a long time, and to verify whether the damage observed in recent years is the result of a temporary outbreak or whether environmental conditions are favorable to its constant presence at damaging levels.

KEY WORDS: xylophagous beetles, *Quercus suber*, Mediterranean forests.

ORAL PRESENTATION

SESSION VIII
FORESTRY ENTOMOLOGY

***Anoplophora chinensis* and *Anoplophora glabripennis* (Coleoptera: Cerambycidae)
in Italy: traps for interception and protocols for eradication**

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Among invasive species, insects are one of the most successful groups. Moved by international trade (which is constantly increasing) and facilitated by climate change (due to the tropicalisation of temperate regions), more and more alien insect species are introduced to new countries every year. *Anoplophora glabripennis* and *Anoplophora chinensis* (Coleoptera: Cerambycidae) are Asian two long-horn beetles that are particularly relevant due to their extreme polyphagy and, consequently, the severe damage they can cause in new countries. Thanks to their wide range of host plants, these insects can spread rapidly in newly introduced countries, mainly in urban or agricultural environments, feeding on ornamental, agricultural and forestry trees. In recent decades, these species have been introduced in several North American and European states, including Italy. The development of new interception techniques and eradication protocols are therefore essential for a rapid response. Studies conducted in Lombardy have evaluated different types of commercially available lures (Synergy, Witasek and ChemTica), trap models and their location in the territory in order to improve the effectiveness of these detection tools for *A. chinensis*. This study, which was carried out in three macro-areas of the Region using 270 traps, showed that black cross-vane traps placed directly in the tree canopy were more effective than other combinations (more than twice as many captures as traps placed on wooden poles in clearings). The three blends tested, on the other hand, provided similar performance, although there were significant differences in combination with the trap model used (the Synergy and ChemTica lures in combination with cross-vane traps were the most effective). The next step after the interception of a harmful exotic species is its eradication. This process was conducted in Veneto (Treviso province) for the eradication of the outbreak of *A. glabripennis* identified in the Cornuda municipality (TV) and surrounding municipalities since 2009. During the eradication, which was successfully completed in 2020, every six months (summer-winter) all susceptible plants present in the area of the infestation (36,361 trees in total) were individually monitored to search for symptoms of colonisation, with felling of infested plants and those present within a 100 m radius (2361 trees felled, of which 1157 were infested); restoration actions were also carried out (with 217 plantings of new non-susceptible species) and dissemination and information for the population; finally, monitoring protocols were followed with specific traps and lures. The results of these studies can make a useful contribution to the eradication of infestations on the national territory, and to the early interception of any accidental introductions of the two pests.

KEY WORDS: Long-horn beetles, Invasive species, Monitoring.

ORAL PRESENTATION

SESSION VIII
FORESTRY ENTOMOLOGY

Predators and parasitoid guild of *Toumeyella parvicornis* (Cockerell, 1897): a review

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Scale insects are considered one of the most commonly transported groups of insects in the plant trade and one of the most successful invasive insects. *Toumeyella parvicornis* (Cockerell, 1897) (Hemiptera: Coccidae) is a soft-scale insect native to North America accidentally introduced in the Caribbean region and in the EU in the last decades. In Italy, it has been present since 2014. The invaded landscape consisted mainly of reforested areas, and isolated plants or groups used as ornamental trees in urban areas.

Toumeyella parvicornis feeds exclusively on *Pinus* species, particularly on needles and twigs, causing dieback of shoots and branches, leading in the worst case to plant death. Additionally, honeydew excreted by the insects enables the growth of black sooty molds which cover leaves and branches, hindering photosynthesis, causing phyloptosis, and reducing plant growth. *Toumeyella parvicornis* is univoltine towards the northern limit of its range in North America, but is able to complete five generations per year in the Caribbean region; in Italy, it has up to three generations per year and fertilized females overwinter.

Among the predators of *Toumeyella parvicornis*, in the area of origin, there are several species of coccinellids that predate young scales and eggs such as *Brachyantha ursina*, *Chilocorus bivulnerus*, or several other species belonging to genus of *Coccinella*, such as *C. novemnotata* or *C. transversoguttata* and species belonging to genus of *Hyperaspis*. *Hyperaspis congressis* is reported to be the most abundant coccinellid associated with this scale. Also, the larvae of the predatory pyralid moth *Letilia coccidivora* can significantly reduce infesting populations. Lacewing, mirids, spiders, and birds have also been reported as predators. Among the parasitoids, some species have been recorded as attacking the scale including the aphelinids *Aphytis* sp., some species belonging to the genus *Coccophagus* and the encyrtid hymenoptera *Microterys fuscicornis*. Investigations are ongoing in the area of *T. parvicornis* origin and in the newly invaded areas to collect species already reported as active on this scale insect and to identify additional natural enemies, evaluating also their potential role as biological control agents.

This work has received funding from the project PROTEGGO 1.5 - Collaboration agreement between CREA and MASAF aimed at the implementation of the activities of the "Strategic Activities Plan" as well as activities aimed at strengthening the control system, data collection, and the development of an information system for plant protection".

KEY WORDS: alien invasive species, natural enemies, soft-scale insect.



SESSION VIII

FORESTRY ENTOMOLOGY

Exploring *Ips typographus* (Coleoptera: Curculionidae, Scolytinae) vision to improve trapping strategies

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Aggressive bark beetle species, such as the Eurasian spruce bark beetle *Ips typographus*, play a crucial role in forest ecosystems. Their infestations kill large amounts of trees, promote the opening of forest gaps, increase dead-wood availability significantly affecting nutrient cycling and soil structure. Along with their ecological role and importance for biodiversity, aggressive bark beetle can also become pests leading to extensive forest mortality and massive economic damage during outbreaks. So far the vision system of *Ips typographus* was not studied because priority was given to chemical cues, either plant or insect semiochemicals. Understanding the visual system could open the way to more effective trapping methods, particularly important during the switch from an endemic to an epidemic phase. In this project, we started to investigate eye morphology and anatomy with optical and electronic microscopes. The preliminary results indicate that the eyes are approximately oval with a concave indent in the middle rostral part; the central parts contains enlarged ommatidia, indicating the presence of a possible acute zone. Ongoing studies include electroretinography, which will allow to characterize the light spectrum perception of *Ips typographus*. Acquired information will be then used to tailor specific field studies. First, different trap colors will be compared as in several wood borers (Buprestidae, Cerambycidae and Scolytinae) the use of colored traps combined with semiochemicals may allow to catch a significantly higher number of species and/or individuals than the commonly used black traps. Second, traps mimicking the spectral reflectance of the bark of the main host species will be developed.

The study will be carried out within the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

KEY WORDS: *Ips typographus*, bark beetle, vision, eyes, traps, visual traps.

SESSION VIII
FORESTRY ENTOMOLOGY

Webgis-based mobile apps and participatory approach for ground mapping of *Ips typographus* outbreaks in the Alps

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Bark beetle outbreaks are affecting coniferous forests worldwide in the last decades. In Europe, several thousands of hectares of Norway spruce (*Picea abies*) stands are killed every year by the Eurasian spruce bark beetle *Ips typographus* (Coleoptera, Curculionidae, Scolytinae). In the Italian southern Alps, the windstorm Vaia in October 2018 predisposed the forest to the beetle outbreak, and we are currently facing the largest outbreak ever recorded in the region.

Monitoring effort is needed to follow the infestation year by year and to map tree mortality. In the last years, remote sensing tools have been used for detecting declining or dead trees over large geographical areas. Besides the use of remote sensing techniques, ground surveys are still important source of data. Georeferenced reports can be used as ground-truth data for algorithm training of remote sensed data, and they also provide detailed information on the symptoms of the affected trees and the stage of the attacks. However, ground surveys are extremely time demanding. Here, we present a participatory approach using GIS mobile apps and webgis system aiming at collecting ground data. Real-time ground data on the *I. typographus* outbreaks in the Veneto region were collected and visualised on a webgis platform. Integration of remote sensing tools and collection of ground data through webgis framework can greatly improve the understanding of the status of the current bark beetle outbreak.

KEY WORDS: *Ips typographus*, remote sensing, participatory approach, smartphone app.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

Management strategies to control the pine processionary moth outbreaks in North-Western Italian Alps

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Insect pest outbreaks are known to be one of the major threat in forestry. The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera, Notodontidae) is considered one of the main insect defoliators of conifers in Southern Europe and North Africa. The moth's geographic range is constrained by its requirement for sunshine in winter and its susceptibility to both cold winter and high summer temperatures, but in the last 30 years a further spread has been recorded, mainly related to an increase in the winter temperature and annual solar radiation. The PPM caterpillars are severe defoliators, causing significant pine tree health decline. Larvae feed on a wide range of *Pinus* species, especially *Pinus nigra* Arnold and *P. sylvestris* L. From the 3rd instar onwards, they possess urticating hairs having serious social impacts. Moreover, associated health problems in humans and domestic and farm animals in urban and suburban areas have been reported. The Interreg V-A Italy-Switzerland 2014/2020 cooperation program "Linee Guida per il monitoraggio e la gestione delle emergenze fitosanitarie nelle foreste delle Alpi centro-occidentali (MONGEFITOFOR)" is a project having the aim to provide management guidelines to contain PPM outbreaks. Surveys were carried out in the 3-year period 2020-2022 in Aosta Valley (NW Italy) to assess PPM population by using pheromone-baited funnel traps and to compare the effectiveness of different control strategies. In particular, *Bacillus thuringiensis* var. *kurstaki* (Btk), mating disruption, and trunk injections were performed. Their effectiveness was evaluated by assessing the number of nests and larval mortality versus control plots. Trunk trap devices were investigated as well, using adhesive barriers and collars to catch larvae moving down along the trunk. For most of the control strategies, results have shown significant reductions of nests and increased larval mortality in treated sites. Regarding trunk trap devices, whereas adhesive barriers were easily overcome by larvae, only collars proved to be effective in trapping a high number of caterpillars during the descent along the trunks. These results provide useful data for the management of PPM in forestry and urban environments.

KEY WORDS: *Thaumetopoea pityocampa*, seasonal flight activity, control strategies, *Pinus* spp., forest insect pest.

POSTER



SESSION VIII
FORESTRY ENTOMOLOGY

Toughness evaluation of the galls of *Dryocosmus kuriphilus*

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Gall-maker insects are responsible for the formation of cecids, better known as galls, occurring mainly on the leaves, buds and shoots of many cupuliferous species. Cynipids (Hymenoptera: Cynipidae, Cynipini) represent the richest group of species capable of determining their formation, thanks to the substances introduced by oviposition or produced with their trophic activity. Among them, particular importance has been assumed in the last two decades by the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, first reported in Europe in 2002 in Italy and effectively contained through the release of the biocontrol agent *Torymus sinensis* Kamijo. This parasitoid lays eggs in the fresh galls of *D. kuriphilus* and the ectoparasitic larva feeds on the host larva, allowing effective control of the pest.

Gall-maker insects have developed many strategies to reduce the attack of natural enemies. Several studies have shown how the hardness of the gall wall can influence the success of parasitism.

With the aim of assessing the degree and evolution of hardness of *D. kuriphilus* galls, 60 galls were collected monthly from April to December 2021. The galls were tested for penetration strength at a depth of 1 mm using a Texture Analyzer equipped with a specific needle probe. Moreover, further investigations were carried out to confirm how the time window of oviposition of *T. sinensis* is optimal in the first months of galls formation, not only in relation to the phenological stage of the host, but also to the degree of hardness of the galls themselves.

The results showed that the penetration strength (expressed in N) was lower in the case of galls collected in April and May, and then gradually increased over time, with a more pronounced increase as early as June. In controlled conditions, oviposition was recorded on all the galls collected in May and June, and no difference was found in the number of events observed. In contrast, no oviposition was ever observed on the galls collected in July. When comparing the number of oviposition events by *T. sinensis* and the hardness of the galls, a negative correlation was found.

KEY WORDS: *Dryocosmus kuriphilus*, *Torymus sinensis*, galls, toughness, oviposition.

POSTER

SESSION VIII
FORESTRY ENTOMOLOGY

Variation of *Torymus sinensis*'s phenology and impact on the effectiveness of biological control

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The biocontrol agent *Torymus sinensis* Kamijo is considered as one of the most successful examples of classical biocontrol programs. This parasitoid represented a feasible option to obtain a sustainable and long-term control of the Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu. Recently, an unusual presence of galls was recorded in a few chestnut orchards of Northern Italy, although *T. sinensis* was released in previous years. Investigations were performed in the five-year period 2018–2022 in six chestnut orchards, selected according to the ACGW infestation level, and divided in two categories: non-critical sites (negligible presence of ACGW galls) and critical sites (high presence of galls with suppression of bud growth). We evaluated *T. sinensis*'s parasitism rate and phenology by dissecting chestnut galls, comparing non-critical sites versus critical sites. Temperatures were monitored in all the orchards with data loggers. In non-critical sites the parasitism rate by *T. sinensis* was stable and/or growing in all years, accounting from 77% to 99%. Conversely, the parasitism rate in critical sites experienced a severe decline, reaching values under 50% in all sites in 2021–2022. Specifically, the parasitism rate was reduced on average by 44% when comparing 2019 versus 2022. The dissection of the galls recorded in non-critical sites in winter (February) highlighted that on average most *T. sinensis* were larvae and immature pupae (about 70%). Conversely, in critical sites most of the individuals were mature pupae (76%), and the presence of newly formed adults (12%) was recorded, as well. In 2019–2022, the mean temperature recorded in critical sites was 2.72 °C and 2.34 °C higher in January and February respectively, when compared to non-critical sites. Moreover, in critical sites the early emergence of the biocontrol agent (late February-early March) was recorded when current-year ACGW fresh galls were not available. Our results suggest that the asynchrony between *T. sinensis* and the ACGW heavily affected the role of the released agent in the suppression of the pest's outbreaks. Future studies are needed to clarify whether this event is increasing in relevance, also in a climate change perspective.

KEY WORDS: *Torymus sinensis*, phenology, biocontrol, climate change.

ORAL PRESENTATION



SESSION VIII
FORESTRY ENTOMOLOGY

***Rhynchophorus ferrugineus* Olivier and *Paysandisia archon* Burmeister: two potential destructive pest in *Chamaerops humilis* L. natural populations of Sicily**

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Chamaerops humilis L. is the only native palm in Europe, it has a high naturalistic value as the last wreck of tropical vegetation of the Tertiary. *C. humilis* plays an important ecological role in Mediterranean maquis, has a great adaptive ability and grow in harsh conditions, on rocky soils, in areas lashed by wind and marine aerosol, as well as high resistance to fire. In Italy it is widely spread in the Tyrrhenian coast and especially in Sardinia and Sicily. The disappearance in Sicily of *Phoenix canariensis* palms as a result of the infestations of *Rhynchophorus ferrugineus* (Olivier), commonly called red palm weevil, has led curculionid to search for alternative hosts among them *C. humilis*. At the same time since 2010 the presence of *Paysandisia archon* Burmeister (palm borer moth) on *Phoenix robellinia* and in the nursery on *C. humilis* has been recorded in Sicily.

Based on these critical issues, from 2016 until 2020 a monitoring plan was carried out in order to evaluate the infestation of the two phytophagous in the natural population of *C. humilis* most representative in Sicily and in particular nature reserves and parks. For western Sicily, monitoring was carried out in the Zingaro, Monte Cofano, Capo Rama, Capo Gallo nature reserves and in the Archaeological Park of Segesta. Other areas included the Torre Salsa (Agrigento) and Vendicari (Siracusa) nature reserves.

The infestation of *P. archon* was high in the Monte Cofano Reserve (80%), followed by the Zingaro Reserve where infestation levels were from 40% to 60%. In eastern Sicily, Nature Reserve of Vendicari were recorded. In the Archaeological Park of Segesta an infestation level was from 1% to 20% and the ratio of 1:1 between the larvae of the two phytophages inside the stems of the palms was recorded. Moreover, in all sites a high preference of male plants for both *P. archon* and *R. ferrugineus* was recorded.

In Mount Cofano Reserve the destructive action of *P. Archon* led to a loss over 60% of the plants present and consequently it changed the landscape. For these reasons, further studies are needed to control the infestation of *P. archon* and *R. ferrugineus* in the natural dwarf palm populations of Sicily. Therefore, preserving *C. humilis* is essential for the preservation of its habitat and the survival of both the arthropod communities that inhabit it and the wildlife and birdlife that, at certain times of the year, feed exclusively on its fruits.

KEY WORDS: dwarf palm, red palm weevil, palm borer moth.

POSTER



SESSION VIII
FORESTRY ENTOMOLOGY

Attacks of *Pityophthorus juglandis* on Walnuts in central Italy

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Thousand Cankers Disease (TCD) is a complex disease involving the ascomycete fungus *Geosmithia morbida* and the vector *Pityophthorus juglandis* (Coleoptera Curculionidae Scolytinae), a bark beetle known to attack plants of the genera *Juglans* (*J. ailantifolia*, *J. californica*, *J. cinerea*, *J. hindsii*, *J. major*, *J. mandshurica*, *J. microcarpa*, *J. mollis*, *J. nigra*, *J. regia*, and hybrids *J. hindsii* X *J. regia*, *J. nigra* X *J. hindsii*, *J. cinerea* X *J. ailantifolia*, *J. nigra* X *J. regia*) and *Pterocarya* (*P. fraxinifolia*, *P. rhoifolia*, *P. stenoptera* and ***Pterocarya* spp.**). In Italy, after its accidental introduction, the species rapidly spread in various regions reaching Tuscany to the south, mainly on Black Walnut plants and more sporadically on Common Walnut. To date, it is difficult to assess the actual economic damage caused to walnut trees due to the slow progression of the disease. In addition to this, as investigations progressed, peculiar behaviour of this Scolytid in newly introduced territories were observed, thus raising particular concerns over the future of these Juglandaceae plantations, in both plantations for wood production and fruit trees. Observations carried out in Tuscany in the provinces of Arezzo and Florence in mixed Black Walnut and Persian Walnut plantations, frequently evidenced that this scolytid may attack and colonise not only tree branches and twigs but even spread on stems of up to 30-40 cm in diameter, leading to plant death in 3-5 years from the appearance of the first symptoms. Further bio-ethological observations are in progress to define the attack behaviour of the beetle and its potential association with other pests.

This work has received funding from the Tuscany region, Grant/Award number DGR. 1426—23/11/2020, MONI.TOSC 2022 “scientific collaboration agreement between the Tuscany region and the Council for Agricultural Research and Economics to carry out of joint activities in the field of entomology and nematology of trees, shrubs, ornamental and forest plants.

KEY WORDS: *Geosmithia morbida*, *Juglans nigra*, Thousand Cankers Disease.

SESSION VIII
FORESTRY ENTOMOLOGY

***Phloeosinus armatus* (Reitter) (Coleoptera Curculionidae Scolytinae): new updates on its distribution in Italy**

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Phloeosinus armatus, the largest scolytid of the Gen. *Phloeosinus*, associated with the Mediterranean cypress (*Cupressus sempervirens*) in its native areas, and reported in several countries such as Turkey, Greece and Cyprus, was initially reported in Liguria in the early 1990s, and it is slowly but progressively spreading in the provinces of Pistoia, Florence, Siena and Arezzo.

Surveys carried out in urban environments and forest ecosystems evidenced an increasing number of outbreaks in the last five years, involving both adult and young Mediterranean cypress plants. This trend has been reported not only in forest ecosystems, where attacks have also affected natural regeneration, but also in urban contexts (e.g. parks, avenues) and in areas of particular historical and landscape value. In this context, in addition to the disruption of the landscape, major operational problems due to the complexity of felling operations near protected buildings were frequently reported. In some cases, the attacks of this bark beetle were so sudden and massive as to exclude any possibility of colonization of the affected plants by the native *Phloeosinus* species typical of conifers, namely, *P. aubei* and *P. thuje*. Investigations in the territories colonised by *P. armatus* have been recently extended to include research on fungi associated with the Scolityds, in relation to both what is being recorded in other Mediterranean geographical areas affected by an increase of infestations, and following the recent discovery in central Italy of associations of this beetle with pathogenic fungi of the genera *Geosmithia*, *Pestalotiopsis*, and *Penicillium*.

This work has received funding from the Tuscany region, Grant/Award number DGR. 1426—23/11/2020, MONI.TOSC 2022 “scientific collaboration agreement between the Tuscany region and the Council for Agricultural Research and Economics to carry out of joint activities in the field of entomology and nematology of trees, shrubs, ornamental and forest plants.

KEY WORDS: Scolitidae, *Cupressus sempervirens*, xilophagous.

SESSION VIII
FORESTRY ENTOMOLOGY

Correlation between leaf damage, leaf area index, and remote sensing spectral indices in monitoring infestations of *Tortrix viridana* L. on deciduous oaks

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During periodic outbreaks, the larvae of the green oak leaf roller moth (*Tortrix viridana* L.) are able to defoliate large areas of woodland with important impacts on the health status of oak forests in the Mediterranean region. The availability of data on the intensity of defoliation provided by direct methods, combined with spectral indices derived from high resolution satellite data, are becoming a useful tool to support the development of models to estimate and predict the spatial and temporal distribution of outbreaks and intensities of attacks.

In this study, we aimed to evaluate the correlation between the spectral index NDVI (Normalized Difference Vegetation Index) derived from Sentinel-2 remote sensing images, the Leaf Area Index (LAI) measured by the Li-cor LAI 2000 instrument, the direct observation of the canopy transparency level and the degree of leaf damage in deciduous oaks infested by the tortricid.

The surveys were carried out on four pubescent oak forest stands in northwestern Sardinia between April and June 2021. On each stand, from oaks budburst phase, weekly measurements of LAI and estimates of the overall canopy transparency index and the defoliation level visually assessed were collected on a set of 5 to 10 single oaks, or groups (consisting of 2 to 4 trees) per stand. The NDVI spectral index trend was obtained for each sampled point and for the same temporal window. Moreover, during May-June 2021 and 2022, trends of NDVI were collected together the degree of defoliation on an additional set of one hundred pubescent oak stands located in the same forest district and representative of the main phytoclimatic conditions.

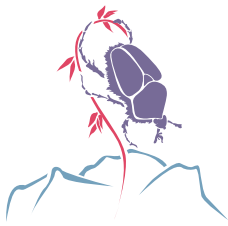
Experimental results obtained at the four forest stands showed a good correlation among the canopy transparency index and both LAI (R^2 from 0.68 to 0.82) and NDVI index (R^2 from 0.55 to 0.85). Canopy development and the related variations of LAI and NDVI showed a linear increase on not infested trees and stands until maximum leaf expansion; whereas, in the infested trees and stands, damages caused by the larvae resulted in a slowdown, stop or inflection on canopy transparency and LAI and NDVI values.

The analysis of trends of spectral indices obtained on stands at the forest district area level confirm that the rate of change of NDVI on defoliated stands showed values equal to zero or negative during the peak defoliation window. The correlation between leaf damage caused by the pest and trends of the spectral index allows the extension of the values of defoliation, collected at level of monitoring stand, to landscape scale, providing a quantitative mapping of defoliation also for wide areas characterized by similar bioclimatic conditions.

These maps could provide useful support both for estimating the extent of damages and for explore time series of attacks carried out by the tortricid. Finally, this methodology can provide spatialized input data for the development and calibration of predictive models of *T. viridana* infestations based on the main bioclimatic drivers affecting the development of the pest, useful for planning monitoring and management activities.

KEY WORDS: green oak leaf roller moth, *Quercus pubescens*, defoliation, satellite data, LAI, NDVI.

POSTER



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P A L E R M O

XXVII Congresso Nazionale
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Session IX

SESSION IX

INSECTS FOR BIOCONVERSION

**Evaluations of durum wheat former foodstuffs in diets for *Tenebrio molitor* (L.)
(Coleoptera: Tenebrionidae)**

Ferdinando Baldacchino¹, Flutura Lamaj²

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The environmental sustainability of *Tenebrio molitor* (L.) farming can be improved by using feed that does not compete with the food supply chain. To this end, the attention of researchers is focused on by-products of the agri-food chain. However, a further increase in sustainability can be achieved through the bioconversion of potential waste into new ingredients for the agri-food/feed supply chain. This would be possible by recycling scraps and "former foodstuffs" before they are classified as "waste", contributing to the goal of halving food waste and reducing waste production.

Tenebrio molitor is generally reared on bran-based diets, bioconverting it into new food (novel food) and processed animal protein (PAP) for feed. In line with its ethology and the principles of the circular economy, even the "former foodstuffs" derived from wheat could be usefully valorized in the same way. The aim of the work was the preliminary evaluation of the various "former foodstuffs" of the durum wheat supply chain in diets for *T. molitor*.

The study compared diets based on: bran (G or Control), bran and zootechnical yeast at 5% (O), semolina (L), semolina bread (M), semolina bread with oil (N), semolina pasta (B), fresh pasta "orecchiette" (C) and pasta with egg "lasagna" (A). The matrices were used as they were, but crushed and screened on a sieve (2 mm mesh) to reduce their granulometry. Diet was available ad libitum and the moist supplement was provided twice a week with pieces of pumpkin. The tests were performed in a climatic room (28±0.1°C, 70±5% RH, 0:24h (L:D) and 10 replicates from 20 one-month-old larvae were used for each diet. After 28 days of growth, the larval survival, the biomass increase and the biomass/frass ratio were measured. The results showed a high larval survival, not significantly different between diets, with values between 98% and 100%. The best increase in larval biomass was obtained with diets A and O, although not significantly different from L and Control (G). Only in the diet based on semolina pasta (B) were increases in biomass significantly lower than in the Control. The analysis of the biomass/frass ratio highlighted significant differences between diets, predicting a different efficiency of the diets tested. The lower values in bran-based diets (G and O) could depend on the higher fiber content of this by-product.

In conclusion, this preliminary study demonstrated that the durum wheat processing industry is a source of multiple derivatives ("former foodstuffs" and potential waste) suitable for diets for *T. molitor*. The good results obtained, often superior to the classic bran-based diet, are attributable to the agri-food transformation processes which generally reduce the fiber content. Egg pasta was particularly performing, thanks to its richer amino acid composition. Further investigations on the conversion efficiency of the diets are needed for a better valorization of the tested matrices.

PAROLE CHIAVE: yellow mealworm, edible insects, bioconversion, by-products.

ORAL PRESENTATION



SESSION IX

INSECTS FOR BIOCONVERSION

Use of tomato peels in diets for *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae)

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Bioconversion of agri-food by-products using *Tenebrio molitor* (L.) is poorly applied than the more advantageous use of the saprophagous *Hermetia illucens* (L.). One reason is the possibility for *H. illucens* to use wet matrices, characteristic of many agri-food by-products. Conversely, for *T. molitor* breeding dry diets are used, limiting the range of choice of suitable by-products. However, new matrices are now available due to efficient transformation processes, or to the preliminary drying required in some by-products recovery paths (thermal energy, feed, etc.). In this work, the possible use of agro-industrial waste from tomato processing is investigated as a growth substrate for *T. molitor* larvae.

Dried tomato peels were used both alone and within two isoproteic diets based on 50% bran, tomato peels (27% and 41%) and spent grain/yeast. A bran-based diet of equal protein content was used as a control. The diet was provided ad libitum and the wet supplement was distributed twice a week with pieces of pumpkin. Tests were performed in a climatic room at 28±1°C, 70±5% RH and 0:24h (L:D). Ten replicates with 20 six-week-old larvae were used for each thesis. At the formation of the first pupa, the diets performances were measured considering the following parameters: larval survival, growth time, live weight increase, average larval weight, diet conversion index and conversion efficiency of the digested diet.

Results showed a larval survival close to 100%, with no significant differences between diets. The use of tomato peels in purity significantly increased the larval growth times compared to the control diet, with growth performance resulting the lowest among the tested diets. The two diets enriched with tomato peels provided results comparable to the control diet in most growth parameters. These results show that the use of tomato peels is suitable in the formulation of multi-component diets. This increases range of potential agro-industrial by-products that can be bioconverted by *T. molitor*. Further investigations are needed to formulate the best performing mixture and evaluate the nutritional composition of the larvae grown on such innovative diets.

KEY WORDS: yellow mealworm, edible insects, bioconversion, by-products.

SESSION IX

INSECTS FOR BIOCONVERSION

Wet supplement based on *Opuntia ficus-indica* (L.) in diets for *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae)

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Generally, the breeding of edible insects for feed and food is considered sustainable, due to the lower consumption of resources for the same amount of protein produced. Much emphasis is placed on the reduced water requirement of *Tenebrio molitor* (L.), since its larvae are able to recover water from the diet and from atmospheric humidity. However, this ability requires energy with a consequent lower diet conversion rate; in fact, the best consumption curves of the diet are obtained in the presence of higher atmospheric humidity. In order to avoid the development of molds and mites, dry diets and relative humidity of 60-70% are used in farms; this defeats the possibility of rearing without an additional source of moisture. In fact, it is common practice to supply pieces of vegetables (potato, pumpkin, carrot, etc.) twice a week as supplementary wet feed. The management of vegetables (acquisition, transport, storage and handling) affects the economic sustainability of the rearing farm; moreover, the availability of vegetables can be a limiting factor in marginal environments or in hot-dry climate areas. The search for alternatives has focused our attention on cladodes of prickly pear (*Opuntia ficus-indica* (L.)). This plant is widespread in temperate-warm areas and often also for non-productive purposes (e.g. defensive hedges, windbreaks, etc.), making cladodes available throughout the year.

The aim of the work was to evaluate the replacement of some vegetables with prickly pear cladodes, as a source of water in the *T. molitor* rearing. The study tested one-year-old cladodes in comparison with potato tuber and carrot root. The cladodes were collected from plants present at the C.R. ENEA della Trisaia – Rotondella (MT), while vegetables purchased from local suppliers. The collected cladodes were brushed and stored at room temperature. All the vegetables tested were sliced at the time of use and supplied with the standard diet (bran supplemented with 5% zootechnical yeast). Potato slices represented the standard or control thesis. Supplementary wet feeds (1 g/replicate) were provided weekly; an ad libitum thesis with a double dose of cladode was also planned. The tests were performed in a climatic room (28±0.1 °C, 60±5% RH, 0:24h (L:D)) and 10 replicates of 20 one-month-old larvae were used for each thesis. After 28 days of growth, larval survival and growth ratio were recorded.

The results showed a non-significantly different larval survival between theses ($p \leq 0.05$). The growth rate values obtained with cladodes were all significantly higher than the control or potato (3.31 ± 0.73). No significant difference was recorded between the use of carrot (3.75 ± 0.08) and cladode (3.79 ± 0.09), at the same dose. The thesis with double dose of cladode did not report a further significant improvement in the growth rate and showed the absence of mold on the uneaten portions. This last observation suggests investigations on the "self-life" of the cladode under rearing conditions. A better "self-life" could allow for a more deferred distribution, with a reduction in labor costs.

In conclusion, *Opuntia* cladodes represent a valid alternative to the tested vegetables, as a water supplement, and more performing than the control (potato).

KEY WORDS: yellow mealworm, rearing edible insect, water, cladode, prickly pear.

POSTER

SESSION IX

INSECTS FOR BIOCONVERSION

Insect-mediated bioconversion for organic waste valorization: understanding and exploiting the digestion capability of *Hermetia illucens* larvae

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Insect-mediated bioconversion of organic waste and by-products from agri-food supply chains can represent an innovative strategy to valorize low-value biomass and obtain sustainable bioproducts according to circular economy criteria. To this purpose, one of the most promising agents for bioconversion is the Black Soldier Fly (BSF) (*Hermetia illucens*; Diptera: Stratiomyidae), whose saprophagous larvae can grow on a wide variety of low-quality organic matter. In particular, we are investigating the biotransformation efficiency of the Organic Fraction of Municipal Solid Waste (OFMSW) by BSF larvae, with the final aim to obtain biobased materials with low environmental impact and high technological value, such as bioplastics and biodiesel (from insect proteins and lipids respectively).

As the midgut plays a fundamental role in the insect-mediated bioconversion processes, we evaluated the digestion capability of BSF larvae reared on two substrates with different nutritional content that mimic the composition of OFMSW. Our results showed that the larvae can compensate variations in nutrient composition of the substrate by post-ingestion responses, through the regulation of gene expression and activity of digestive enzymes. Indeed, despite differences in diet composition, we observed similar larval performances, and similar larval and pupal chemical compositions.

To evaluate in depth the lipid profile we performed a lipidomic analysis on larvae and pupae reared on the two diets. The results indicated that the rearing substrate composition affects lipid composition in both developmental stages. These data set the stage to evaluate how the developmental stage and the rearing substrate affect BSF metabolism and lipid composition, and can also be useful to assess if the lipids from *Hermetia illucens* reared on OFMSW are suitable for biodiesel production.

The study was funded by Fondazione Cariplo (Project title: Turning Rubbish Into biobased materials: a sustainable CHain for the full valorization of organic waste (RICH), protocol n. 2020-0900).

PAROLE CHIAVE: *Hermetia illucens*; insect-mediated bioconversion; midgut; lipidomics.

ORAL PRESENTATION

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INSECTS FOR BIOCONVERSION

Valorization of the municipal solid waste by *Hermetia illucens* larvae for the production of protein-based biomaterials

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The management and disposal of food waste represents a serious concern. It is in fact estimated that 1.3 billion tons of the organic fraction of the municipal solid waste (OFMSW) are globally produced every year. One of the most promising approaches recently proposed to relieve this global emergency is represented by the insect-mediated bioconversion of this waste, to obtain proteins and lipids that can be used for different purposes. To this aim, the saprophagous larvae of the black soldier fly (BSF), *Hermetia illucens*, are efficient decomposers of organic waste and can thus help to develop innovative applications aimed at reducing and valorizing the OFMSW through the creation of high value and environmentally friendly bioproducts.

In this study we investigated the growth performance of BSF larvae and their efficiency in the reduction of OFMSW. Moreover, we evaluated the exploitation of their proteins for the production of bioplastics.

The analysis of waste bioconversion indexes demonstrated the high efficiency of BSF larvae to reduce two OFMSW substrates with different nutritional composition. In particular, although the growth rate of larvae reared on both substrates was comparable, significant differences in the reduction of waste and conversion efficiency into insect body mass were observed. In addition, larvae and pupae obtained from the bioconversion of both substrates revealed a similar amount of proteins and lipids. At the end of the bioconversion process, proteins were extracted from larvae and pupae for biofilm preparation. Biofilms revealed to be thermally stable and of high quality, but differences in terms of resistance and flexibility were detected according to the developmental stage of the insect.

The results obtained so far indicates that the biotransformation of OFMSW into insect-derived bioproducts can really contribute to generate a circular economy supply chain with a significant impact on economic and environmental global issues.

The study was funded by Fondazione Cariplo (2020-0900).

PAROLE CHIAVE: *Hermetia illucens*, biobased materials, insect-mediated bioconversion, food waste.

SESSION IX

INSECTS FOR BIOCONVERSION

Immune responses of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) reared on catering waste

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The insect-based bioconversion represents an economically viable solution for agri-food by-products management. The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), larvae have been proposed as a promising solution to recycle these biomasses thanks to their ability to feed on a wide range of organic substrates, characterized by a different nutrient content. In a circular economy perspective, it is interesting to consider the possibility of recovering local readily available substrates, such as catering waste. However, waste-based diets could be nutritionally unbalanced and even have deficiencies that could lead to a weakening of the immune system. Therefore, the effect of different catering waste on the growing performances of larvae and on the immunity of *H. illucens* prepupae has been evaluated. Insects were reared on five diets: one based on feed for laying hens, as control, and four based on catering waste containing (i) fruits and vegetables; (ii) fruits, vegetables and bread; (iii) fruit, vegetables, bread and dairy products; (iv) fruits, vegetables, bread, meat and fish. For each diet, the average daily gain, the final weight, the survival rate and the final larval biomass were evaluated. The gene expression of two antimicrobial peptides, one defensin and one cecropin, was assessed. Moreover, the hemolymph inhibitory activity against two bacteria, *Escherichia coli* DH5 α and *Micrococcus yunnanensis* HI55, was evaluated using diffusion assays in solid media. Among the different catering waste, a higher growth rate and a higher final larval biomass were recorded in insects fed a protein-rich diet, so the ones containing dairy products or meat and fish. A higher up-regulation of both antimicrobial peptide encoding genes was observed in insects fed a dairy product-added diet. All hemolymph samples showed inhibitory activity against both bacteria, affecting the colony size and number. The results open new perspectives for an optimal re-use of various catering waste in insect mass rearing. Moreover, the possibility of modulating antimicrobial peptide expression levels through the diet opens up new perspectives in the management of insect health in mass rearings.

PAROLE CHIAVE: Circular economy, antimicrobial peptides, defensin, cecropin, hemolymph, diet.

ORAL PRESENTATION

SESSION IX
INSECTS FOR BIOCONVERSION

Characterization of RNASET2 in the Black Soldier Fly, *Hermetia illucens*

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Hermetia illucens (Diptera: Stratiomyidae), known as Black Soldier Fly (BSF), is a saprophagous insect used for the bioconversion of organic waste and by-products of different agro-food chains. Since the larvae of this insect grow on organic waste rich in potential pathogens, they have likely evolved a complex array of antimicrobial compounds. Thus, in addition to the classical antimicrobial peptides (AMPs) largely represented in insects, it is worthy to investigate the presence in this dipteran of antimicrobial, complex molecules, that can play multifunctional roles during the immune response.

This study aims at investigating RNASET2, an acid hydrolase belonging to the T2 RNase family that plays key roles in immunity both in invertebrates and vertebrates, in *H. illucens* larvae following bacterial infection.

Bioinformatics analysis on *H. illucens* RNASET2 indicated the presence of the two specific active sites, namely CAS I and CAS II, which are involved in the housekeeping catalytic activity and are extremely conserved in Rh/T2/S ribonucleases. The three-dimensional structure was determined using I-TASSER software to visualize the conserved α and β motifs. Moreover, the AMPA software identified an amino-acid sequence with potential antimicrobial activity.

The expression of RNASET2 in the fat body and hemocytes, two tissues that are involved in the immune response of insects, was analyzed. Immunostaining, qRT-PCR, and Western blot analyses indicated the circulating cells as the primary site involved in the synthesis of this ribonuclease. Further assays are in progress to address if RNASET2 is associated to the lysosomes, as suggested by the bioinformatic analysis, and could thus have a role in phagocytosis.

Future studies will aim at investigating the antimicrobial role of this ribonuclease against selected bacterial strains.

POSTER

SESSION IX

INSECTS FOR BIOCONVERSION

FLIES4FEED – New animal feed from insects using agricultural by-products and biogas plants

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In a climate change scenario where the human activities entail high CO₂ emissions, circular economy could be the right answer in order to reduce the environmental impact related to these activities. In this sense, using insects for bioconversion could be promising. Among these insects, Black Soldier Fly (BSF) *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae) is one of the most promising species. The great voracity and polyphagy of the larvae, their rapid development and the relative ease of mass rearing make this species a useful ally for the valorisation of a wide variety of biowaste.

The GOI “Flies4Feed” project is set in a circular economy perspective and uses BSFL for the upcycling of agro-industrial by-products for the purpose of producing insect-meal for aquaculture and pet birds. In particular, the project aims to install an insect-producing facility on a farm equipped with a biogas plant, in order to exploit the excess heat for the rearing, suppression and drying of the larvae.

Six different types of mill waste were tested in the project for BSFL mass rearing, using Gainesville diet as control. The by-products were tested both individually and in different combinations. The optimal combination of by-products for BSFL rearing was identified (50% soft wheat middlings, 20% bran, 15% durum wheat middlings and 15% corn middlings). This combination of by-products was the best in terms of substrate conversion efficiency, reduced growth times and lower larval mortality. Several cycles of mass rearing were therefore carried out using this mix of substrates, obtaining several kg of larvae. The BSFL meal was then obtained from the dried larvae, which together with other commercially available insect meals was characterized and subjected to NIRs (Near Infrared Spectroscopy) analysis in an attempt to obtain initial statistical data for the purpose of creating a calibration curve.

The results of the GOI project “Flies4Feed” confirm that among the seven species of insects authorized for feed use, *H. illucens* is among the most promising and that the implementation of structures for BSFL rearing on agro-industrial by-products, especially if combined to biogas plants, represents a sustainable path in the production of protein meals. Overall, this goes in the direction of leading Europe to achieve greater protein independence, with a view focused on circular economy and sustainability.

“FLIES4FEED – New animal feed from insects using agricultural by-products and biogas plants” is a project financed by Emilia-Romagna region, PSR 2014-2020 Op. 16.1.01-GO PEI-Agri - FA 3A, coordinated by CRPA.

KEY WORDS: Bioconverter insects, black soldier fly, *Hermetia illucens*, biogas plants, insects as feed.

POSTER

SESSION IX

INSECTS FOR BIOCONVERSION

The exploitation of residual biomass through Black Soldier Fly (*Hermetia illucens* L.) bioconversion

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In a Circular Economy perspective, by-products and left-over from the agro-food sector have a potential added value as raw material in other processes. The application of the CORS (Conversion of Organic Refuse by Saprophages) technology mediated by the black soldier fly (BSF), *Hermetia illucens* L. (Diptera, Stratiomyidae: Hermetiinae) represents an important innovation that can significantly contribute to the development of supply chains within the green economy and green chemistry. BSF larvae (BSFL) can grow on various organic substrates (vegetable matter, manure, catering waste), which are reduced by 50-70% and transformed into biomass with high added value. Mature larvae can provide proteins and fats used in animal feed, pupae and adults provide chitin for textile, cosmetic and pharmaceutical industries. In addition, the residue resulting from the bioconversion process can be used as a soil conditioner in the agricultural and nursery sector. The activity was carried out within the framework of the Hermes project (POR FESR Lazio 2014-2020, Det. Reg. no. G09493 140721) and was aimed at verifying the suitability of bioconversion by BSFL of fruit and vegetable (FV) left-over and by-products from the agro-industry. The Hermes project involves the set-up of the bioconversion process and the design of a modular prototype for process automation. The base substrate was FV left-overs provided by a large-scale local retailer. We assessed the FV left-overs produced during a calendar year to analyse how the larval development varied with seasonal variability. The FV left-overs were used as they are or mixed with bakery scraps, bran or brewers' spent grain (BSG) in a ratio of 80:20 to reduce the moisture content of the base substrate and to increase the carbohydrate and protein content. Six-day-old BSFL, fed until then on Gainesville diet, were used for the tests, carried out 'in batch', supplying the material required for larval maturation only once, at the beginning of the rearing, in controlled climatic conditions. The trials tested two different amounts of substrate (2 kg or 10 kg) and two different feeding levels (1,25 g/larva and 2 g/larva). The process took 12-20 days, depending on the composition of the substrate. Preliminary results showed that mixtures gave better results than the FV alone, in terms of final larval weight and lipid and protein content. Larval growth and substrate consumption were also influenced by the different composition of the FV left-overs (driven by the seasonal availability) and by the different feeding levels. Both the FV left-overs variability and the different type of mixtures influenced the moisture content of the substrate. The moisture content plays a key role in the automation of the process, especially in the sieving phase to separate the mature larvae from the residue. Further investigations are in progress to optimise the substrate composition for both the larval growth and the two fractions separation.

KEY WORDS: Black soldier fly, *Hermetia illucens*, Bioconversion, Modular prototype, Agro-food left-overs, Green economy, Green chemistry.

POSTER

SESSION IX

INSECTS FOR BIOCONVERSION

Evaluation of the effect of alternative vegetable sources of water and nutrients on the sustainability and quality of flour obtained from *Tenebrio molitor* (L.) larvae.

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The steady and rapid increase in the world's population and the increasingly evident climate change entail a growing demand for protein for human needs on the one hand, and a need to produce such protein in a sustainable manner on the other. Insects can be a viable alternative to traditional sources of protein, perfectly in line with the precepts of the circular economy, although they cannot stand as the ultimate solution to global food shortages. In this context, the production of protein meal from *Tenebrio molitor* larvae (TMLs) is a well-liked solution, especially after the approval of this beetle as the first Novel Food insect in Europe (2021). The challenge is to make TMLs rearing sustainable on a large scale, even in countries with semi-arid climates.

Our research group has been studying the possibility of replacing, in TMLs rearing, the usual sources of water and nutrients (carrots, potatoes) with more sustainable alternatives: we have obtained important data, at least for our latitudes, using cladodes of *Opuntia ficus indica* (OFI). Based on encouraging preliminary data, we tested the "storability" of these cladodes in terms of resistance to dehydration and mould growth, as well as their palatability for larvae.

In addition, we have adapted and developed a protocol for obtaining flours from TMLs that can be used both as feed and as food, and we are testing the applicability of the protocol on larvae fed on different diets or different sources of water and nutrients. The water activity in the flours obtained from larvae reared on a standard diet with diversified wet supplements is between 0.36 and 0.41. This range makes the obtained flours suitable for long storage without moulting or losing organoleptic characteristics.

Preliminary data show a protein content, measured by Kjeldhal, of 40% in the flour obtained from TMLs fed standard diet (95% bran + 5% yeast), which is in line with values reported in the literature. This content appears to be unaffected by the plant matrix added as a source of water and nutrients (OFI, potato or carrot). Data obtained from this preliminary study show how the use of OFI as a source of water and nutrients leads to increased sustainability of both TMLs rearing and flour production. Further studies will focus on the potential effects of OFI use on flour quality.

PAROLE CHIAVE: *Tenebrio molitor* rearing, *Opuntia ficus indica*, Alternative protein sources, Insect meal, Circular bioeconomy.

ORAL PRESENTATION

SESSION IX

INSECTS FOR BIOCONVERSION

Inbreeding depression in *Hermetia illucens*: from mate acceptance to transgenerational fitness

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The black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) is a promising species used as protein source for feed, which has been also proposed as biological tool for managing organic wastes. Although many studies investigated these aspects, the biology of this insect species is still needing deeper investigations to optimize mass rearing and bioconversion efficiency. Inbreeding depression can play a crucial role for productive farms, also impairing the successful use of *H. illucens* for bioconversion.

This study aimed to investigate inbreeding in *H. illucens*, firstly exploring the ability for mate recognition among siblings and then evaluating progeny outcomes. Concerning mate recognition, females could not distinguish courtship of sibling (acceptance rate: 55%; copula: 34 ± 3.1 min) and non-related male (acceptance rate: 58%; copula: 36.2 ± 2.8 min). However, female offspring generated from a sibling mate (F1-sib) was less inclined to accept courtship both from a sibling partner (acceptance rate: 38%) as well as from a non-related one (acceptance rate: 43%), showing also longer copula durations when mating with inbred males (copula: 52.2 ± 6.5 min). Similar to mating, no differences were recorded in the rate of mated females which produced and oviposit egg masses (non-related mates: 95.6%; sibling mates: 95.5%), while only 73.3% and 88.2% of F1-sib females lay eggs when mating sibling or non-related males, respectively. On the other hand, egg fertility was reduced from 95.5% to 85.7% due to inbred mating in parental generation and from 80% to 63.6% in first inbred generation.

Considering fecundity and progeny outcomes, every female produced an average of 262.4 ± 39.8 larvae with a weight of 0.193 ± 0.007 g/larva. Despite larvae produced from inbred mates were in average 277.6 ± 61.3 , their median weight (0.168 ± 0.011 g/larva) was lower compared to larvae obtained from mating between unrelated specimens. The progeny was consistently reduced in F1-sib females, mated either with sibling partners or not-relatives, which produced 100 ± 25.6 and 194.9 ± 46 larvae, respectively. Furthermore, inbreeding affected both the adult emergence rates and the progeny sex ratio.

PAROLE CHIAVE: mating behavior; reproduction; fecundity; offspring; sex ratio; bioconversion.

SESSION IX

INSECTS FOR BIOCONVERSION

Effect of seasonality and pre-treatment of OFMSW on the production of *Hermetia illucens* larvae and their digestibility in vitro

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Hermetia illucens (Diptera: Stratiomyidae), also known as the black soldier fly, has received particular attention due to the ability of its larvae to rapidly convert numerous organic matters into larval biomass mainly rich in proteins and lipids. The larvae produced represent a source of feed for some farmed animals or can find various industrial applications.

The organic fraction of municipal solid waste (OFMSW), produced in large quantities, is still rich in nutrients, mainly represented by carbohydrates, proteins and lipids. In the present work, part of the SMARTFEED project (Sustainable Model for Agroenergy and Feed production by Urban Waste Recycling and Treatment), the growth of *H. illucens* larvae was evaluated on untreated or pulped OFMSW, collected during four seasons in Lombardy. The starting substrates were characterized, and the nutritional value of the larvae produced was analyzed. Finally, the in vitro digestibility of the obtained larvae was evaluated.

Larvae grew on all substrates tested, with high survival rates and an average of nine days to reach the prepupal stage from the start of the experimental trial. Although variability was observed between the parameters measured in the different experimental trials (survival, larval biomass, substrate reduction, indexes of conversion), only in a few cases statistical differences were found, that could be attributed to the treatment of the OFMSW or its seasonality.

Regarding the chemical composition of the larvae, those grown on pulped FORSU showed a lower lipid content compared to the larvae reared on the unprocessed OFMSW. The protein content was lower than reported in other studies, while ash was high. No significant differences were observed in the in vitro protein digestibility values due to the treatment, while the energy digestibility level was significantly higher in the case of larvae grown on the OFMSW provided as-is compared to the pulped OFMSW. The OFMSW can be considered a suitable substrate for the growth of *H. illucens* larvae without any noticeable effects due to season of collection, while the homogenization treatment affected lipid content and energy digestibility. The bioconversion of these wastes by the larvae into products with a high added value allows their valorization contributing to the recycling of nutrients in a circular economy perspective.

PAROLE CHIAVE: organic fraction of municipal solid waste, black soldier fly, bioconversion, circular economy.

ORAL PRESENTATION

SESSION IX

INSECTS FOR BIOCONVERSION

Lipidomic analysis and morphofunctional characterization of the fat body of *Hermetia illucens* reared on the organic fraction of municipal solid waste

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Waste management represents one of the most pressing challenges of the twenty-first century and plays a key role in sustainable development. In particular, it is estimated that the organic fraction of municipal solid waste (OFMSW) will globally increase by 70% in the next 30 years. In this context, the use of saprophagous insects, and especially of the black soldier fly (BSF), *Hermetia illucens*, for the bioconversion of organic waste into biobased products can represent a valid solution to overcome this issue.

The present study is part of the RICH (Turning Rubbish Into biobased materials: a sustainable CHain for the full valorization of organic waste) project that aims at developing a circular supply chain based on the use of BSF larvae for the bioconversion of the OFMSW to produce protein-based biomaterials. In addition to the exploitation of proteins, RICH aims at valorizing insect lipids for biodiesel production. To this purpose, we undertook a lipidomic analysis of larvae and pupae of *H. illucens* grown on two experimental substrates that reproduced the composition of the OFMSW, as well as a morphofunctional characterization of their fat body. The lipidomic analysis of BSF reared on the two substrates revealed quantitative and qualitative differences in lipid composition according to the developmental stage of the insect. Moreover, lipid profile was significantly affected by the rearing substrate, too. This evidence on lipids was corroborated by differences in the morphology and histochemical pattern of the fat body, the main site of lipid accumulation in insects. A transcriptomic analysis is in progress to get insights into the molecular mechanisms underpinning lipid accumulation in this insect.

The study was funded by Fondazione Cariplo (2020-0900).

SESSION IX

INSECTS FOR BIOCONVERSION

The immune response of *Hermetia illucens*: a deep characterization of the cellular and humoral responses

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Hermetia illucens, also known as Black Soldier Fly (BSF), is one of the most promising saprophagous insects used for organic waste management. As the larvae grow on various decaying substrates, they have likely evolved a sophisticated immune system. In this context, the knowledge on the immune mechanisms and the physiology of the insect is of primary importance to monitor its health status during the bioconversion process.

In this study we analyzed timing and features of the humoral response activated following bacterial infection, characterized in depth *H. illucens* larvae hemocytes, and investigated the processes in which these cells are involved (i.e., phagocytosis, encapsulation, and nodulation) to fill the gap of knowledge on BSF cellular immune response.

Our results demonstrate that five hemocyte types (i.e., plasmatocytes, lamellocyte-like cells, granulocytes, crystal cells, and adipohemocytes) carry out the immune response triggered by the infection. Differently from other insect species, only plasmatocytes are able to phagocytize foreign bacteria present in the hemolymph. Moreover, they are also involved in the nodulation and encapsulation processes together with granulocytes, lamellocyte-like cells, and crystal cells, which are responsible for melanin production. The combined action of the cellular processes, which intervene more promptly, and humoral mechanisms, which are activated later, leads to the complete removal of the Gram-negative and Gram-positive bacteria from the insect body.

The study was funded by Fondazione Cariplo (2020-0900).

PAROLE CHIAVE: Black soldier fly, insect immunity, bacterial infection, hemocytes, antimicrobial peptides.

SESSION IX
INSECTS FOR BIOCONVERSION

The armour of a black soldier fly

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The larvae of different subfamilies of Stratiomyidae and Xylomyidae (Stratiomomorpha) show mineralised cuticle. The presence of calcium carbonate in the integument of stratiomyiid larvae and their puparia has been mentioned in very old papers but surprisingly no detailed investigation is available on the biomineralised cuticle of these insects, in spite of the large number of articles published in the last years on *Hermetia illucens*, the 'crown jewel' insect as feed industry species. Here we describe in detail the ultrastructure of the cuticle of the larva of *H. illucens* at different developmental instars using scanning and transmission electron microscopy (SEM, TEM), we characterise its chemical composition with confocal laser scanning microscope (CLSM) and energy dispersive X-ray microanalysis, analyse the crystalline structure of calcium carbonate with X-ray diffraction and characterize the cuticular hardness and elasticity with nanoindentation measurements. Amorphous calcium carbonate (ACC) accumulate in the epithelial cells in the form of spherical granules, pass through pore canals in the cuticle, aggregate on the epicuticle just after the moult and crystallize as blocks of calcite in the pupal cuticle. CaCO₃ is already present in the cuticle of young larval instars, but it is mainly in the form of ACC, while the amount of calcite is reduced and increases during larval development. Larval and pupal cuticle contain large amount of resilin which guarantees cuticle flexibility necessary for larvae, when moving inside soil and rotten material and for prepupae, when crawling to seek shelter to pupate. On the other hand, the presence of calcite reduces elasticity and hardens the cuticle. Mineral precursors of ACC are stored in two specialised Malpighian tubules representing "lime gland". These last represent conspicuous storage structures analogous to those present in terrestrial crustaceans, like Isopod sternal plates, and could be regarded as an adaptation to calcium deficient environments, thus revealing possible convergent evolution in these systems in terrestrial crustaceans and insects. *Hermetia illucens* ability to cyclically elaborate and resorb biomineral composites requires further investigations. Knowledge on the structure and biomechanical properties of the cuticle of black soldier fly larvae can help to widen the basic knowledge on arthropod biomineralization, an aspect intensively studied so far only in Crustacea and almost unknown in insects, and add important information on the use of *H. illucens* as mineral source in animal diet.

PAROLE CHIAVE: cuticle, calcium carbonate, calcite, *Hermetia illucens*, pupa.

ORAL PRESENTATION

SESSION IX

INSECTS FOR BIOCONVERSION

Effect of feed amount, adult density and sex ratio on the quantity of eggs laid by *Tenebrio molitor* L. reared for mass production

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Mass rearing conditions of the yellow mealworm (*Tenebrio molitor* L.) adults during mating and oviposition have a great impact on laid eggs. After mating, females burrow into the food substrate to oviposit and eggs, are glued to the bottom of the tray, allowing their collection. In order to maximize the production of eggs for mass-rearing, experimental trials were conducted to: (1) identify the optimal amount of feeding substrate for oviposition; (2) define the optimal density of adults per unit area; and (3) evaluate how variations in the sex ratio (usually 1:1) affect fecundity.

A total of 1270 pairs of newly eclosed males and female beetles were reared for 4 weeks in plastic trays of 75 cm² (7.5x10.0x5.0 cm) and fed on a wheat bran substrate under laboratory conditions. The trays were replaced weekly and the eggs sticking to the bottom were counted. In each trial (feed amount, adult density and sex ratio), each treatment was replicated 5 times.

To define the optimal amount of feed substrate, eggs laid by 4, 8 and 16 pairs of adults on three different amounts of wheat bran of 5, 10 and 20 grams/tray, respectively were compared. A significantly higher (P-value=0.036) average number of eggs (2.2 eggs/cm²) was observed in trays containing 10 grams of feed substrate (equaling to 0.60 cm thickness) than in trays containing 5 and 20 grams of wheat bran (1.4 and 1.1 eggs/cm², respectively). In the density trial, eggs laid by 1, 2, 3, 4, 8, 12, 16, and 24 pairs of adults were compared. The total number of laid eggs significantly increased (P-value=0.0005) until it reached a plateau, at around 16 pairs/tray (0.43 individuals/cm²), along with a maximum oviposition of 12.1 eggs/cm². Comparing different sex-ratios (SR) of 1:1, 2:1, 3:1 and 4:1, eggs laid by 6, 8, 10, 12 and 16 females showed a reduction, although not significant (P-value=0.555) when SR were lesser than 1:1. Compared with SR of 1:1, the reduction in the number of eggs laid was 16, 25 and 19% for SR of 2:1, 3:1 and 4:1, respectively.

The results of trials provided useful information to optimize egg production of *T. molitor* for mass rearing purpose. A substrate thickness of at least 0.5-0.6 cm allows insects to feed adequately and females to easily reach the bottom of the trays to oviposit; conversely, a higher bran layer results in greater difficulty to stick eggs to the tray. The density of 0.4-0.5 adults/cm² appears to be the optimal condition in terms of egg production per area unit, whereas higher densities do not result in a significant increase. Finally, a less favorable ratio of males to females does not significantly compromise mating even when the number of males is very low. Instead, the greater mating effort required of males may result in lower fecundity of females.

KEY WORDS: yellow mealworm, insect farming, sex ratio, oviposition.

POSTER

SESSION IX
INSECTS FOR BIOCONVERSION

Bioconversion of distillery by-products by *Tenebrio molitor* mealworm

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Agricultural and industrial waste represent valuable starting materials to create novel products with economic value added. In this perspective, also driven by global warming and the increasing demand for food and feed, we wanted to check the possible use of distillery by-products as possible supplements to feed *Tenebrio molitor* larvae (TML).

In our study, we have exploited the larvae of *T. molitor* as an unconventional disposal system and enhancement of the by-products of the oenological chain through the transformation into larval biomass, a product of high biological, ecological and economic value.

Winery industry represents an important economic sector in Italy, which produces tons of by-products every year. [1] Grape pomace (GP) is used in distillery to produce ethanol bringing to some by-products (grape marcs (GM), grape skin pulp (GSP), grape seeds (GS) and winery waste sludge (WWS)). [2] Distillery by-products are usually used to obtain tartrates, bioactive compounds or biogas. [3] In our project these by-products were used to enrich insect feed. Larvae were raised on a standard diet composed of brewer's yeast (0.5%), wheat flour (49.75%) and oat (49.75%) and the same diet added (10%) with the various by-products of the wine industry after a first exploitation in the distillery.

Waste materials were also analysed for their antioxidant activity and fatty acid (FA) profile before the experiment confirming an interesting functional and nutritional potential. Even if subjected to multiple processing, by-products had an important content of antioxidant compounds, in particular polyphenols, flavonols, flavonoids and condensed tannins. Moreover, the high amount of polyunsaturated fatty acids and the low percentage of saturated fatty acids found make them useful feed supplements. Herein has been disclosed that their use as TML feed material was well tolerated over TML development, with a significant mean weight gain respect to control of about 25% and no effect on survival rate. Surprisingly, the best results in term of larvae growth were obtained with WWS, the final product of the distillery, currently used only for the biogas production or soil amendment.

With all the by-products total antioxidant activity and FA profile of the TML ameliorate significantly, suggesting that distillery by-products can be used to extend their shelf-life and ameliorate their nutraceutical properties, with possible application in controlled dietary regimens. This work confirmed that by-products largely produced in Europe can be used as TML feed materials, simplifying waste management and reducing rearing costs.

1 - Pomarici, E., Corsi, A., Mazzarino, S. and Sardone, R., 2021. The Italian wine sector: evolution, structure, competitiveness and future challenges of an enduring leader. *Italian Economic Journal* 7: 259-295.

2 - Bustamante, M.A., Moral, R., Paredes, C., Perez-Espinosa, A., Moreno-Caselles, J. and Perez-Murcia, M.D., 2008. Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. *Waste Management* 28: 372-380.

3 - Arvanitoyannis, I.S., 2008. Waste management for the food industries. *Waste Management for the Food Industries*. Elsevier, Amsterdam, the Netherlands.

KEY WORDS: Bioconversion, oenological chain, anti-oxidant activity, fatty acids.

POSTER

SESSION IX

INSECTS FOR BIOCONVERSION

Use of agricultural waste to feed *Tenebrio molitor* mealworms and evaluation of their nutraceutical properties

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Circular economy principles aim to maintain resources in the economic circle. Accordingly, the use of agricultural waste and its transformation into novel products is a smart approach that creates benefits for the environment, industries and consumers. Herein, we conjugated the principles of circular economy with the search for novel sustainable functional foods, transforming agricultural food chain by-products into feed supplements for *Tenebrio molitor* larvae (TML) feeding.

Accordingly, tomato peels and seeds (TPS) stemming from the tomato sauce production, as well as olive (*Olea europaea*) and mastic (*Pistacia lentiscus*) leaves (OEL and PLL) from pruning were finely pulverized and used as feed supplements. Larvae were raised on a standard diet composed of brewer's yeast (0.5%), wheat flour (49.75%) and oat (49.75%) and the same diet added with 10 % of the various pulverized by-products. All the diet supplements efficiently supported larval growth, offering optimal values of larvae mean body weight and survival rate.

Interestingly, both total phenol content and antioxidant activities increased compared to the control, thanks to the accumulation of active compounds with hydrophilic or lipophilic characteristics. In addition, the fatty acids composition was determined, revealing a beneficial reduction of omega-6/omega-3 ratio in TML fat. The most relevant nutritional indexes were calculated, with small differences between control and groups that received feed supplements. Nevertheless, all the nutritional indexes were very compelling, being comparable with the ones reported for healthy foods like fish, chicken and brown algae. This result highlights that the lipidic portion of TML has a valuable nutraceutical value that coupled with the availability of different anti-hypertensive peptides (Brai et al., 2022; Pessina et al., 2020) and the improved antioxidant activity makes the supplemented TML a novel functional food. Furthermore, the increased antioxidant activity represents an additional added value, due to the possibility to prolong TML shelf life and decrease oxidative stress.

Taken together, our results strongly support TPS, OEL and PLL use as smart breeding supplements, able to increase the antioxidant activity and ameliorate fatty acid profile of TML with important applications in human and animal nutrition.

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Pessina, F., Frosini, M., Marcolongo, P., Fusi, F., Saponara, S., Gamberucci, A., Valoti, M., Giustarini, D., Fiorenzani, P., Gorelli, B., Francardi, V., Botta, M. and Dreassi, E., 2020. Antihypertensive, cardio- and neuro-protective effects of *Tenebrio molitor* (Coleoptera: Tenebrionidae) defatted larvae in spontaneously hypertensive rats. *PLoS ONE* 15: e0233788.

KEY WORDS: food chain by-products, tomato, olive, mastic.

POSTER

SESSION IX

INSECTS FOR BIOCONVERSION

Plastics, polystyrene, *Alphitobius diaperinus*, insect rearing, larval stages, GC-MS, micro-FTIR, microbiome, metabarcoding

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Industrial production of plastics has grown rapidly from the 1950s to present days. Due to its properties (high plasticity, versatility of performance, ease of processing), the plastic has become one of the most widely used materials in the world, especially in the packaging industry. Plastic pollution is among the major environmental problems of the new millennium. In recent years, there have been numerous attempts to research environmentally sustainable methods for the disposal of plastic materials such as polyethylene, polypropylene and polystyrene. Possible approaches include the study of 'plastivorous insects' that seem to feed on and degrade different types of plastics. As for polystyrene, the most studied group is that of tenebrionids, especially those species belonging to *Tenebrio* and *Zophobas* genera. Nevertheless, the beetle *Alphitobius diaperinus* also seems to be involved in plastic material digestion. Previous studies, in fact, attribute the possible role of polymer degradation to the intestinal bacterial flora of this species. Some authors, after comparing insects fed with a polystyrene-based diet with those fed with a classical diet, found significant differences in the gut microbiome. Although a high mortality rate was found in the plastic-fed rearing, it was possible to identify three bacterial strains capable of growing on polystyrene biofilms (*Klebsiella aerogenes*, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*). Therefore, this work has, as its first goal, the standardization and the improvement of the rearing conditions described in previous works. After careful observations, it was seen that only the last larval stage, the pre-pupal one, is involved in polystyrene ingestion. By measuring the size of the cephalic capsule, it is possible to determine to which larval stage it belongs. With these new considerations, rearing conditions of *A. diaperinus* were optimized. Moreover, in order to obtain further informations on polystyrene degradation rate, chemical analyses were initiated on insects' frass. The analyses are based on Fourier Transform Infrared Spectroscopy (micro-FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) techniques. Preliminary data show the presence of by-products derived from polystyrene polymer metabolism. In addition, a metabarcoding screening is underway with the aim of identifying differences in the gut microbiome between different larval stages and between different portions of the gut of late-stage larvae (foregut, midgut, hindgut). These data will be essential for the development of new research lines, primarily focused on the identification of bacterial enzymes involved in the polystyrene degradation process.

KEYWORDS: Plastics, polystyrene, *Alphitobius diaperinus*, insect rearing, larval stages, GC-MS, micro-FTIR, microbiome, metabarcoding

ORAL PRESENTATION



CNiE²⁰₂₃
P A L E R M O

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Session X



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Development of new food products using after-silking worms as a promising ingredient

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In Thailand, silk industry is one important sector, contributing to an economic and social impact since long time. Before the COVID-19 crisis, the export value of silk products was approximately 13-16 M€ annually. Recently, there is an emerging innovation of silk fiber sheet products from silkworm (*Bombyx mori* L.). Once weaving the silk thread, after-silking worms are left as by-products in a large quantity. Therefore, in this research we aimed to develop products using after-silking worms as the main ingredient. The silkworms were processed in different ways: vacuum-fried, snack bar, sausage, instant cream soup, and mixed with peanut butter. Prior to the product development, after-silking worms were subjected to analyses of physical properties, proximate analysis, and amino acid and fatty acid compositions in comparison with silkworm pupae. The proximate analysis results showed that after-silking worms and pupae were significantly different except protein content. All the nine essential amino acids were present in both after-silking worms and pupae. Lipid analysis of both after-silking worms and pupae evidenced the presence of saturated, monounsaturated and polyunsaturated fatty acids. The consumer acceptance towards the developed products were carried out using 9-point hedonic scale (1= dislike extremely, 5 = neither like nor dislike, and 9= like extremely) with the consumers (N=50, not the same group) who used to eat insects. The consumers showed positive interest on those products and rated the overall liking scores of instant silkworm cream soup, silkworm sausage, silkworm - peanut butter, vacuum-fried silkworms and silkworm snack bar as 7.7 ± 0.7 , 7.4 ± 0.5 , 6.8 ± 0.8 , 6.7 ± 1.2 and 6.5 ± 1.4 , respectively. The research demonstrated that after-silking worms can not only be consumed in traditional ways, but they can also be used as promising ingredients for newly-developed food products.

KEY WORDS: Silkworm; edible insects; silkworm snack, consumer evaluation, food innovation, *Bombyx mori*.

SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Smart Urban Forest Monitoring Project for the development of a remote sensing system of anomalies linked to pest attacks in urban-forest areas

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The climate and environmental changes that characterize this historical period are increasingly demanding actions to enhance available resources and contain ongoing regressions. These actions also include the protection and conservation of forest heritage, which is necessary to counteract climate change, reduce biodiversity loss, and safeguard the socio-economic systems connected to such heritage. In this context, digitization is emerging as an innovative tool for the sustainable management of these ecosystems. In particular, remote sensing, i.e. the acquisition of information and monitoring of physical characteristics in specific areas at distance, has become a priority tool. The process is carried out through the measurement of reflected and emitted radiation, typically from satellites or airplanes.

The Smart Urban Forest Monitoring (SUFM) Project fits into this context by expanding the application of remote sensing to the field of forest protection from pathogens and pests. SUFM is a monitoring program designed to detect alterations in vegetation indices caused by parasitic attacks on trees and forests in an urban context using remote sensing of spectral anomalies from the PRISMA satellite platform. RGB-NIR multi/hyperspectral and SAR images will be validated on the ground by the Greenery Scanner developed at the Massachusetts Institute of Technology, a system of hyperspectral and thermal sensors mounted on a vehicle; additional validation will come from aerial images, Tree Talkers sensors, and traditional visual inspections for evaluating the degree of infestation. All these data will merge in a Data Lake where models of different types of data analysis (neural networks, physical models, statistical analysis) will be developed and calibrated to correlate the images to the degree of infection/infestation.

The first case study of the project involves the development of a remote sensing system to detect the infestation of the pine tortoise scale, *Toumeyella parvicornis* (Hemiptera: Coccidae), on *Pinus pinea* in urban parks and forests in Rome (Italy). Since 2018, *T. parvicornis* has spread throughout the city, reaching a high population density and drastically modifying the urban landscape. Endotherapy was the only short-term control strategy suitable in densely inhabited environments. The current availability in Rome of both treated and untreated pine plants, constitutes the starting point for developing a methodology that can correlate images of the *P. pinea* crowns to the symptoms caused by the scale at different levels of infestation. This tool will potentially allow to promptly identify and map possible new outbreaks in areas that are still pest-free but at risk of introduction. With a broader horizon, SUFM aims to develop a remote sensing system of spectral anomalies that can be used for the early and non-destructive monitoring of other types of biotic or abiotic stresses of forest systems in both urban and non-urban contexts.

KEY WORDS: spectral images, PRISMA satellite platform, hyperspectral and thermal sensors, *Toumeyella parvicornis*.

POSTER

SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Preference and behavior of *Trichogramma evanescens* (Westwood) and *Habrobracon hebetor* (Say) against two storage products pests

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To comply with binding and voluntary regulations (Reg. (UE) 2018/848, Reg (UE) 528/2012, BRCGS, IFSFood) and to respond to the growing sensitivity of consumers towards the quality and safety of food, pest control in the food industry tends to move towards the application of integrated pest management programs (IPM). Biological control, which involves the use of natural antagonists reared in laboratory against pests, finds growing interest in this context.

Stored-product moths are among the most harmful and common species in the food industry (Fam. Pyralidae). These insects are generally polyphagous, the larvae can attack various stored products such as cereals, legumes and dried fruit, contaminating them with excrement, silky burrs and exuviae with consequent loss of product and possible equipment damages.

In this work, we studied the behavior and the preference of a larval parasitoid *Habrobracon hebetor* (Say) and an oophagous parasitoid *Trichogramma evanescens* Westwood, reared in laboratory, against *Ephesia cautella* (Walker) and *Plodia interpunctella* (Hübner).

One mature larva of *E. cautella* and one of *P. interpunctella* were simultaneously exposed to a mated female of *H. hebetor*. In the first hour, the time elapsed since exposure to parasitization and paralysis of the hosts was recorded. After 1, 4 and 24 h the eggs laid on the larvae were counted.

Regarding *T. evanescens*, 5 females of the parasitoid, 50 eggs of *E. cautella* and 50 eggs of *P. interpunctella* were exposed simultaneously for 24 hours. Subsequently, the parasitization rates of the host species and the emergence rates of the newly emerged parasitoids were calculated.

Although there were no significant differences between the timing of aggression in the case of *H. hebetor* against the two hosts, the number of eggs laid on *E. cautella* was significantly higher both after 4 hours (0.67 ± 0.04 eggs on *P. interpunctella* and 2.72 ± 0.07 on *E. cautella*, $p < 0.05$) and after 24 hours (3.67 ± 0.10 eggs on *P. interpunctella* and 6.16 ± 0.11 on *E. cautella*, $p < 0.05$). The oophagous parasitoid *T. evanescens* parasitized significantly more eggs of *E. cautella* (*P. interpunctella*: 18.7 ± 3.3 , *E. cautella*: 35.6 ± 3.4 , $p < 0.05$) which resulted in an equally significantly higher emergence rate (*P. interpunctella* 17.9 ± 3.2 , *E. cautella* 33.1 ± 3.2 , $p < 0.05$).

These results can contribute to the improvement of biological control programs in the food sector, but also to the optimization of mass rearing in view of increasing demand for the two antagonists.

KEY WORDS: biological control, *Habrobracon hebetor*, *Trichogramma evanescens*, tignole delle derrate.

ORAL PRESENTATION



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Susceptibility and attractiveness of old and modern wheat varieties to the granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae)

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The granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae), is one of the main damaging primary pest of stored cereals worldwide. Development of resistant wheat varieties to insect attacks during the wheat grain storage is one of the most promising low impact alternatives to insecticides in management of stored grain pests. In recent years, the interest for old cereal varieties is increased due to the consumers' demand for typical local products, their nutritional properties, and adaptability to marginal agricultural areas. However, very little is known about their susceptibility and attractiveness to *S. granarius*. In this study, the susceptibility and attractiveness of 3 old (Saragolla Antica, Dauno III, Senatore Cappelli) and 3 modern (Ofanto, Svevo, Faridur) *Triticum durum* varieties, and 1 modern (Mec) *T. aestivum* variety towards granary weevil adults were investigated. Moreover, volatile organic compounds (VOCs) emitted by intact kernels of each genotype were extracted by head-space solid-phase micro-extraction (HS-SPME) and identified by gas chromatography coupled with mass spectrometry (GC-MS).

In susceptibility tests, intact kernel samples (60 g) of each variety were infested with *S. granarius* adults (n=12). After 15-day exposure, insects were removed, sexed and the number of dead specimens was recorded. The F1 progeny was monitored until no adults emerged for 5 consecutive days. The Dauno III and Senatore Cappelli varieties showed the significantly ($P < 0.05$, Tukey test) lower number of F1 progeny and longer mean development period compared to the modern variety Faridur, indicating differences among varieties in the susceptibility to granary weevils. The olfactory responses of granary weevil adults to kernel odours were investigated in two-choice pit-fall bioassays. The test arena was a steel container with two diametrically opposed holes (diam. 3 cm) connected with two glass flasks (500 ml), respectively left empty or filled with 200 g of wheat kernels as stimulus. There were 10 replicates for each wheat variety. For each experiment a response index (RI) was calculated. All varieties induced positive and significant ($P < 0.001$, t-test) mean RI indicating actual attraction. However, significant differences between the mean RIs recorded in response to different varieties ($P < 0.05$, Tukey test) were found with Faridur being the most attractive. Finally, the GC-MS analysis of HS-SPME kernel extracts highlighted the presence of 33 VOCs with some quantitative and qualitative differences among varieties. The identified compounds mainly included alcohols, aldehydes and alkanes, and to a lesser extent, alkenes, terpenes, organic acids, benzene derivatives, lactones and ketones. Studies are in progress to clarify the physical, chemical and biochemical factors possibly involved in the lower susceptibility and attractiveness of some of the old wheat varieties assessed.

KEY WORDS: Curculionidae, storage pests, durum wheat, varietal resistance, progeny, VOCs, GC-MS.

ORAL PRESENTATION



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Investigation on *Necrobia rufipes* (De Geer) (Coleoptera: Cleridae) food preferences in dry food for pets

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Necrobia rufipes is a species known since a long time in forensic entomology as a useful indicator for the evaluation of the post-mortem interval of a corpse. It is also known as harmful pest in storage areas for animal products, oilseeds, nuts and pet shops. Moreover, this species is reported as facultative predator of other insects and in particular of *Piophilus casei*, *Lasioderma serricorne*, *Oryzaephilus* spp., *Carpophilus dimidiatus*, *Tribolium castaneum*. *Necrobia rufipes* has a worldwide distribution range and its presence has been recorded in many countries of South, Central and North America, Asia and Oceania. In the last decade, *N. rufipes* has gained significant importance consequent to the increasing number of reports in the pet food industry, especially in Europe between 2015 and 2017 when it was signaled in pet food bags in warehouses and shops, contributing to spread the infestation throughout the entire supply chain. In particular, reports from manufacturers indicated that dog food is preferred by far by this species, compared to the other types of pet food.

In relation to the evident polyphagia of *N. rufipes*, and its increasing spread in Italy and in Europe in the pet food chain, laboratory investigations were carried out to examine in-depth the knowledge on food choices of this insect, comparing some dry dog food selected from different brands.

In addition, considering the highlighted predatory attitude of *N. rufipes*, the attractiveness of a sample of dry dog food already infested by *Oryzaephilus surinamensis* or *T. confusum* was assessed in order to deepen the interaction of *N. rufipes* adults with other pest of the food industry.

In all cases, tests were performed using a single adult per replicate placed in a dual choice arena for 5 days. The data did not show a marked feeding preference for test substrates. The results on dry dog food already infested by other coleoptera confirmed the predatory attitude of *N. rufipes* mainly on *O. surinamensis*.

POSTER

SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Biological risk assessment at the ambrosiana library in Milan

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The purpose of this work was the evaluation of biological risk at the Veneranda Biblioteca Ambrosiana in Milan, based on an entomological monitoring and on the measurement and analysis of environmental parameters.

Integrated Pest Management (IPM) is an effective system for reducing damage and infestation management costs, as it allows to minimize corrective interventions, making them increasingly targeted and effective.

The IPM program follows specific principles:

- assessment of the biological agent activity (regular visual inspections at pre-established intervals);
- risk assessment in relation with the location and intrinsic vulnerability of the cultural heritage (measurement of the factors influencing the risk, the importance of the potential damage and the probability that it will occur, in relation to the different materials and conservation conditions);
- insect monitoring through passive glue traps, placed near windows and light points;
- recording of monitoring data and choice of the best measure to reduce pest development and diffusion of biological agents and corrective measures, favoring non-toxic methods;
- internal staff training and education.

Entomological monitoring was activated on 8/02/2022 using 46 passive glue stations distributed in the library, near windows or light points, in order to detect the insect activity during the year. Monitoring activity was accompanied by the detailed visual inspection carried out on shelves and volumes.

In one of the library rooms, a continuous monitoring of the environmental parameters was activated through the installation of a sensor network made up of 8 Bluetooth Dataloggers Mod. Tz-Bt04. The sensors were placed in various points of the room, in order to obtain information on the trend of temperature and humidity over time.

The analysis of the data collected with the sensors highlighted the presence in the room of microclimate conditions suitable for the development of humidity, and therefore potentially favorable for fungi and insects. Collected data evidenced the need to optimize the environmental conditions, by developing auxiliary ventilation systems and optimizing the regulation of the air conditioning and forced ventilation system.

In conclusion, the risk analysis of Veneranda Biblioteca Ambrosiana in Milan is linked to structural and management issues which, thanks to monitoring data, can be managed to improve or resolve the reported problems. The collected data allow to carry out an effective risk analysis, allowing more specific and targeted corrective actions for the issue management linked to the pest presence or environmental conditions that are unsuitable for the conservation of cultural heritage.

KEY WORDS: IPM, passive glue traps, Datalogger Bluetooth.

POSTER



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Application of chitosan enriched with essential oils to protect fresh foodstuffs from the attack of dipteran pests.

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Each year, from 12 to 20% of worldwide food production is lost during post-harvest and post-production stages due to biotic factors, including several insect pest species. Fruit flies pose a threat to both the yield in the field and the handling and retailing of fruits, while blowflies and cheese skippers are dangerous to meat and ripening cheese products in places where hygienic conditions are scarce and in the event of failures along the cold chain network.

From a food sustainability perspective, this work aimed to evaluate the protection given by essential oils (EOs) and chitosan against the oviposition of fresh foodstuff dipteran pests, specifically, *Ceratitis capitata* (Tephritidae) on kumquat fruits (*Citrus japonica*, Rutaceae), *Drosophila suzukii* (Drosophilidae) on blueberries (*Vaccinium myrtillus*, Ericaceae), *Calliphora vomitoria* (Calliphoridae) on raw beef meat, and *Piophilidae casei* (Piophilidae) on cheese and hams during curing. EOs raise great interest as an eco-friendly alternative with low toxicity to synthetic repellents and insecticides, although, currently, their actual use in food protection is limited due to their high volatility and the potential alteration of foodstuffs' organoleptic qualities. Chitosan, a renewable and biodegradable polysaccharide already known as a preservative in the food industry, is proposed here as a stabiliser for the volatile component of EOs to prolong and/or enhance their bioactivity.

Through oviposition deterrence trials specifically adapted to the four dipteran species, we tested and compared, under laboratory conditions, the protective action of some EOs (concentrations from 0.5 to 2.0% in ethanol), chitosan solutions (from 0.5 to 2.0% w/v in aqueous solution), and chitosan formulations enriched with the correspondent EOs (same chitosan concentration and EO content). The EOs were selected by a trained panel of sensory judges who found the best combinations with the different foodstuffs and were then chemically characterised through GC-EIMS. For kumquat fruits and blueberries, the chosen EOs were *Cinnamomum verum* (Lauraceae, main component (*E*)-cinnamaldehyde 58.7%) and *Citrus reticulata* (Rutaceae, limonene 83.6%); for meat products and cheese, they were *Laurus nobilis* (Lauraceae, 1,8-cineole 28.1%) and *Piper nigrum* (Piperaceae, β -caryophyllene 45.7%).

All treatments produced a significant reduction in oviposition compared to the untreated control samples, with a prolonged protective action over time from the EOs-enriched chitosan solutions. The results of the trials will be discussed based on the different biology and oviposition behaviour of the four dipteran species, the potential repellent or attractive effect of the employed EOs, and the possible practical applications of the chitosan formulations enriched with EOs in fresh foodstuffs protection.

KEY WORDS: oviposition deterrence, *Calliphora vomitoria*, *Ceratitis capitata*, *Drosophila suzukii*, *Piophilidae casei*.

ORAL PRESENTATION



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Three years of investigations on *Toumeyella parvicornis* (Cockerell) infesting *Pinus pinea* owned by EUR SpA in Rome: the results of trunk injections, biological control and recovery of pine ecosystem services

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The spread of *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae) in urban areas is considered a major threat deeply related with the survival of the Italian stone pine (*Pinus pinea*) as iconic landscape element in cities like Rome and Naples. The threat posed by the pine tortoise scale forces authorities and agencies to be involved in the protection of this important tree species. In Rome the agency EUR SpA maintains 70 hectares of urban greenery with 40 tree species including about thousand *P. pinea* trees. To protect this pine species from the infestations of pine the tortoise scale EUR SpA and the Department of Agricultural Sciences - University of Naples Federico II activated a research collaboration. Through a multiple approach to the phytosanitary problem ecological aspects, natural enemies, and chemical approach have been evaluated in order to optimize the control of *T. parvicornis* aiming at the recovery of pine ecosystem services.

In 2020-2021 the efficacy and the persistence of trunk injection trials, carried out in spring and during fall, have been comparatively evaluated. Results showed that application of authorized insecticide products delivered with Arborjet® technique significantly reduced the infestation levels, allowing the trees to recover their vegetative capacity for at least 12-15 months, before a new outbreak of the scale insect was recorded. A sort of initial delay relative to translocation in the tree crown of the injected mix has been observed with low efficacy rate allowing the reproduction of the pest. In May 2022 a comparative trial started with the use of two endotherapeutic techniques (Nuovo Metodo Corradi®-NMC and Arborjet®) to assess the efficacy against the spring generation of the coccid. 70 days after treatment a clear difference between the two methods has been recorded. NMC controlled completely the evolution of the first generation of the tortoise scale with an efficacy rate (Henderson-Tilton) of 99.6% against a value of 60.8% showed by Arborjet®. At 170 days after treatment, the control rate remains high for NMC (95.7%) and an interesting growth in efficacy rate has been recorded for the second technique (88.4%). Investigations with Pressler drill have been carried out on pine tissues to observe if trunk injections or insecticide dose rate might have damaged tree conducting vessels. Long term monitoring of environmental climate parameters with data loggers is in progress to evaluate the impact of *T. parvicornis* on pine ecosystem services. A first inoculative biological control against the pine tortoise scale has been carried out during 2022 with the release of adults of *Cryptolaemus montrouzieri* Mulsant and *Exochomus quadripustulatus* (L.) without success. This negative result could be probably related with a high rate of dispersal flights showed by the predators. In alternative the release of larval stages of both ladybirds should be considered to enhance predatory behavior.

KEY WORDS: invasive species, pine the tortoise scale, urban green, trunk injections, biocontrol.

POSTER

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SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Contact toxicity of phthalate esters against *Sitophilus granarius* (L.) adults

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Phthalate esters (PAEs) are widely used as additives in many fields of industry due to their role as plasticizers; thus, PAEs can be found in a huge number of products as medical devices, toys, food containers, cosmetics, shampoos, and perfumes. This wide and increasing employment rises a serious environmental concern since PAEs can be easily released in the atmosphere, soil, and water. Moreover, PAEs exert significant biological activities being endocrines and nervous disruptors in mammals, besides showing phytotoxic and antimicrobial activities. However, little is known about PAEs effects on insects. Thus, in this study we investigated the contact toxicity of nine phthalates against adults of *Sitophilus granarius* (L.) (Coleoptera, Curculionidae), used as a model.

To this aim, dimethyl-phthalate (DMP), diethyl-phthalate (DEP), diisobutyl-phthalate (DiBP), di-n-octyl-phthalate (DnOP) dibutyl-phthalate (DIBP), dioctyl-phthalate (DOP) diisodecyl-phthalate (DiDP), benzylbutyl-phthalate (BzBP), diisononyl-phthalate (DINP) were solubilised in acetone (concentration range 37.5-600.0 mg/ml) and toxicity tests were performed by topical application (0.5 µl) of acetone solutions on the pronotum of adult weevils in thanatosis. For each compound, three replicates of 10 insects were used. Control consisted of acetone alone. Mortality values were registered 48 h after application and LD50 -LD90 were calculated for each PAE.

Except for DMP, all PAEs exerted toxic activity at the concentration range tested, although that of DEP was very faint (mortality < 30%). On the contrary, PAEs with longer hydrocarbon chains almost reached 100% mortality at the highest concentration tested, with significant mortality values starting from 75 g/adult for DOP, DnOP, DINP, DiDP, and DiBP. The calculated LD50 values were very similar among PAEs ranging from 65.2 g/adult for DOP to 89.6 g/adult for DiBP; the only exception was BzBP which showed a higher LD50 value, 127.0 g/adult. LD90 values ranged from 160.7 g/adult for DOP to 281.5 g/adult for DiBP; also in this case BzBP showed the highest LD90 values which was 416.7 g/adult.

In conclusion, these results showed that several PAEs have contact insecticidal activity toward adult granary weevils. Despite LD50-90 values were 15-20 folds higher than that of pyrethrin extract, the adulticidal effect of PAEs cannot be neglected, especially in the light of the increasing presence of these compounds in the environment. In this regard, their effect on beneficial insects (i.e., pollinators etc.), as well as the mechanism(s) of action and the evaluation of other ways of application (ingestion, inhalation) for PAEs represents an interesting item to be checked in future studies.

KEY WORDS: phthalate, biological activity, pollutants, granary weevil.



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Inhalation toxicity of *Eriobotrya japonica* (Thunb.) Lindl. seeds flour against *Sitophilus granarius* (L.) adults

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The seed of *Eriobotrya japonica* (Thunb.) Lindl. (Maloideae, Rosaceae), commonly named loquat, accounts for 20-30% of the fruit weight and represents a significant waste-product of its transformation process. In a circular economy view, this by-product could be used as a source of bioactive molecules against crop and stored-product pests. To evaluate the insecticidal activity of flours obtained from loquat seeds, inhalation toxicity tests were carried out against *Sitophilus granarius* (L.) adults, one of the main primary pests of stored cereals worldwide.

To this end, different flours were prepared by milling whole or peeled seeds, fresh or after drying at atmospheric pressure (40°C, 7 days) or under vacuum (40°C, -670 Torr., 2 days).

Flour aliquots (0.125 - 1.000 g) were placed in closed Petri dishes (polystyrene, diam. 9.0 cm x 1.5 cm; V= 85.1 cm³) containing adult insects (n = 20), wheat kernels as food, and a picric acid (0.6% v/v) plunged paper (35x27 mm) to quantitatively detect hydrocyanic acid formation. Three replicates for each sample were prepared. Toxicity values were determined by counting dead insects after incubation in the dark at 28±1°C for 24 h.

No insect mortality was measured for any flour in the absence of further addition. On the contrary, the addition of sulphuric acid (1.5 ml of a 0.01 M H₂SO₄ solution) to the flours resulted in dose-dependent mortality of the insects and HCN formation as shown by the progressive staining (R/G ratio, Apple digital colorimeter) of the paper inside the chamber. The released biological activity proved to depend on the method applied for obtaining the flour: in fact, the highest toxicity was registered for seed dried under normal pressure (LC₅₀ 373.33 mg/L; LC₉₀ 558.13 mg/L), whereas toxicity values were reduced for samples dried under vacuum (LC₅₀ 450.66 mg/L; LC₉₀ 636.80 mg/L) and completely negligible for flours obtained from ground seeds. Interestingly, toxic activity of dried seed flours remained unchanged 40 months after storage.

In conclusion, although in vitro experiments need to be confirmed under field conditions, results reported here strongly suggest that the flour of loquat seed could be an interesting source of bioactive compounds and a promising pest control tool. In particular, its use as a powder appears interesting to treat pests living in confined areas as well as in the soil (i.e., nematodes and arthropods).

KEY WORDS: loquat, inhalation toxicity, hydrogen cyanide, circular economy, storage pests, soil pests, botanicals, IPM.

POSTER



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Pests in edible insect rearing systems: state of art and possible treatment options

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In Europe, the insect farming industry as food and feed is exponentially growing and producers have to ensure the best farming practices.

However, pests can represent a hazard during the production process of insect farming, infesting and damaging reared insects, insect feed and substrates, infrastructures, materials and equipment of the insect facility. For this reason, every facility must have an efficient pest control program and management, targeting the specific pests of insect production (e.g., other insects, mites, spiders, rodents).

Among insect pests, mite infestation caused by different species (e.g., *Acarus sirus*, *Dermanyssus* spp., *Lakerbaueria* spp.) represent an emerging problem in different insect facility worldwide (Belgium, Czechia, Italy and Israel). Mites could cause both nuisance and stress in reared insects and allergy reactions in insect producers and could transfer pathogenic microorganisms through the facility to both insects, humans and insect-derived products.

In this context, few pest control methods (physical, environmental and chemicals) are effective and most of them are not allowed to face mite infestation. New treatment options and protocols need to be developed. Ozone (O₃) treatment has been applied in recent years as a reliable and sustainable sanitization tool. It offers the advantage of leaving no residues, due to the quick decomposition of its molecule.

Here we present the application of O₃ treatment to control mite infestation in farmed cricket (*Acheta domesticus*) oviposition substrate (soil) under laboratory conditions, used in an Italian insect facility (Italian Cricket farm). This experiment aimed to determine the efficacy of O₃ as mite killer, while preserving cricket eggs hatchability.

The fumigation with O₃ at the concentration of 10 ppm was performed in two applications (day 1 and day 5) under controlled environmental conditions (T° and RH), to kill the newborn mites that eventually hatched after the first treatment. Two different exposure times were applied: 8 hours and 5 hours on 10 substrates each. Each substrate was sampled in different points, both on the surface and in the depth. Each substrate, obtained by coring, was sampled at four time points: 1) before 2) after treatment 1, 3) before 4) after treatment 2.

Two different mite species were found, *Acarus sirus* and *Lakerbaueria* spp., infesting 95% of the substrate analyzed before O₃ treatment (>100mites/g). Two treatments of 8 hours each were needed to eliminate the *A. sirus* infestation. After cricket hatching, *Lakerbaueria* spp. specimen, more resistant to the O₃ treatment, were observed only on their surface. No adverse effects were observed on crickets hatching.

This study demonstrates that O₃ has the potential to be an efficient and safe method to control mite infestation from *A. domesticus* oviposition substrate without a negative impact on cricket hatching.

Further researches are needed to: i) list all the harmful insect pests; ii) identify all the effective control and treatment options; iii) assess the long-term effect of O₃ on insect progeny.

KEY WORDS: *Acheta domesticus*, mites, ozone, control methods.

POSTER



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Enhancement of *Lasioderma serricorne* (F.) pheromone traps through the use of new attractive molecules

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The cigarette beetle, *Lasioderma serricorne*, is an insect that represents a serious threat to food preservation, the tobacco industry, museums, and herbariums. The capture of this insect is usually carried out with glue traps lured with the sexual pheromone. The studies presented here aimed to identify practical co-attractants to be used in an industrial setting for more accurate monitoring and mass capture. Considering that chili powder (*Capsicum annuum*) is capable of exerting a clear attractive effect on *L. serricorne* adults, tests were conducted, both in the laboratory and in food industry, to characterize the volatile substances responsible for this attraction, with the aim of identifying the bioactive molecules. The powder from *C. annuum* fruits was therefore extracted in hexane for behavioral bioassays and analysis through gas chromatography techniques. The results of these experiments showed that the insect's attraction is particularly determined by the α -ionone and β -ionone molecules. Subsequent tests with the two molecules conducted in a food industry demonstrated that the addition of β -ionone to the pheromone allows for an increase in trap captures compared to traps baited with the pheromone alone. These results support the potential applicability of this molecule as a co-attractant for industrial use.

KEY WORDS: β -ionone, α -ionone; cigarette beetle; serricornin; semiochemicals.

SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Using *Acheta domesticus* powder for the bread and fresh pasta production

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In recent years, the interest in using insects for human consumption has become increasingly important worldwide in the search for alternative animal protein sources that can meet the growing demand of the population. The cricket *Acheta domesticus* is one of the species recently authorized for production and commercialization by European legislation. This species, due to its nutritional composition, is a good source of protein, as well as fatty acids, minerals and vitamins. The consumption of insects in Western countries is currently very limited due to consumer neophobia and low acceptability, but their inclusion within other foods, instead of the consumption of the insect as it is, could help to increase its acceptability and propensity to consume.

In the present study, we evaluated the technological properties of a wheat flour with different levels of inclusion of cricket powder for the production of fresh pasta and bread. In recent years, the search for new formulations to increase the nutritional profile, and in particular the protein profile, of certain foods is the subject of much research.

To characterize the dough, *Acheta domesticus* powder produced at DeFENS was mixed with wheat flour at different percentages: 5%, 10% and 20%. Hydration, foaming, emulsifying and rheological properties were evaluated.

Fresh pasta ("tagliatelle" type, length: 200 mm; width: 13.5 mm; thickness: 2.0 mm) was prepared using 5% or 10% cricket powder and 100% fresh wheat pasta was used as a control. Once cooked for 90 seconds, water absorption, cooking losses and firmness were evaluated. With regard to bread production, two levels of cricket powder inclusion were tested, 10% and 20%, and dough development, carbon dioxide production and retention, and crumb firmness were measured.

The inclusion of cricket powder in the dough, regardless of the percentage of enrichment, resulted in a reduction in both dough development time and mixing stability. Furthermore, with the addition of the cricket powder, an increase in the tenacity index and a reduction in extensibility were observed, thus leading to increased dough stiffness. The fresh pasta enriched with cricket flour had a higher water absorption and cooking loss than the control. With regard to pasta firmness, the 5% substitution level did not affect this index, whereas it decreased at the 10% substitution level. In the bread, the presence of cricket powder reduced the loaf specific volume, especially at the 20% inclusion level. When it was present at the lowest percentage (10%), on the other hand, the crumb softness was not affected either in fresh bread or during its storage. In conclusion, cricket powder can be used in the preparation of fresh pasta or bread without drastically worsening the quality of the final products, but it is important to appropriately define the optimal inclusion percentages, which may vary depending on the final food.

KEY WORDS: Entomophagy, crickets powder, rheological properties, impasto.

POSTER



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Residual toxicity and selectivity of anise and mint essential oil-based nano-formulations against stored grain pests

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Rhyzopertha dominica (F.) and *Sitophilus zeamais* (Motschulsky), are two key pests of stored grains able to induce extensive damage mainly to stored cereals. To date, conventional control of these pests is based on chemical control, but this is often the cause of negative effects on the environment and the health of the consumers.

Research has increasingly focused on the development of new natural solutions that meet criteria of eco-sustainability, low cost, good persistence and efficacy, and lower risk of resistance development. Biopesticides are substances that share all these advantages. These are classified as Biochemical Substances, of natural origin that can be applied for pest control and monitoring. These include essential oils (EO).

Essential oils (EOs) are secondary metabolites produced by plants with acknowledged insecticidal properties. These compounds, when formulated in nano-emulsions can increase their insecticidal efficacy, stability and persistence of the active ingredient.

Investigating innovative biological control techniques that are environmentally sustainable and with a reduced impact on human health, the experimentations aimed at the development of two nano-insecticides (nano-emulsions) based on anise (*Pimpinella anisum*) and mint (*Mentha x piperita*) essential oils for the control of *R. dominica* and *S. zeamais*. The bioactivity of the developed nano-emulsions was tested in residual toxicity trials, simulating the insecticidal treatments that are usually applied in real operative conditions. This application method was also used to assess the toxicity against a non-target insect, *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), a parasitoid wasp which can parasitize both the target species *S. zeamais* and *R. dominica*.

Both the developed nano-emulsions exhibited excellent physical characteristics in terms of droplet size and polydispersion index. Results of residual toxicity tests highlighted that the two developed formulations were effective in controlling both *R. dominica* as well as *S. zeamais*, despite the same also presented a toxic effect toward the non-target insect *T. elegans*.

The results confirmed the efficacy of the developed formulations against the target insects but, at the same time, the low selectivity towards the non-target insect poses some difficulties whether the two formulations might be used safely in proposed biological control programs.

ORAL PRESENTATION



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Attacks of *Kaloterмес flavicollis* in Palermo and associated *Aspergillus* species

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Among the termites of the Kalotermitidae family, one of the most primitive of the Isoptera and typical of damp wood, the species *Kaloterмес flavicollis* is known as the 'yellow necked dry-wood termite'. Recently, several nests of *K. flavicollis* have been found in public and private buildings and in two churches in Palermo, associated with attacks on both structural parts and artefacts. Their lucifugous behavior has made it difficult to identify the infestations early, underestimating the risk, up to the manifestation of damages. The identified colonies consisted of about 400-500 individuals, while the nuptial flights of males and females both fertile and winged were recorded in the months of October and November of the years 2019-2021.

During the survey, several dead individuals with evident fungal efflorescence were found. Therefore, a study aimed at the isolation and identification of these microorganisms was conducted. For this purpose, from the nests with infected individuals, samples of wooden material and insects were taken to be submitted to the usual laboratory analyses.

The first observations under the stereoscopic microscope have highlighted the presence of yellow-ochraceous conidic heads of *Aspergillus*, brought by long conidiophores emerging from the body of the insects. Therefore, direct and indirect isolations (serial dilutions) were carried out on the agarized nutrient substrate PDA. The grown fungal colonies were bred in purity and observed under an optical microscope for their morphological identification on the basis of macro- and microscopic features. The most recurrent colonies were subjected to DNA extraction and amplification of the ITS and β -tubulin genes.

Morphological and molecular analyzes allowed the identification of *Aspergillus nomius*, *A. subramanianii* and *A. tamarii*. In the literature, *A. nomius* has been reported on termites of the *Coptotermes formosanus* species, both as a saprophyte and as a facultative parasite, on insects subjected to some form of stress, while *A. tamarii* has been identified in association with those of the *Psammotermes hypostoma* species. In this study *A. subramanianii* is reported for the first time in association with termites. Further investigations will be able to provide useful indications on the possible ecological role that these fungi play, both singly and in consociation, on the vitality of *K. flavicollis* colonies, also with a view to developing targeted biocontrol strategies.

KEY WORDS: termites, *K. flavicollis*, *Aspergillus*.

POSTER

SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Quality control of *Pachycrepoideus vindemiae* (Hymenoptera Pteromalidae) and *Muscidifurax raptor* (Hymenoptera Pteromalidae) as pupal parasitoids for biological control of *Piophilidae casei* (Diptera Piophilidae) in ham productions.

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Ham products play a fundamental role in the Italian economy, and attention to the problems of this sector is essential. The products of this sector can be attacked by parasites, including arthropods, which can cause direct and indirect damage. The production of these foods takes place in environments characterized by constant thermo-hygrometric conditions, close to the optimum of many pest organisms. There is almost unlimited availability of pabulum with a high nutritional value that can favor the development of pests. For example, the curing of hams takes place in temperature-controlled rooms, between 14 and 25 °C, which is a favorable microclimate for insects. An example is *Piophilidae casei* (L.) (Diptera: Piophilidae) a parasite of cheese and meat, which is currently responsible for critical hygiene problems in ham factories. This pest is an excellent invader of food industries due to its characteristics: resistance to a wide range of temperatures, many generations per year, and high fecundity. The trophic activity of the larvae of this pest on the products causes serious direct damage. Unintentional human ingestion of the larvae can potentially lead to gastric and intestinal myiasis. Moreover, the adults of the fly are strongly attracted to protein substrates, even in an advanced state of decomposition. For this reason, *P. casei* is a potential vector of pathogens such as *Clostridium botulinum*.

Piophilidae casei management has relied on conventional methods like fumigation and residual spray treatment of the premises. Recent restrictive regulations on the use of chemicals for pest control in the food industry have left few alternatives for satisfactory control. The methods of control against *P. casei* must have certain requirements such as: having a low cost and that do not affect the taste and organoleptic qualities of the product.

In order to contain *P. casei* infestations, interest in developing biological control programs could raise. In particular, biocontrol with parasitoids against *P. casei* could represent a safe and sustainable method in comparison with pesticides. On the other hand, concerns have been raised about the potential contamination of stored products with living organisms, due to restrictive regulations and potential negative perceptions of consumers and processors. In this study, to further avoid contamination problems, we decided to focus on the biological control of pupal parasitoids, because of the behavior of *P. casei* to pupate outside the food. In this study, we investigate quality parameters such as the success rate of parasitism (SP), degree of parasitism (DP), sex-ratio (SR), and life-span (LS) of two pupal parasitoids of Diptera: *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae), currently the only known pupal parasitoid of *P. casei*, and *Muscidifurax raptor* (Girault and Sanders) (Hymenoptera: Pteromalidae).

Our research confirmed *P. vindemiae* efficacy to parasitize *P. casei* and reported, for the first time, *M. raptor* as a pupal parasitoid of this Piophilidae. The parameters investigated can be considered useful for pre-release evaluation and crucial for mass-rearing programs.

KEY WORDS: hams, parasitoids, biological control, quality control, stored product protection.

ORAL PRESENTATION



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Preliminary notes on entomological monitoring in institutions of Piacenza and Cremona (northern Italy) preserving items pertaining to cultural heritage

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The cultural assets kept by public and private bodies consist of a wide range of materials which when of organic origin are subject to the attack of hexapods which can significantly contribute to their deterioration. Other arthropods (parasitoid Hymenoptera, spiders), which are scarcely or not at all harmful, or indeed are potentially useful as natural biocontrol agents, are permanently established in the same protected environments, and still others are occasionally found as temporary or accidental presences. The knowledge of harmful organisms in the diversified environments in which this conspicuous heritage is kept is an indispensable premise for conservation primarily based on prevention, partly through constant monitoring, rather than on defense implemented by means of non-targeted disinfestation.

For this purpose, a qualitative-quantitative monitoring plan has been launched in the buildings of private institutions preserving items of cultural heritage, one located in Piacenza and two in Cremona (northern Italy). The three selected museums and archives hold tapestries, paintings, wooden artifacts, ancient books, historical documents on paper, parchment or other material, zoological collections. The research was agreed with the heads of the respective institutions with the additional aim of evaluating in advance the types of devices to be adopted as required, considering both the safety and protection of the goods present and on display, and the public. The investigation, carried out with the aim of ascertaining the presence of hexapods and other arthropods, followed the criteria set out in the European standard EN 16790:2016 – Conservation of Cultural Heritage – Integrated Pest Management (IPM) for protection of cultural heritage. In the two-year period 2021-22, adhesive traps and pheromone traps were placed in the rooms where the collections are kept to monitor Zygentoma, Blattodea, Psocodea, Coleoptera Dermestidae and Anobiidae, Lepidoptera Tineidae, arachnids as a whole. The positioning of the traps, and their periodic checking and replacement, when envisaged, were carried out regularly, including during the winter months, mostly on a fortnightly basis. This contribution illustrates the preliminary results for the monitoring of Zygentoma, carried out by means of small adhesive traps (adhesive surface 6 x 3.5 cm) placed on the floors and periodically replaced. Over the two-year period, these traps caught a total of about 500 arthropod specimens, including over 200 Zygentoma Lepismatidae (adults and juvenile forms), about 130 Psocodea (adults and juvenile forms), and about 20 Dermestidae (adults and larvae), as well as about 50 larval exuviae of the latter. Spiders, pseudoscorpions, mites and other hexapods (Collembola; Blattodea; Hemiptera Pentatomidae; Hymenoptera Chalcidoidea, Bethyloidea, Vespidae; Coleoptera Carabidae, Anobiidae, Ptinidae; Lepidoptera Tineidae; etc.) were far less frequent. Although most of the monitored rooms are not heated during the cold season, there were catches along the entire time frame of the samplings, although with lower numbers of specimens caught in winter.

KEY WORDS: museums, archives, cultural heritage pests, insects, Zygentoma, adhesive traps, Italian private bodies.

POSTER

XXVII ITALIAN NATIONAL CONGRESS OF ENTOMOLOGY

12 - 16 JUNE 2023, PALERMO

SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Pest control companies and bed bugs: a survey through interviews

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The bed bug has returned to being an increasingly aggressive pest in a diverse range of environments in recent decades. The control it is still a consistent commitment and we wanted to verify the state of the art by investigating the attitude and techniques used by the various companies carrying out questionnaires and interviews. At the moment, 22 companies distributed throughout the national territory have responded. The companies have stated that they have 3 to 22 years of bedbug control experience, with an overall average of 9.5 years. The company budget relating to these interventions ranges from 1 to 40%. The environments treated see in first place hotels (73% of cases per company), private houses (55), nursing homes (32), motor vehicles (18), reception centres (14), residences for the elderly (9), trains (9), dormitories (5) and hospitals (5%). Among the control tools, the use of steam with chemical product in specific points dominates (45%), followed by the use of only chemical products (36%), the primarily use of chemical and steam in specific situations (8), liquid nitrogen (9), mechanical treatments (5) and only steam (5%). Only 64% use post-intervention monitoring, mainly through traps. 36% of companies consider the use of dogs to trace bedbugs useful and report that in the majority of cases there is no seasonality in the request of interventions o it is linked to the arrival of tourists to hotels. 68% of the technicians have followed specific courses for bedbug control and 90% consider other updates important, especially on modern monitoring techniques and the use of low-impact products and techniques and more successful approaches. Results of the interview are therefore varied and all of them point to an increase in cases and to the need to increasingly less impactful but also increasingly successful way to work.

KEY WORDS: Bed Bugs, Pest Control Company, Survey.



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Moth monitoring results for a commercial dairy in northern Italy (Lepidoptera: Tineidae, Oecophoridae)

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Among the insect pests infesting dairy premises, some species of tineid, oecophorid and pyralid moths may be significant in long-term ripening rooms. With regard to the ripening of Grana padano DOP - like other dairy products with medium-long ripening - the significance of the tineid *Nemapogon granellus* (Linnaeus, 1758) seems to have increased in northern Italy especially in the last two decades in our experience; this species is often also reported to be harmful in the rooms where cured meats and sausages are seasoned because of its larval attacks on the product. Another species observed in the same dairy areas, though perhaps a little less widespread than *N. granellus*, is the oecophorid *Endrosis sarcitrella* (Linnaeus, 1758).

In order to further our knowledge of the ecology of these two species in these environments, we carried out research concerning above all annual trends in catches in the ripening room of a Lombard dairy located on the eastern Bergamo plain. The ripening room (T = 15-16°C, R.H. = 72-78%) houses exclusively Grana Padano DOP. The investigation was based on the examination of the yellow glue plates, which were replaced on monthly basis, of UV light traps (about 10) placed in the room at three different heights above the ground (floor-level; 1.5 m; 2.5 m). These are used for monitoring flying insect pests and to control the populations of these moths. The counts concerned the adhesive surfaces replaced between 2018 and 2021; overall, over 150 glue plates were examined. Given the similarity between the two species and the non-optimal conservation conditions of the moths on these surfaces, the identification of the adults (species and sex) was carried out on numerically significant samples by means of microscopic preparations of the abdomen.

The research revealed that: a) the only moth species identified were *N. granellus* and *E. sarcitrella*; b) the catches of adults of the two species, albeit with quantitative fluctuations (decrease in the winter months), were distributed without interruption throughout the entire year, with overlapping of their 'flights'; c) the number of catches of the two species was much greater with traps placed at floor level, which alone retained more specimens for each month than traps placed at greater heights considered jointly; d) the adults of *E. sarcitrella* were clearly numerically prevalent compared to *N. granellus*, with an overall ratio of about 2:1. Their prevalence was particularly high in catches with floor-level traps, where *Endrosis* percentages were recorded equal to 62-88% of the total adults of the two species.

Despite the prevalence mentioned above, larval attacks on ripening Grana padano DOP were only ascertained for *N. granellus*, whose larvae do not limit themselves to feeding on the surfaces of the wheels but dig short tunnels deep inside, with significant damage. The behavior of *E. sarcitrella* larvae and their possible harmfulness in the examined environment require further study. The research has highlighted the considerable utility of placing light traps at floor level for a more effective control of the adults of the two species.

KEY WORDS: province of Bergamo, ripening room, cheese, Grana padano DOP, *Nemapogon granellus*, *Endrosis sarcitrella*, light traps, yellow glue plates.

POSTER



SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

Integrated pest management of *Nemapogon granellus* using mating disruption

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The European grain moth, *Nemapogon granellus* (L.) (Lepidoptera: Tineidae), can infests several vegetal stored foodstuffs as grains, bulbs or dried mushrooms, and also stored animal products such as meats, sausages and cheeses. Recently it has also been reported on the corks of wine bottles.

Traditional treatments with chemical products cannot be carried out in the presence of maturing products, so alternative control methods are needed. In this study, the efficacy of mating disruption technique for the control of *N. granellus* populations in ham factories was evaluated.

The trials were conducted in two factories located in Northern Italy, where the lepidopteran species were present in the storage warehouses. Two warehouses were selected in both factories: in one (test) dispensers loaded with 10 mg of pheromone were positioned, at a density equal to 1 unit/22.5 m³ (factory A) and 1 unit/25 m³ (factory B), while the other (control) was not treated.

The efficacy of mating disruption was evaluated on the reduction in the number of mated females, captured in a plastic container filled with water up to a level of 8 cm, placed in the control and test warehouses. The results showed a significant reduction of mated females in the test warehouses compared to the control warehouses, in both ham factories. The total number of mated females was greater than 90% in untreated warehouses, less than 50% in warehouses with mating disruption. These results indicate that the use of pheromones to reduce the couplings is a technique that can be successfully applied in the integrated management of *N. granellus* in ham factories.

KEY WORDS: Stored-product pests, pheromones, ham factories, IPM.

ORAL PRESENTATION

SESSION X

COMMODITY TREATMENT AND URBAN ENTOMOLOGY

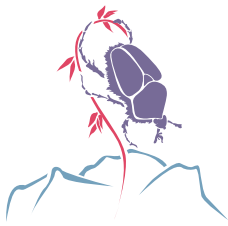
Changes in the distribution and pest risk of stored-product insects in Europe due to global warming

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Global warming affects the distribution of stored-product pest insects across Europe in a way comparable to field crop and orchard pests. Nevertheless, stored-product research has been neglected in Europe and detailed monitoring is lacking. This paper aims to illustrate current knowledge about the movement of storage pests up north today triggered by altered environmental conditions. In addition, it stresses the need for a pan-European surveillance to monitor the distribution, movement and spreading of stored product pests in a rapidly changing environment. Global warming and a growing number of extreme weather conditions may influence on climate and can negatively affect global food security, especially in the case of durable commodities, which are of fundamental importance for human nutrition. It is thus suggested that the distribution of stored-product pests within Europe is uniformly monitored and studied by a joint initiative. Furthermore, for additional food safety the World Food Program should receive more support to fund research needed and provide larger food storages in regions prone to agricultural instability. It is also suggested that the missing quarantine/regulated status for the most serious stored product and invasive pests should be re-evaluated in the EU.

KEY WORDS: Global warming, insect distribution, stored-products.



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P A L E R M O

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Session XI



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

PS1-70 and PS1-120 peptides reduce the damages produced by *Phthorimaea absoluta*, (Lepidoptera, Gelechiidae) infestation in tomato plants

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The leafminer, *Phthorimaea absoluta* Meyrick (Lepidoptera: Gelechiidae) is currently considered as one of the most devastating invasive lepidopteran pest of tomato crops, in both greenhouse and outdoor production. Native to South America, this Microlepidoptera has invaded more than 113 countries outside of South America becoming a serious threat to tomato production worldwide. *P. absoluta* larvae destroy all aerial parts of plants (leaves, stems, fruits, buds, and flowers), resulting in severe yield losses. In the Mediterranean basin, the most widespread control strategy against *P. absoluta* relies on chemical insecticides. Continuous overuse of pesticides may lead to adverse effects on non-target organisms, environmental contamination and to the development of resistance in populations, which is relatively common in this species. In the present context of growing demand of sustainable agriculture practices and healthy food, plant resistance inducers acting by stimulating plant defense mechanisms, are seen as an eco-friendly and promising option to conventional pesticides. Two protein fragments biologically active against different pests, namely PS1-70 and PS1-120 have been recently identified in the N-terminal region of Prosystemin (ProSys), the protein precursor of tomato Systemin, a very well-studied defense-signaling peptide. Here, we report the ability of these fragments to confer protection against *P. absoluta*. We demonstrated that plants treated with foliar spray of PS1-70 and PS1-120, were less damaged by *P. absoluta* attack. Larvae fed with treated leaves were severely impaired in their survival rate. This phenotype was associated with the upregulation of genes associated with the octadecanoid signaling pathway that is known to play a central role in plant defense against a wide range of biotic stressors. Moreover *P. absoluta* oviposition preference was significantly reduced by PS1-70 treatment. Such behavioral observation appeared to be associated with VOC profiles of PS1-70 treated plants. Our results showed that PS1-70 and PS1-120 are effective in the stimulation of tomato immunity and the consequent protection of the plants against *P. absoluta*.

KEY WORDS: Gene expression analysis, crop protection, peptide, *Solanum lycopersicum*, biotic stress, resistance inducers, leafminer, *Phthorimaea absoluta*.

POSTER



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Application of RNA interference, an innovative approach for controlling the invasive pest *Aethina tumida*, a new threat for European beekeeping

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The small hive beetle *Aethina tumida* is a species native to South Africa, endemic to sub-Saharan Africa, where its populations have African subspecies of *Apis mellifera* as natural hosts without serious consequences. In newly introduced areas where the hosts are the European subspecies of *A. mellifera*, it causes serious damage to beekeeping (destruction of combs, fermentation of honey, colony collapse), as a result of its biological cycle taking place within apiaries.

A. tumida was declared exotic invasive species of European Union (EU) concern according to EU Regulation 2016/429. In Italy it was first detected in the Calabria region in September 2014, where it is still present, though limited to the provinces of Reggio Calabria and Vibo Valentia. Due to its active and passive movement capabilities it poses a serious threat to neighboring territories, and to the entire EU. To contain its spread, restrictive measures were immediately applied by EU and are still in force [CID (EU) 2023/110], establishing a ban on the shipment of products to other areas of the Union (e.g., honeybees; bumblebees; unprocessed apiculture by-products; beekeeping equipment; honeycombed bee products for human consumption). Italy is obliged to conduct surveillance of hives and apiaries and epidemiological surveys and notify the Commission about the results. Additional appropriate measures might be implemented according to the outcomes of the surveillance plan. Although necessary, these measures lead to an economic burden on beekeeping activities, adding to the considerable damage caused by the infestations.

The purpose of this study was to test an innovative approach to control the spread of *A. tumida*, based on the mechanism of interfering RNA (RNAi), which, due to its inherent characteristics, can ensure efficacy and selectivity while avoiding negative effects on the bees themselves.

Double strand RNAs (dsRNAs) synthesized to interfere with the expression of two genes essential for the development of *A. tumida* (Ribosomal protein S13 - RPS13 and V-ATPase) were tested on young larvae. Doses of 3.7 µg (RPS13) and 4 µg (V-ATPase) administered orally via artificial diet induced antimetabolic effects by affecting beetle survival, development and reproduction. Specimen that survived dsRNA feeding showed a significant decrease in growth rate, a slowing of the biological cycle, and a significant reduction in fertility.

Topical administration of the dsRNAs at lower doses (370 ng of dsRNA against RPS13 and 517 ng of dsRNA against V-ATPase) did not cause the same negative effects on larval development; however, a significant reduction in fertility was recorded in surviving adults after treatment with each dsRNA.

To check for possible target sites in the bee, a bioinformatic analysis was performed by comparing the sequences of the two dsRNAs used for the experiments with the bee genome. The results indicated the absence of homology for sequences of length > 21 base pairs, the range required to effectively activate silencing.

KEY WORDS: gene silencing, Small hive beetle, *Apis mellifera*, bee health, biopesticides.

ORAL PRESENTATION

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

***Galleria mellonella* the plastic-eater insect, source of molecules useful in the biodegradation of polyethylene and polypropylene**

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One of the major environmental issues facing modern society is the growing amount of plastic waste and their accumulation in the environment. Finding an environmentally friendly solution is one of the main challenges humankind is bound to face in the 21st century since the actual methods for plastic disposal (mostly mechanical recycling, incinerators and landfilling) are not viable in the long way run. Insects could represent a new green way to dealing with plastic waste and could be used as potential plastic degraders due to some Coleoptera and Lepidoptera larvae's ability to break down plastics like polyethylene (PE), polypropylene (PP) and polystyrene (PS). One of the quickest insects known to date in degrading plastics is represented by the larvae of the lepidopteran *Galleria mellonella*, popularly known as greater wax worms, which may chemically change PE by oxidizing the polymer within a few hours of exposure. According to the previous studies, two hexamerin/phenoloxidase superfamily enzymes discovered in wax worms' saliva were found to be active on PE by oxidizing it. Those molecules were renamed PEases and are now known as Demetra and Ceres. In order to better investigate the effects of these active molecules on the PE and their role in the development of the insect larvae, we decided to silence these genes using the method of RNA interference (RNAi); this is a well-known phenomenon whereby long dsRNAs specifically suppress the expression of a target gene. So, by injecting this specific dsRNA directly into the larvae hemocoel we can under-express the genes responsible for the production of these PEases. Effective silencing is confirmed by qPCR, using 18s gene as housekeeping gene and an Odorant Binding Protein (H.ill_OBP_C11107) from *Hermetia illucens* as negative control. Saliva fluid is then collected by placing a mouth pipet and glass capillary at the buccal aperture and applied on PE films in order to evaluate the biodegradation effect.

POSTER



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Gene-family analyses for the invasive species *Popillia japonica* (Coleoptera, Scarabaeidae) obtained from de novo sequencing of specimens introduced in Italian localities

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Popillia japonica Newman (Coleoptera: Scarabaeidae) is a beetle endemic of Japan unintentionally introduced in North America (1916), the Azores Islands (~1970) and, recently, northern Italy (2014) and Swiss Ticino (2017) (EPPO, 2022). *Popillia japonica* is an A2 quarantine organism for EPPO (EPPO, 2022) and a potential Union quarantine pest by EFSA's criteria since it feeds on over 700 plant species causing serious injury (EFSA Panel on Plant Health, 2018).

The aim of our project includes genome and transcriptome analysis of the pest. The genome analysis consists in extraction, sequencing, and de novo assembly of the whole beetle genome with the aim to studying specific gene families which may be functional for the species invasion. In particular, major efforts have been directed to study insecticide resistance genes, sexual differentiation genes and odorant receptor genes. The final genome assembly has a total length of 578 Mb with a N50 of about 0.89 Mb, composed of 28,437 single genes encoding for 31,483 splice variants. The assembly has a BUSCO completeness of 95.7% (single 94.5%, duplicated 0.8%), 1.7% fragmentation and 2.6% missing genes. These results were compared with other sequenced beetles.

The transcriptome analysis, in addition to sequencing and de novo assembly of the whole RNA component, will help, in the near future, the study of gene expression in different life stages (larvae, pupae, male and female adults) and in treated versus untreated specimens. Differentially expressed genes (DEGs) across stages were preliminarily identified using the quasi-likelihood (QL). The principal component analysis (PCA) showed similarities between the replicates and differences between the four life stages. The gene ontology analysis reveals that most of the genes that we obtained are typical of Scarabaeidae and Coleoptera and classifies the resulting genes according to biological processes, cellular components, and molecular functions. At the time of writing, the genome assembly is in a draft stage and annotation is very preliminary.

However, from the combination of genome and transcriptome results, the sequences of the genes involved in the gene regulatory network (GRN) of sexual differentiation (*dsx*, *sxl*, *tra* and *tra2*) were extrapolated. Primers for reverse transcription-polymerase chain reaction (RT-PCR) (already designed) will allow us to identify how these genes differentiate in the two sexes.

Cytochrome P450 family gene associated with resistance to pesticides in other invasive beetles has been identified in the *P. japonica* genome and will soon be analysed with the same approach applied for sexual differentiation genes.

KEY WORDS: *Popillia japonica*, Invasive Species, Genome, Transcriptome, de novo sequencing, Gene Ontology, Gene families, Cytochrome P450, Sexual differentiation.

ORAL PRESENTATION

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Salivary gland transcriptome of *Varroa destructor* reveals suitable targets for dsRNA-mediated control of the mite

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Varroa destructor is a parasitic mite of honey bees and a major driver of honey bee colony losses. By feeding on the host's body fluids, this obligate ectoparasite has a strong impact on honey bee physiology, causing the reduction of weight and longevity, and the spread of viral pathogens. Despite its importance, many aspects of *Varroa* lifestyle are still obscure. During feeding activity on the host, the mite injects salivary secretions only partly identified so far. Here we performed a differential expression analysis between the transcriptome of salivary glands and the rest of the body of *V. destructor*, identifying 12 transcripts encoding putatively secreted proteins. Using databases such as InterPro, UniProt and NCBI, we functionally annotated this secretome, which includes proteins belonging to cysteine protease, serine protease, endochitinase and phospholipase families. Selected genes were subsequently studied from a functional point of view, by analyzing their impact on mite survival following gene silencing. Mites were soaked in dsRNA solutions targeting chitin-binding, kazal serine protease inhibitor and papain cysteine protease, for a few hours and maintained on honey bee pupae under laboratory conditions. The effect on mortality was checked daily, while gene silencing was assessed at 72 hours after treatment, by qRT-PCR. The dsRNA mix targeting the three genes simultaneously resulted effective in reducing mite survival compared to controls. Further functional characterization of these salivary components will offer new knowledge on the molecular basis of *Varroa*-bee interactions, on which to develop innovative and eco-sustainable mite control strategies.

KEY WORDS: RNAi, honey bee, host regulation, colony losses.

POSTER

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Plant immunity inducers: new peptide motifs from Prosystemin to face plant stressors

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Chemical pesticides represent a pivotal tool in agriculture to control pest agents and secure both quality and yield in plant production. Nevertheless, their extensive use in crop protection led to a widespread toxicity that involved also non-target organisms as well as the surrounding environment, endangering ecosystems sustainability. Therefore, the increasing demand for food products low or free in pesticide residues, spurred the scientific community to develop more effective, sustainable, and eco-friendly solutions for pest control. Promising biotechnological strategies include molecules that act as plant immunity inducers, known as elicitors. Among them, tomato Systemin (Sys) has often been the focus of attention as a key component of plant resistance phenotype towards a wide range of stressors. This 18-amino acids peptide is released upon wounding from the C-terminal end of its 200 amino acids precursor called Prosystemin (ProSys), activating an array of local and systemic defenses. However, ProSys is not only a mere scaffold for the Sys peptide, but itself contains other biologically active regions. In addition, the defence mechanism underpinned by the protein is likely linked to the intrinsic disorder of its sequence that may have its own biological function beyond the release of Sys. Indeed, our recent findings suggests that ProSys hides other functional peptide motifs able to activate multiple stress-related pathways. To contribute to this knowledge, we identified and produced different synthetic peptides derived from ProSys precursor that when exogenously supplied to tomato plant, induce defense-related genes protecting plants against necrotrophic fungal pathogens such as *Botrytis cinerea* and chewing insects like *Spodoptera littoralis* larvae. Larvae fed on treated leaves are unable to reach the required weight to moult, dying either during moulting or shortly afterwards. It is intriguing to notice that dying larvae exhibit a strange "albino" phenotype, remaining contracted and trapped in the old cuticle and lacking sclerotization of the new cuticle. Since peptides act only on plant defenses rather than directly on larvae, the observed unusual phenotype is possibly linked to the induction of genes that promote the production of substances with insect hormone-like activity that impact on larvae development. Overall, our data open new perspectives on ProSys functionality, since it contains multiple biologically active sequences that may be novel exploitable tools in crop protection programs.

KEY WORDS: small peptides, sustainable agriculture, biotic stress, plant elicitors, *Spodoptera littoralis*.

POSTER

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Activation of anti-herbivore defences in tomato plants against *Halyomorpha halys*

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Plants can recognise attacks by invasive arthropod herbivores and activate appropriate defences to reduce damage. After an oviposition event or feeding by adults, elicitor or effector compounds induce direct defences (antixenosis and antibiosis), with possible effects on offspring development. However, the degree of plant response and the type of activated pathway after a herbivore attack can vary with the plant species. Therefore, understanding how plant species differently modulate herbivore-induced defence responses is important for the development of management strategies based on improving plant defences. *Halyomorpha halys* is an invasive stink bug now established in many European and American agroecosystems, where it is responsible for severe crop losses. Attacked broad bean plants can recognise *H. halys* presence and activate direct and indirect defences against the herbivore. Here, we investigated whether tomato plants attacked by *H. halys* females, ovipositing or feeding on the plant, can impair the development of offspring through the activation of induced direct defences. We then challenged attacked and control plants with newly emerged *H. halys* nymphs, and found a lower weight in nymphs developing on plants previously subjected to *H. halys* female feeding or oviposition, compared to those that developed on control plants. Molecular analyses revealed a higher expression of two jasmonic acid-dependent genes in plants exposed to oviposition or feeding and subsequently challenged by nymphs. Expression of proteinase inhibitor genes increased only in plants with an oviposition event after 72 hours since nymph feeding. Our results indicate the activation of direct defences in tomato plants exposed to *H. halys* oviposition or feeding with consequences on offspring development.

KEY WORDS: induced plant defence, stink bug, invasive species.

ORAL PRESENTATION



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

RNAi-mediated silencing of an immune gene of *Spodoptera littoralis* (Lepidoptera, Noctuidae) alters its embryonic development

Gennaro Volpe¹, Ilaria Di Lelio¹, Andrea Becchimanzi¹, Niccolò Baranzini², Daniele Bruno², Gianluca Tettamanti², Silvia Gigliotti³, Francesco Pennacchio¹

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The immune gene named 102 is highly expressed in haemocytes and encodes a precursor protein that gives rise to amyloid fibrils essential for the cellular immune response in both *Heliothis virescens* and *Spodoptera littoralis* noctuid moths (Lepidoptera, Noctuidae). RNAi-mediated silencing of this gene (SI102) in *S. littoralis* larvae results in an immunosuppressed phenotype that increases the susceptibility of this pest to pathogen infections and provides the basis for developing new insect control strategies. Here, we investigate the effects of SI102 gene silencing on embryonic development, by soaking *S. littoralis* eggs in a dsRNA solution. Time-course analysis showed that the SI102 gene is expressed throughout embryonic development, from egg laying to hatching, and the transcript levels are strongly reduced after treatment with specific dsRNA molecules (dsSI102), compared to control embryos treated with dsRNA targeting GFP (dsGFP). Interestingly, the gene silencing is associated with a drastic reduction in egg hatching and a very high mortality rate of newly hatched larvae. Structural and ultrastructural analyses showed a dramatic delay in the development of silenced embryos, which presented morphological alterations that point out an important role played by the SI102 gene during embryogenesis. This study suggests that targeting eggs for disrupting embryogenesis represents a promising strategy for Noctuidae control. Further studies will be aimed at understanding SI102 role in embryogenesis and developing a proper delivery system for dsRNA targeting lepidoptera eggs.

KEY WORDS: noctuid moths, insect control, dsRNA, morphological alterations, embryogenesis.



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Evaluation of antimicrobial activity of lipids extracted from *Hermetia illucens* reared on different substrates

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As the issue of antimicrobial resistance is constantly getting worse, there is a new interest in antimicrobial solutions deriving from natural sources, especially those made from cutting-edge and environmentally friendly materials. Insect lipids can be categorized as naturally occurring antimicrobial compounds. Indeed, due to their fatty acid composition, they may represent a new source of antimicrobial molecules in response to the need to find and use new molecules to fight antibiotic resistance. In order to assess the antibacterial efficacy of *Hermetia illucens* lipids, this component was extracted from the larval stage and characterized. Particularly, lipids from larvae fed on different substrates were extracted, also in order to determine if and how the feeding substrate affects the antimicrobial activity. Following the extraction by Soxhlet method, antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria. As differences in antimicrobial activity were detected among larvae fed on different substrates, the specific fatty acid composition was analyzed by gas chromatography, in order to understand if this component varies according to the substrate. Statistically significant differences were detected in five fatty acids between lipids showing antibacterial activity and lipids not showing any activity. Literature data reports undeniable antibacterial activity of these fatty acids, confirming the hypothesis that they can contribute to the antimicrobial activity of *H. illucens* lipids.

ORAL PRESENTATION



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

**Evaluation of antimicrobial activity of lipids extracted from Yellow Mealworm
*Tenebrio molitor***

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Antimicrobial products deriving from natural sources, especially those made from new sources and environmentally friendly ingredients, are gaining more and more attention since the problem of antimicrobial resistance has become worse in recent decades. Due to the nature of their fatty acids, insect lipids can be categorized as naturally occurring antimicrobial substances. The purpose of this study was to investigate the antibacterial characteristics of crude insect fat of the coleopteran *Tenebrio molitor*. Lipids were isolated by Soxhlet extractor from *T. molitor* larvae, fed with standard diet (wheat bran and fresh vegetables for hydration), and adults and tested against *Escherichia coli* and *Micrococcus flavus*. Lipids from larvae showed antibacterial activity against *Micrococcus flavus*, whereas no samples had any activity on *Escherichia coli*. In order to understand if the specific fatty acid composition can influence the different antimicrobial activity, samples were analyzed by gas chromatography. The antibacterial activity shown during the trials, could be attributed to the most detected fatty acids (oleic, linoleic and stearic acids), that are reported to have antibacterial effects alone or combined with each other.

POSTER



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Use of chitosan nanoparticles obtained from the bioconverter insect *Hermetia illucens* for the delivery of controlled-release drugs

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Chitin, the structural component of the arthropod exoskeleton and the cell wall of fungi, can be deacetylated into chitosan, its most soluble derivative. Due to their biodegradability, biocompatibility, non-toxicity, antioxidant and antimicrobial activities, chitin and chitosan are used in various fields. The main source of chitin is the waste from the fishing industry, chiefly crustacean shells. Insects are a valuable alternative source to crustaceans, and in particular the bioconverter dipteran *Hermetia illucens*. Chitosan, with its high versatility related to better solubility and enzymatic degradation resistance, is a very safe, available and economical coating biopolymer for the production of nanoparticles (NPs). The increasing progress of nanotechnology has affected all fields of science and the great interest raised by NPs in the scientific sphere is related to their structural, chemical, mechanical, magnetic, electrical and biological properties. NPs can be defined as submicron colloidal drug carrier systems, composed of natural or artificial polymers of 10-100 nm. They allow continuous and controlled drug release, site-specific targeting and high surface-to-volume ratio, improving drug efficacy and safety. Microemulsion, ionotropic gelation, emulsion solvent evaporation and emulsion solvent diffusion are common methods for obtaining chitosan-based NPs. The molecular weight and degree of acetylation influence the NP size and surface charge. Some of the mechanisms involved in trapping drugs in the polymer matrix are electrostatic interaction, hydrogen bonding and hydrophobic interactions. Drug loading and release are not the only fundamental characteristics. The envisioned use of the NPs and the physiological environment at the site of administration must be taken into consideration. Among various polymers, chitosan-based drug delivery systems are gaining increasing interest as vehicles capable of releasing their active ingredients at the desired rate and body site. Chitosan-coated NPs can incorporate, through different preparation methods, different types of drugs and be used to transport small molecules, proteins, peptides, vaccines, genes, and oligonucleotides. Chitosan NPs are soluble in acidic aqueous solution, biodegradable, stable, non-toxic, biocompatible and easy to prepare. They do not interfere with the active ingredients they carry and have no side effects. In this perspective, chitosan produced from the innovative and sustainable source *H. illucens* can be used for the production of chitosan NPs. Preliminary experiments were carried out by dissolving chitosan produced from *H. illucens* in a solution of acetic acid, adding tween, glycerol and sodium tripolyphosphate (TPP). The formation of chitosan-TPP NPs proceeded through the cross-linking mechanism of TPP. This technology is of particular interest and importance for the use of NPs in the biomedical and pharmaceutical fields.

POSTER

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Interactions between leafhopper vector and phytoplasma membrane proteins affect Flavescence dorée acquisition

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Phytoplasmas are phloem-limited plant pathogenic bacteria that cause diseases in hundreds of plant species, producing severe economic losses to crops worldwide. Phytoplasmas are transmitted by hemipterans, belonging to the suborder Auchenorrhyncha (Fulgoromorpha and Cicadomorpha) and the family Psyllidae. Once acquired by an insect, phytoplasmas must cross the midgut membrane, spread into the hemolymph, and colonize the salivary glands to be transmitted with saliva to another plant. Since phytoplasmas are wall-less bacteria, membrane proteins are involved in the internalization process within the host cell. In the present study, the interaction of the immunodominant membrane protein (Imp) of Flavescence dorée phytoplasma (FDp) with vector proteins was investigated. The FDp is a grapevine quarantine pest and a major threat to the viticulture of several European regions. Control of the disease relies on several measures including compulsory insecticide treatments to reduce vector population, roguing of infected plants and pruning of symptomatic vegetation to minimize the inoculum source, hot water-treated grafted cuttings to replace missing plants. These strategies are costly, affect the health of the environment and of wine growers, and raise concerns on insecticide residues in the final product. *Scaphoideus titanus* is the main natural vector of FDp to grapevine, whereas *Euscelidius variegatus* is commonly used as laboratory vector. The external domain of FDp Imp selectively interacts with proteins from vector species rather than those from non-vectors. Nevertheless, the identity of the insect vector proteins interacting with FDp Imp is still unknown. Imp C-terminus domains of two FDp strains (16SrV-C and -D), covering the whole portion of the protein expected to be exposed outside of the bacterial cell, were synthesized together with His-tag, using an optimized *Escherichia coli* expression system. Pull-down assays with the newly synthesized FD Imps (as bait) and extracts of insect gut proteins identified similar interacting partners for both vector species. The interacting insect proteins were analysed by mass spectrometry and identified based on the appropriate *S. titanus* and *E. variegatus* protein datasets, according to NGS transcriptomes. Five *E. variegatus* proteins interacting with Imp were further characterized by measuring expression of their corresponding transcripts in different insect tissues and in healthy vs infected insects. In the next step, the same genes were silenced by abdominal microinjection of cognate dsRNAs, to evaluate any effect on phytoplasma acquisition. The specific silencing of two of them, namely legumain and natterin-4-like, resulted in significant reduction of phytoplasma multiplication in treated insects fed on infected plants. This work is a step forward to elucidate mechanisms of FDp infection of the vector, and identifies potential targets to disrupt transmission, providing a new tool for the integrated control of FD.

KEY WORDS: *Euscelidius variegatus*, *Scaphoideus titanus*, immunodominant membrane protein.

ORAL PRESENTATION



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Evaluation of the biological activity of two attacins from the stick insect *Carausius morosus*

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Insects produce a wide repertoire of antimicrobial peptides (AMPs) as crucial effectors of the innate immunity. AMPs from insects include several families which can be classified according to their structure and/or function. Attacins are a rather heterogeneous group of AMPs, rich in glycine residues, and active mainly against Gram-negative bacteria. Herein, sequences of two putative AMPs, named Cm3610 and Cm3021, belonging to the attacin family, were identified *in silico* from the transcriptome of the stick insect *Carausius morosus*. After *in vivo* immuno-stimulation with bacterial and fungal elicitors, qPCR analysis indicated significant higher expression of these peptides in *C. morosus* fat bodies, Malpighian tubules, brain, gut and hemocytes, compared to unstimulated specimens. The Cm3610 and Cm3021 mature region was synthesized, and the specific bioactivity evaluated *in vitro*. The membranolytic activity of the peptides was investigated on Gram-negative (i.e., *Escherichia coli*) and Gram-positive (i.e., *Bacillus cereus*) bacterial models: Cm3610 altered the permeability of membrane in *B. cereus*, while Cm3021 was not effective on either *E. coli* or *B. cereus*. Neither peptide showed any haemolytic activity against mammalian erythrocytes. Moreover, the individual and combined effects of the peptides was evaluated towards primary human fibroblast (FB789) and murine melanoma (B16F10) cell lines. Of note, Cm3610 peptide exhibited significant cytotoxic activity against the B16F10 cells, without affecting FB789 viability. Otherwise, Cm3021 peptide significantly decreased the viability of both cell lines. The combined exposure of cells to Cm3610 and Cm3021 peptides did not cause any synergistic cytotoxic effects. These results open interesting perspectives on the possible application of Cm3610 peptide for biomedical applications.

KEY WORDS: antimicrobial peptides, *Carausius morosus*, Attacins.



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Evaluation of the antitumoral activity of *Hermetia illucens* hemolymph

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The use of conventional chemotherapy agents is often associated with deleterious side effects caused by involuntary damage to healthy cells and tissues. Moreover, cancer cells often become resistant to chemotherapy due to increased expression of detoxifying enzymes and drug carriers and defense factors involved in reducing the concentration of intracellular drugs, alterations in the interactions between the drug and its target, increased ability of the cell to repair DNA damage or tolerate stress conditions and defects in apoptotic pathways. Some general solutions to drug resistance are based on: early detection of tumors; adaptive monitoring during therapy; addition of new drugs and improved pharmacological principles that result in deeper responses, as well as the possibility of combined therapy; integration of clinical-genomic data and computational modelling; the use of molecules with specific and targeted activity on cancer cells to reduce any long-term and off-term toxic effects of therapies on healthy cells. Since 1957 the interest in the study of peptides with anticancer activity has increased exponentially and numerous studies have shown that some of the antimicrobial peptides (AMPs), have a wide spectrum of cytotoxic activity even against cancer cells and are called ACPs, anticancer peptides. AMPs are small molecules of the innate immune system of a wide range of organisms characterized by a sequence of amino acids usually from 5 to 50 residues, high hydrophobicity and the net charge ranging from -5 (anionic) to + 10 (cationic), the most widespread. Like bacteria, cancer cells have a net negative charge due to their high expression of anionic molecules compared to unprocessed cells, allowing electrostatic interactions between ACPs and the surface of many cancer cells. In addition, the fluidity of the membrane and the presence of microvilli on it are typically increased in cancer cells compared to their healthy counterparts, which can facilitate the destabilization of the membrane of cancer cells and the greater anchoring surface by the ACPs.

In addition, the mitochondria of eukaryotic cells are negatively charged and have a highly negative transmembrane potential and once inside the cell, ACPs can disrupt the integrity of mitochondrial membranes resulting in the release of several apoptosis stimulating proteins into the cytosolic compartment. Antimicrobial peptides are produced by all organisms, but insects are among the richest and innovative sources of AMPs. The aim of this work is to identify, starting from the insect *Hermetia illucens* (Diptera, Stratiomyidae), pharmacologically active principles of natural origin, to be used for the development of alternative anticancer drugs or in support of conventional therapies already in use.

The hemolymph was extracted from the larvae of *H. illucens* and precipitated with organic solvents in order to separate the peptides of interest from proteins of higher molecular weight. The activity of the hemolymphatic extracts was evaluated by experiments on colorectal carcinoma cell lines treated with different dilutions of samples.

POSTER



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Insecticide activity of a polydnavirus protein

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Polydnaviruses (PDVs) are symbiotic viruses of ichneumonid wasps parasitizing lepidopteran larvae, that are co-injected with the egg and venom into the body of the host insect during parasitization. Viral particles infect host' tissues, without undergoing replication, and express virulence factors such as peptides and proteins that alter host physiology to increase its suitability for the developing parasitoid progeny. We have extensively studied the PDV associated with the parasitoid *Toxoneuron nigriceps* (Hymenoptera, Braconidae) and discovered that one of its virulence factors (TnBVANK1) is orally toxic to *Spodoptera littoralis* (Lepidoptera, Noctuidae). Indeed, this viral protein sticks on the surface of the midgut epithelium and interferes with absorption of nutrients. Here we further explore the oral insecticide activity of this protein on a sap feeder, the aphid species *Acyrtosiphon pisum* (Hemiptera: Aphididae). Recombinant TnBVANK1 was produced in the yeast *Pichia pastoris*, purified by affinity chromatography, and administered to aphids *ad libitum*, via a liquid diet offered in parafilm sachets. A significant increase of aphid mortality was observed over time, showing a clear dose-dependent response. The morphological analyses conducted by means of optical and transmission electron microscopy (TEM) showed an extensive alteration of the midgut epithelium, characterized by severe symptoms of cellular disruption. The mechanism of action is currently being explored. These results indicate that TnBVANK1 is a good candidate for the development of new bioinspired technologies for aphid control, which at the present are very limited.

KEY WORDS: virulence factor, *Toxoneuron nigriceps*, ankyrin, biopesticides, aphid control.

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

The role of *Aphidius ervi* venom in regulating host nutritional suitability

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Parasitoid wasps have evolved complex strategies to exploit and regulate the physiology of their hosts and enhance their suitability for the development of their progeny, which are modulated by host regulation factors both of maternal (venom) and embryonic (teratocytes) origin. The pea aphid, *Acyrtosiphon pisum* (Homoptera, Aphididae), and its endophagous parasitoid wasp, *Aphidius ervi* (Hymenoptera, Braconidae), represent a unique model system to investigate the molecular mechanisms underlying the interactions between parasitoids, their hosts and primary symbionts. In this study, we focused on the functional role of Ae- γ -glutamyl transpeptidase (Ae- γ GT), the most abundant component of *A. ervi* venom which is known to induce host castration. Using RNA interference technique, we knocked down Ae- γ GT gene in newly emerged female wasps, using microinjections of double-stranded RNA (dsRNA) in the pupal stage of the parasitoid. These females were used to observe phenotypic changes in parasitized hosts and the parasitoid's progeny, as affected by a venom blend lacking Ae- γ GT. We found that Ae- γ GT silencing prevented the aphid castration and enhanced the growth of both the host and parasitoid, which was supported by a higher load of the primary bacterial symbiont *Buchnera aphidicola*. However, emerging adults exhibited reduced survival and fecundity, suggesting a trade-off with body size. This demonstrates the primary role of Ae- γ GT in host ovary degeneration and suggests that this protein counterbalances the proliferation of *Buchnera* likely triggered by other venom components. Our study offers a new approach to unravel the complexity of aphid parasitoid venom in vivo and sheds light on a previously unknown role for Ae- γ GT in the host regulation process.

KEY WORDS: Host-parasitoid interaction, RNAi, Biological control, *Acyrtosiphon pisum*.



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Identification and Functional Characterization of *Toxoneuron nigriceps* Ovarian Proteins Involved in the Early Suppression of Host Immune Response

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Toxoneuron nigriceps (Viereck) (Hymenoptera, Braconidae) is an endoparasitoid of the larval stages of *Heliothis virescens* (Fabricius) (Lepidoptera, Noctuidae), the tobacco budworm. During oviposition, *T. nigriceps* injects into the host body the egg, the venom, the calyx fluid, which contains a Polydnavirus (*T. nigriceps* BracoVirus: TnBV), and the Ovarian Proteins (OPs). OPs, originating from the ovarian calyx cells, are involved in the induction of precocious symptoms in the host immune system alteration. Indeed, although viral gene expression in the host reaches detectable levels within a few hours, the host metabolism and immune system are disrupted shortly after parasitization. To functionally characterize the OPs, we carried out two approaches. Firstly, incubating hemocytes with OPs in toto, detecting several alterations on host cells: the OP injection induced an extensive oxidative stress and a disorganization of actin cytoskeleton. These alterations can explain the loss of haemocyte functionality (the reduction in encapsulation ability by the host) and the high-level of haemocyte mortality. Then, we evaluated the effect of HPLC fractions deriving from in toto OPs. Among the 28 analyzed fractions, two fractions caused a reduction in hemocyte viability and were tested to detect changes in hemocyte morphology and functionality. In accordance with previous results, obtained with in toto OPs, the two fractions caused severe oxidative stress, actin cytoskeleton disruption, loss of hemocyte encapsulation ability and high mortality rate. Moreover, a transcriptome and proteomic approach was applied to identify the proteins and particularly the proteins of the two fractions: eight proteins that might be involved in the observed host hemocyte changes were detected. Our findings will contribute to a better understanding of the ovarian components and their role in parasitic wasp strategy to escape the host immune responses.

ORAL PRESENTATION



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Usage of insect-based chitosan for the preservation of fresh cherry tomatoes

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Chitin, the second most abundant biopolymer on Earth, is the main structural component of the arthropod exoskeleton and the cell wall of yeasts and fungi. Nowadays, the main commercial source of chitin, the crustacean exoskeleton, is no longer a sustainable source. For this reason, insect-derived chitin has received increasing attention, as an alternative and more sustainable source. Among insects, the Diptera *Hermetia illucens* is a very promising source of this biopolymer as larvae can feed on organic waste and bioconvert it in larval biomass, and at the same time, breeding waste (pupal exuviae and dead adults) can be used as source of chitin. Chitosan, its main deacetylated derivative, due to its antimicrobial, antioxidant and film-forming capacity, is one of the most promising natural polymers for use as edible coating. Chitosan-based coatings reduce dehydration and retard microbial and fungal spoilage by acting as barriers, able to delay ripening and senescence. These coatings produced by chitosan crustaceans have already been successfully used on a variety of fresh fruit and vegetables.

Due to its content of healthy compounds, tomato (*Solanum lycopersicum*) is one of the most consumed and cultivated fruits worldwide. The packaging of tomatoes is a key element in their post-harvest preservation, as they are perishable climacteric fruits. Indeed, tomatoes, in the post-harvest period, continue to undergo biochemical ripening and respiration mechanisms, which affect their deterioration and thus lead to their senescence.

In this work, chitosan was produced from *H. illucens* pupal exuviae following two different types of deacetylation methods: heterogeneous and homogeneous. Chitosan, at concentrations of 0.5% and 1 %, was applied by spraying and by dipping on the tomatoes; then, they were stored at two temperature conditions: room temperature and 4°C. The weight loss, physico-chemical parameters, total phenolics, total flavonoids and variation in antioxidant activity of the tomatoes were studied for a 30-day storage period. All parameters were kept stable with the use of heterogeneous chitosan, in comparison to the homogeneous one. Furthermore, the spraying method, compared to the dipping technique, was more effective in reducing weight loss and pH variation. Chitosan coatings were effective in reducing the pH variation of the tomatoes. No significant differences in the protective action between chitosan derived from *H. illucens* and commercial chitosan were found. These preliminary results are encouraging for the validation of the insect *H. illucens* as a source of chitosan. They provide an encouraging starting point and pave the way for new opportunities in the use of chitosan, an economically interesting biopolymer derived from a waste product of the insect breeding chain.

POSTER

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Usage of insect-based chitosan for the preservation of fresh fruits

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Due to its properties, chitosan is suitable for use as a natural biopolymer coating material to preserve the quality and extend the shelf-life of fresh foods. Chitosan-based bioactive films against microbial contamination and spoilage are successfully used in the packaging of fruits, vegetables, eggs, and meat. Currently, the main commercial source of chitin and chitosan comprises waste streams from the marine food industry, mainly crustacean exoskeletons. However, issues of low availability and sustainability of crustaceans make insects, particularly bioconverters, a promising alternative source of chitin and chitosan. This work is focused on the exploitation of one of the side streams generated from *Hermetia illucens* farming, pupal exuviae, which prove to be the best biomass of insect for the chitin extraction and the production of chitosan. Chitosan from pupal exuviae of *H. illucens* were used for the formulation of coating solutions to be applied to fresh fruit. Particularly, apricots (*Prunus armeniaca*), yellow nectarines (*Prunus persica*) and yellow peaches (*Persica vulgaris*) were coated with 0.5% and 1% bleached and unbleached chitosan and stored at both room temperature and 4°C for the time needed to assess their storage conditions. The effect of the chitosan coating on different fruits were evaluated by determining changes during storage occurred in weight loss, total soluble solids content and pH. The effect of the chitosan-based coating on spontaneous development of mould on the skin of fruit examined was also assessed. The results of this investigation revealed that insect chitosan is as effective as or better than the commercially available crustacean chitosan in maintaining more stable some important post-harvest physicochemical parameters in fresh apricots, nectarines and peaches. The effects are more evident on fruits stored at controlled temperature than on those stored at room temperature. No relevant differences are found between the two tested concentrations of chitosan, nor between bleached and unbleached chitosan. These preliminary results provide an encouraging starting point for validating pupal exuviae from insects, particularly *H. illucens*, for the chitosan production and use in the agri-food industry.

POSTER

SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Antimicrobial properties of the chitosan from different developmental stages of the bioconverter insect *Hermetia illucens*

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Chitin and its deacetylated derivative, chitosan, have various applications in biomedical and pharmaceutical fields. The market need to have these two biopolymers readily available has led to the search for alternative sources to crustaceans, the commercial source on the industrial scale. Insects, and among them the bioconverter diptera *Hermetia illucens*, is one of the most popular, thanks to the possibility of recovering waste materials (pupal exuviae and dead adults) from its breeding to extract chitin and convert it into chitosan. Chitin and chitosan have some important properties such as biocompatibility, biodegradability, non-toxicity, antioxidant, humectant and antimicrobial activity. It is exactly the latter that makes chitosan particularly versatile for pharmaceutical and medical applications.

Some pathogens have acquired new mechanisms of drug resistance, leading to antimicrobial resistance, that makes the human body progressively weaker to fight and deal with common infections. Because of this, antibiotics are becoming more and more ineffective and drug resistance is spreading widely, leading to increasingly difficult-to-treat infections. New antibacterial molecules are needed to tackle this problem. Among them, natural ones can be a safe alternative solution.

After protonation in acid conditions, chitosan can inhibit the proliferation of many bacteria, fungi and yeasts. The mechanism of action involves an electrostatic interaction between the NH₃⁺ groups of chitosan and the negatively charged portions of the membranes of bacteria, both Gram-negative and Gram-positive. The antimicrobial activity of chitosan depends on certain of its chemical-physical characteristics, mainly molecular weight and degree of deacetylation, and on some specific experimental conditions, such as temperature and pH.

The evaluation of the antimicrobial activity of chitosan was carried out through two types of experiment: agar diffusion test and microdilution assay. Bleached and unbleached chitosan from larvae, pupal exuviae and dead adults of *H. illucens* induced the formation of inhibition zones. This data is an indication of the biopolymer's ability to inhibit microbial growth. This important property of all chitosan samples was also confirmed by microdilution assay. By this experiment both against Gram-negative and Gram-positive bacteria, it has been possible to identify the minimum inhibitory concentration (MIC) values, ranging between 0.3 mg/ml and 0.15 mg/ml.

POSTER



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

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

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Antibiotic resistance is globally one of the most crucial health and social emergencies along with resistance to cancer therapies: the use of conventional chemotherapy agents and the indiscriminate and prolonged use of antibiotics has contributed to the development of resistant cancer cells and microorganisms (viruses, bacteria and fungi) resistant to therapies, respectively.

In the search for safe and effective new drugs, more attention has recently been paid to a class of biologically active compounds: antimicrobial peptides (AMPs), small bioactive proteins, naturally produced by all living organisms as components of their innate immune system. These biomolecules provide numerous advantages over conventional antibiotics such as: low levels of resistance, broad-spectrum activity with minimal host toxicity, synergistic effects on antimicrobial activity of antibiotics and rapid killing of the microorganism. Several studies have also shown that some of the antimicrobial peptides have a wide spectrum of cytotoxic activity even against cancer cells and are defined as anticancer peptides (ACPs).

The interactions between microorganism membranes and AMPs depend on electrostatic forces between positively charged AMPs and the membrane of negatively charged micro-organisms due to the presence of teichoic acid on the wall of Gram-positive bacteria and lipopolysaccharide on the wall of Gram-negative bacteria and their mechanism of action can be membranolytic and non-membranolytic.

Similarly to bacteria, cancer cells have a net negative charge due to their high expression of anionic molecules such as phosphatidylserine and mucins O-glycosilate on the outer sheet of the membrane and this allows electrostatic interactions between AMPs and the surface of many cancer cells. In addition, they can more easily destabilize the membrane of cancer cells given its increased fluidity and the greater presence, compared to healthy cells, of microvilli on the cell surface and can, once entered the cell, disrupt the integrity of negatively charged mitochondrial membranes, resulting in the release of are powerful pro-apoptotic proteins.

AMPs are produced by all organisms, but insects are among the richest and most innovative sources. The aim of our research is to identify pharmacologically active principles of natural origin from the insect *Hermetia illucens* (Diptera, Stratiomyidae), to be used for the development of alternative antimicrobial and anticancer drugs or in support of conventional therapies already in use. Peptide component was isolated from the hemolymph of immunized (by Gram-positive and Gram-negative bacteria) and not immunized larvae and tested to evaluate its antimicrobial and anticancer activities. Antimicrobial properties were analysed by agar diffusion assay and microdilution assay against both Gram-positive and Gram-negative bacteria, while anticancer effect was evaluated by MTT assay in different cancer cell lines.

ORAL PRESENTATION



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Optimization of the recombinant production of *Hermetia illucens* antimicrobial peptides in yeast

Carmen Scieuzo¹, Rosanna Salvia¹, Fabiana Giglio¹, Roberta Rinaldi¹, Mariarita Rubino¹, Emine Derin¹, Federica De Stefano¹, Maria Monti², Flora Cozzolino², Alessandro Sgambato³, Daria Nicolosi⁴, Zeljko Popovic⁵, Patrizia Falabella¹

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Antimicrobial peptides (AMPs) are crucial in innate immunity as the first defence against bacteria, fungi and viruses. In addition, their use can help counter the phenomenon of antibiotic resistance, currently considered a significant threat to public health. AMPs are small molecules, ranging in size from 10 to 50 amino acid residues, produced by all living organisms. Due to their high biodiversity, insects represent the richest and most novel source for AMPs. Among them, *Hermetia illucens* shows an extraordinary ability to live in harsh environments, rich in microorganisms, and could be a great source of AMPs. In previous studies, transcriptomes of *H. illucens* were examined in order to identify sequences putatively coding for AMPs. The sequences were further analyzed bioinformatically to characterize them from a chemico-physical point of view and putative functions. This work aims to optimize AMP production in the yeast *Pichia pastoris*, after cloning sequences in a specific expression vector, pPIC9k. Expression in yeast has numerous advantages, including protein processing and folding and post-translational modifications, and ensuring higher levels of expression than common systems for recombinant protein expression.

The expressed peptides will be purified using a poly-histidine tag at the C-terminus by Ni-NTA affinity chromatography. The antimicrobial activity of the peptides will be tested on Gram-positive and Gram-negative strains by agar diffusion assay and other microbiological tests. Further analysis will focus on optimizing the AMP production in bioreactors.

POSTER



SESSION XI

GENETICS, GENOMICS AND BIOTECHNOLOGY

Characterization of chitin and chitosan derived from the diptera *Hermetia illucens* for application in cosmetic and pharmaceutical fields

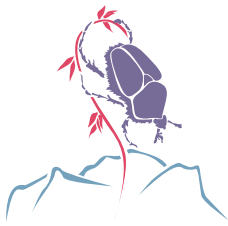
Micaela Triunfo¹, Rosanna Salvia¹, Carmen Scieuzo¹, Anna Guarnieri¹, Dolores Ianniciello¹, Antonio Franco¹, Giovanna Donnarumma², Beatrice Coltelli³, Angela De Bonis¹, Patrizia Falabella¹

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Chitin and chitosan are natural polymers of great technological and economic interest, having numerous applications in different fields. Chitin is a structural component of the exoskeleton of arthropods and the cell wall of fungi and yeasts; due to its insolubility, chitin is converted into its deacetylated derivative, chitosan. Currently, chitin is industrially extracted from fishing waste, mainly crustacean shells. The debate on the sustainability of this resource and the steady increase in market demand for chitin and chitosan have prompted a search for alternative sources. Insects are gaining great interest, particularly bioconverting insects such as *Hermetia illucens*. Currently, *H. illucens* is reared for protein feed production but its farming also generated large amounts of chitin-rich waste biomass, such as exuviae left over from moulting processes and dead adults, that could be exploited as a source for the extraction of this polymer. This work was aimed at developing a suitable procedure for chitin purification and chitosan production from different biomasses generated from the farming of the dipteran *H. illucens*. From larvae, pupal exuviae, and adults, chitin was extracted with yield, chemical characteristics and purity similar to that commercially available from crustaceans. Pupal exuviae were the richest biomass, with the 25.5% of chitin and also the most easily collected from insect farm, thus representing the chosen biomass for the chitosan production. From chitin, chitosan was produced by heterogeneous and homogeneous deacetylation; the two methods showed significant differences in deacetylation efficiency, yield, deacetylation degree and crystallinity degree in support of the heterogeneous method. Spectrometric, diffractometric and morphological characterization of different chitosans confirmed their similarity to the commercial polymer, from which they vary in lower viscosity and molecular weight. The different chitosan samples produced from *H. illucens* exhibited different characteristics dependent particularly on the deacetylation method and chitin decolorization, which lead to a change in deacetylation degree and molecular weight, respectively. The biological properties of chitosan useful for biomedical and cosmetic applications were also evaluated. IC50 values (mg/mL) showed good radical scavenging activity of chitosans from *H. illucens*; all chitosans, particularly heterogeneous ones, were able to reduce the expression of IL-6, IL-8, IL-1 α and TNF- α , proving to be good anti-inflammatory agents already at 6h of treatment. Furthermore, all chitosan samples positively modulated the expression of the antimicrobial peptide HBD-2, demonstrating an indirect antimicrobial activity that can be associated with the direct one already proved. Starting from this study, we will try to relate the specific physical-chemical, morphological and biological features of chitosans from *H. illucens* to specific applications of interest.

ORAL PRESENTATION



CNiE²⁰₂₃

P A L E R M O

XXVII Congresso Nazionale
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12-16 June 2023

Session XII

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Developing an objective landscape monitoring program for pesticide presence and dispersion

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Over the past ten years, pesticide use in Europe accounts for 350 kt/year, among this quantity Italy uses 57 kt/years, being the second largest user after France with 65 kt/year. Due to its intensive agricultural area, Italy is reported of using 6-7 kg/ha of pesticides, although Italy is ranked as the fourth as organic farming area with 15.2%, after Austria (25%), Estonia (22%), and Sweden (20%). In Trentino, apple orchards require up to 30 kg/ha of pesticides, of which 10 kg/ha are insecticides. Recently, several research institutions focus on the reduction of synthetic pesticide use as there is a growing public concern and they are considered to be the major pollutants driving environmental change. This is also in agreement with the recent EU “Farm to Fork” strategy, aiming to promote a healthy environment by reducing the use pesticide by 50% within 2030. All these aspects clearly show the need of an objective measure of pesticide used at landscape level. For this reason our research group has developed a pesticide landscape monitoring strategy using honey bees as a sampling tool of vast areas. Here we reported the achieved results in three alpine valleys of Trentino – South Tyrol: Val di Sole, upper Val Venosta and Valsugana. Trials were done in 2019, in 2021 and in 2022, placing 22 monitoring beehive stations in Val di Sole, 28 in upper Val Venosta and 38 in Valsugana, respectively. Pollen samples were taken in different periods of the growing season from April till September. In all the three valleys, the most frequent insecticide was always Phosmet, found in 17/42 samples of Val di Sole (\bar{x} 147 ppb), in 23/62 samples of upper Val Venosta (\bar{x} 347 ppb) and in 13/68 samples of Valsugana (\bar{x} 58 ppb). The highest three concentrations of Phosmet were found in pollen samples of upper Val Venosta, with 3,250, 2,300 and 1,540 ppb. Similar to the case of Phosmet, the most frequent fungicide was Fluazinam found in 16/42 samples of Val di Sole (\bar{x} 184 ppb), in 47/62 samples in upper Val Venosta (\bar{x} 998 ppb) and in 47/68 samples in Valsugana (\bar{x} 38 ppb). Also in this case, upper Val Venosta showed the highest three Fluazinam concentrations with 8,000, 7,800 and 5,510 ppb. These two pesticides also demonstrated a clear drift out of the apple farming area, up to 10-12 km. Other most frequent detected insecticides were: Acetamiprid, Etofenprox, Methoxyfenozide, Flonicamid, Spirotetramat, tau-Fluvalinate, and Chlorantraniliprole. Among fungicides, the most frequent after Fluaziam were: Dithiocarbamates, Captan, Boscalid, Difenconazole, Fludioxonil, Penconazole, Trifloxystrobin, Cyprodinil and Tetraconazole. Considering the pollen samples together we found a total of 27 insecticides and 56 fungicides. The three most polluted pollen samples were found in upper Val Venosta, containing 24, 23, and 21 pesticides, 9+15, 9+13 and 8+13 of insecticides and fungicides, respectively. Our studies demonstrate a clear correlation between the type of land use and the presence of pesticides all over the area of each of the three monitored valleys. This allowed, for the first time, the possibility of mapping the dispersion of pesticides in a precise and objective manner along relatively large areas.

KEY WORDS: Pollen trapping, pesticide dispersion, environmental quality, honey bees.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Honey bee susceptibility to Varroa and DWV infection varies with age

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The damage caused by parasites to their host doesn't only depend on evolutionary and ecological factors but also on the age of the host at the exposure. Despite the important implications for epidemiology, this aspect is often neglected in studies on host-parasite interactions, particularly in the case of invertebrates.

The western honey bee *Apis mellifera* is affected by a number of parasites and pathogens. The parasitic mite *Varroa destructor* and its associated pathogenic virus DWV play a crucial role causing extensive losses of honey bee colonies worldwide. These two parasites affect both the pre-imaginal and adult stage; the aim of this research is to compare the impact of the mite and the associated virus on different life stages of their host. For this purpose, we artificially infected both bee larvae and adult bees with either one *Varroa* mite or a fixed amount of DWV genome copies and compared the results in terms of survival and expression of a convenient panel of immune genes. We found that honey bee larvae and pupae, are more susceptible to mite parasitization and viral infection than adults. The higher viral load of the infested bees and the genotype of the DWV do not appear to be the drivers of the observed difference which instead appears to be related to the reduced immune-competence of the host's pre-imaginal stages. This evidence supports the hypothesis of a trade-off between immunity and growth making the pupa, which is involved in the highly energy-demanding process of metamorphosis, more susceptible to parasites and pathogens.

The observed trade-off may have important implications for the evolution of virulence and highlight the importance of the host's age and life stage at the exposure in epidemiological studies.

KEY WORDS: *Apis mellifera*, *Varroa destructor*, Deformed wing virus, Immunity, Host age.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Preliminary investigations about pollinators of Mango (*Mangifera indica* L.) in southern Italy

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Native to Southeast Asia, the mango (*Mangifera indica* L. - Anacardiaceae) is widely cultivated in tropical and subtropical areas. In recent decades, mango cultivation has spread to some countries in the Mediterranean basin, including Italy, where it is grown in the coastal areas of Sicily. Although it is a self-fertile species, pollination by insects increases fruit set. Mango pollinators have been widely studied in native areas and other tropical or subtropical countries where it has been imported, however, no studies have ever been conducted in Europe. In this regard, a three-year study started with the aim to identify the pollinators of mango in Italy, assess the insect diversity in this agro-ecosystem, and compare the integrated and organic managements. As the initial part of this study, surveys were conducted in 2022 in two groves in eastern Sicily (integrated and organic). The surveys were conducted during the flowering period with two pollinator sampling methodologies, transects and pan traps. Transects of 200 meters in length were set up, and the collections were done at two different times of the day, in the morning (9:30-11:00) and in the afternoon (14:30-16:00). Insects were also collected during the samplings from wild plants within the transect for an assessment of the diversity of this agro-ecosystem. Honeybees (*Apis mellifera* L.) were collected from the pan traps, and counted during the transect walk. Pan traps were placed in three triplets per grove at 9:00 and removed at 16:30. The results of the first year of surveys highlighted a prevalence of honeybees that represent the 69.4% of the total specimens found (n= 1048 specimens), the other part (n=321 specimens) is represented by insects belonging to Diptera (n= 23), Lepidoptera (n= 30), Coleoptera (n= 37), and Hymenoptera (n= 231) orders. Excluding honeybees, 150 specimens were found in the organic and 171 in the integrated grove, where a greater abundance and richness of wild plants was observed. Overall, about Hymenoptera, the most specimens belong to families Halictidae (n= 64), Andrenidae (n= 61), Colletidae (n= 53), Scoliidae (n= 18), and Apidae (n= 16, excluding honeybees). The most abundant genera were *Hylaeus* (Colletidae, n= 53) collected mainly from mango flowers, *Panurgus* (Andrenidae, n= 47) collected from pan traps and wild plants, and *Lasioglossum* (Halictidae, n= 34) collected from mango, wild plants, and pan traps. Sampling will be carried out in the next years for further evaluation of the pollinators of mango in Italy and the diversity related to this agro-ecosystem, with a particular focus on wild bee species. Sampling methodologies will be implemented to better identify the main pollinators of this tropical crop in the Mediterranean environment.

KEY WORDS: Tropical crop, pollinators, bees, diversity, Sicily.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Using a managed solitary bee and supplementary flowering plants to enhance kiwifruit pollination.

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In species requiring cross-pollination to set fruit, pollination is often a limiting factor for crop production in terms of both quantity and quality. It has been observed that in kiwifruit (*Actinidia chinensis*) a good pollination service is essential to obtain marketable fruits in terms of size and dry matter content. Fruit size is strongly correlated with the number of seeds which directly depends on pollination rate. Several legitimate visits of pollinators are required to have an adequate number of seeds. Nevertheless, the continuous and widespread decline of pollinators affecting in first line agricultural ecosystems makes difficult to maintain an economically sustainable production in this crop. In addition, kiwifruit as exotic crop in Europe may not find in the new area optimal pollinators to fulfil its pollination requirement. The main strategies adopted until now by producers involve pollinator supplementation by *Apis mellifera* or artificial pollination (by hand or spray). The former did not reach the expected results, probably because *A. chinensis* flowers are less attractive for honeybees compared with other flowers due to their lack in nectar production. The second strategy might not be considered a long-term solution, representing a palliative action, economically expensive and time consuming, often with very low outcomes. Artificial pollination also increases the risk of kiwifruit diseases (i.e., *Pseudomonas syringae*). In the present study we assess whether an agricultural ecosystem obtained by combining kiwifruit trees, supplementary flowering cover crops, and the release of a managed population of a solitary bee, *Osmia bicornis*, might represent an innovative and promising solution for pollination deficit in kiwifruit. Our purpose is to increase kiwifruit pollination by i) attracting wild pollinators in kiwifruit orchards by sowing a specific mix of cover crops including high nectar-producing plants; ii) releasing and establishing in the orchards a population of a managed solitary bee species (*O. bicornis*). In our study, kiwifruit production and pollinator activities will be compared in three different setting of agroecosystems: a) orchards with a managed population of *O. bicornis*; b) orchards with a managed population of *O. bicornis* and cover crop patches; c) kiwifruit orchards with a managed population of *O. bicornis* and cover crop patches terminated at the beginning of kiwi flowering. In each experimental field during kiwifruit flowering, data about pollinator density and biodiversity will be collected. Subsequently, we will focus on the evaluation of kiwifruit quality and productivity, collecting data on fruit set, yield, and standard fruit quality parameters. The research activity will be conducted collaborating directly with producers and local cooperatives, shaping together an innovative agroecosystem characterized by a high biodiversity to enhance kiwifruit pollination.

This study received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – ALMA IDEA 2022 – BRIO

KEY WORDS: pollination; kiwifruit; solitary bees; agro-ecosystem.

ORAL PRESENTATION



SESSION XII

SOCIAL INSECTS AND APIDOLOGY

**A field application of RNAi technology reducing *Varroa destructor* in honey bees
(*Apis mellifera*)**

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The honey bee *Apis mellifera* is vulnerable to a broad spectrum of parasites and pathogens that, interacting with other factors, including pesticides and global warming, are responsible for colony losses.

Veterinary medicinal products are often the most rapid and effective control measure to reduce pathogen levels, but seeking alternatives approaches is crucial for more efficient and sustainable bee management. RNA interference (RNAi) technology has shown great potential for honey bee pest control in laboratory assays, but studies to evaluate its effectiveness in the field are still lacking.

We investigated the efficiency and feasibility of an RNAi treatment to improve bee health under natural beekeeping conditions. By integrating honey bee diet with dsRNA against genes of *Varroa destructor*, a mite considered to be the major pest of the European honey bee, we observed a decrease in the mite infestation rates in treated hives. dsRNAs are highly mite-specific and did not result in detrimental effects on honey bees. Beekeepers involved in the project pointed out that this method is easily manageable in the apiary and it does not interfere with production needs.

Further efforts to set up the optimal dosage of dsRNA, the duration, and the best seasonal schedule of treatments to induce more effective gene silencing in mites are needed. Nonetheless, these preliminary results shows that field application of RNAi is a promising approach to sustain honey bee health through the control of mite populations.

The honey bee *Apis mellifera* is vulnerable to a broad spectrum of parasites and pathogens that, interacting with other factors, including pesticides and global warming, are responsible for colony losses.

Veterinary medicinal products are often the most rapid and effective control measure to reduce pathogen levels, but seeking alternatives approaches is crucial for more efficient and sustainable bee management. RNA interference (RNAi) technology has shown great potential for honey bee pest control in laboratory assays, but studies to evaluate its effectiveness in the field are still lacking.

KEY WORDS: RNAi, *Apis mellifera*, *Varroa destructor*.

ORAL PRESENTATION



SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Nest founding strategies in the paper wasp *Polistes dominula*: a potential role of individual immunocompetence?

Federico Cappa¹, Alessandro Cini², Rita Cervo¹

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New spring colonies of the paper wasp *Polistes dominula* can be founded by a single foundress (monogynous founding), or by a small number of associating foundresses (polygynous founding). In the first case, the single wasp carries out all the tasks linked to nest maintenance and brood care until the emergence of the first cohort of workers; in the second, a hierarchy is established among the different co-foundresses, with one female becoming dominant (alpha female) and laying the eggs, while the others become subordinate and carry out all the activities necessary for the development and survival of the colony. Various hypotheses have been put forward to explain the association of foundresses, sometimes unrelated; several factors (e.g. body size, reproductive ability, dominance, possibility of inheriting the colony) underlying the phenomenon have been investigated over the years. However, the possible role played by a fundamental feature of the organism, the immune ability, has never been studied until now. Immunocompetence, an energetically expensive trait, is often linked to the quality of an individual, determining its survival and reproductive success. Thus, in the present study, we aim to investigate the relationship between individual immunity and other indicators of wasp quality - body size, reproductive investment, dominance within the group - in different phases of the life cycle of the species, to evaluate whether this could influence the founding strategy adopted by a female and reflect the hierarchical position and tasks she will perform within the nest. We carried out immune bioassays by injecting wasps with *Escherichia coli* bacterial cells, and analyzing the individual bacterial clearance of *P. dominula* wasps belonging to different categories: overwintering future foundresses, monogynous foundresses, polygynous dominant females (alpha females), polygynous subordinates and beta females promoted to the dominant position after the removal of the alpha female. The comparison among the different groups shows that the wasps' immune response varies among the different phases of the life cycle and seems to highlight an effect of the tasks performed, reflecting the energy cost paid by the wasps, in determining the individual immune response, while no clear link with reproductive investment and dominance behavior was observed.

KEY WORDS: *Polistes dominula*, dominance hierarchy, reproductive investment, immune ability, bacterial clearance.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Effects of seasonality and landscape composition on pesticide residues in pollen collected by honeybees

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In recent decades, pollinator abundance and diversity faced a strong decline due to multiple anthropogenic pressures, including the use of pesticides in agricultural areas and the loss and fragmentation of semi-natural areas. The honeybee (*Apis mellifera* Linnaeus) is the most widespread pollinator species, and its presence is crucial not only for ensuring the reproduction of plant species in natural habitats but also for crop production. Like most bees, the honeybee diet is based on nectar and pollen. The presence of pesticides in the pollen collected by honeybees has potential negative effects on bee health and it can be strongly affected by landscape composition and, in particular, the amount of agricultural areas.

This work aimed to explore how the pesticide residues in pollen collected by honeybees were modulated by seasonality and landscape composition heterogeneity in a mountainous cultivated area. We selected 13 locations in Northern Italy, and at each location, we placed two honeybee colonies from which we collected pollen samples every month during the whole flowering season from 2017 to 2020. For pesticide residue analysis, we searched for more than 400 compounds in pollen samples, including fungicides, acaricides, herbicides, and insecticides. We then calculated for each pollen sample the Pollen Hazard Quotient (PHQ), an index that provides a measure of the potential toxicity of contaminated pollen. Finally, to assess landscape heterogeneity, we determined the cover of the main habitat types in a 3 km radius buffer around the sampling locations and analysed landscape composition using Principal Component Analysis (PCA).

Only 6 pollen samples out of 200 (4%) were pesticide-free, while in the remaining 194 samples, we detected more than 100 compounds, mainly fungicides. PHQ values were high (PHQ > 1000) in 32 samples (16%), medium-high (500 < PHQ < 1000) in 14 samples (7%), medium (50 < PHQ < 500) in 47 samples (24%), and low (PHQ < 50) in 101 samples (50%). The sampling season had a strong effect on pesticide residues in pollen. PHQ was higher between April and July, when pesticide applications in agricultural areas were also higher, and strongly decreased in August and September. On the other hand, the highest number of compounds detected in pollen was recorded in June. Landscape composition differently affected pollen diversity and pesticide residues. Pesticide residues in pollen were also affected by the landscape, as pesticide residues increased with increasing proportion of certain categories of agricultural areas and decreased with increasing proportion of semi-natural areas in the landscape.

Our work highlighted that both seasonality and landscape composition strongly affected the presence of pesticide residues in pollen collected by honeybees, which can strongly negatively impact honeybee health. Also, since it has been shown that pollen quality can influence the response of bees to pesticides, it is therefore advisable for beekeepers to evaluate landscape composition before placing beehives, in order to maximise floral resources around the hives and thus the diversity of pollen collected by bees while minimising the likelihood of high pollen contamination by pesticides

KEY WORDS: Floral resources, Landscape heterogeneity, PCA, PHQ, Pollinators.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Emergency Authorisation of pesticides: an assessment of the risks to bees

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Pesticide effects on bees are of particular interest because of potential reverberation on pollination ecosystem services and therefore food production. The EU regulatory mechanism of pesticide authorisation is required to safeguard the health of bees and the environment. To protect bees and the environment, many pesticides are non-authorised. However, numerous pesticides with a high risk profile are still widely used through an Emergency Authorisation process to control unexpected pests that can not be contained through the standard authorized pest control measures. Unjustified Emergency Authorisations of pesticides granted by some Member States has led the EU Commission to undo some of these authorisations. Some researchers have suggested that the Emergency Authorisation is a loophole to keep in the marked non-authorised pesticides. A comprehensive assessment of which pesticides are Emergency Authorized and the related risks is still however missing. In this work, we analyzed the EU use of Emergency Authorized pesticides using the official data. At first, we assessed if the active substances of emergency authorized pesticides pose a higher risk to bees than regulatory approved active substances. Then, we evaluated the frequency of non-authorised active substances using data from a biomonitoring program using honey bee (*Apis mellifera*) matrices (i.e., beebread and honey) to assess environmental contamination of pesticides.

We demonstrate that EU emergency authorisations are common across the EU with more than 200 active substances per year. For example, the use of the neonicotinoids acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam were granted in 24 EU Member States and renewed for multiple years. We demonstrated that the active substances of emergency authorized pesticides are significantly more toxic to bees (median oral LD50 = 34.46 µg/bee) than regulatory authorized active substances (median oral LD50 = 100 µg/bee). The biomonitoring data indicated that over 35% of the residues of active substances found in bee matrices are included in emergency authorized pesticides. Our results aim at a refined, scientifically accurate and transparent emergency authorisation process of pesticides that better protect bees and the overall environment.

KEY WORDS: ecotoxicology, biomonitoring, *Apis mellifera*, regulation.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

50 years of “selfish herd”: evidences from tropical social wasps

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Understanding why group living is so widespread despite some obvious disadvantages, such as a greater visibility to predators, a greater competition for resources and a facilitated spread of pathogens and parasites, represents a cornerstone of animal behavioural studies. The selfish herd hypothesis, proposed by Hamilton in 1971, has stood out among the various theories that attempt to explain the evolution of social living by its emphasis on individual interest rather than on the benefits of selection among relatives. This hypothesis postulates that groups are formed because individuals, by staying within a group where they basically put other conspecifics between themselves and predators, reduce their risk of predation and/or parasitism. This hypothesis thus predicts a differential fitness based on the position within the group (with the central positions more advantageous than those at the margins), as well as the existence of frequent movements of individuals within the group in order to exploit the different advantages offered by different positions. While the selfish group effect has been extensively studied in many vertebrate taxa and especially in groups of mobile animals (e.g. flock of birds), far less effort has been put into the investigation of the selfish herd hypothesis in insects and, especially so, in large nesting aggregations of social insects (the so called frozen selfish herds). Here we first review the use of the selfish herd concept in the insect scientific literature from its first use in 1971 to the present and, secondly, we assessed whether this hypothesis could explain the presence of extensive colonial clusters in Stenogastrinae, a group of tropical social wasps where large nesting aggregations are common. The presentation will highlight the main biases and knowledge gaps on the selfish group effect in insects and will discuss the experimental evidence supporting the selfish herd as an explanation for cluster nesting in this independent evolutionary origin of eusociality.

KEY WORDS: sociality, selfish herd, predation, aggregation, social wasps.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Chemical analysis of hornet's nest as an effective tool for species identification

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In the fight against invasive alien species, timely reporting is necessary to monitor and curb the invasion. This is particularly evident in the case of *Vespa velutina nigrithorax*, an invasive hornet species with a significant impact on wild honeybees and European apiculture. *V. velutina* colony reports are often late or dubious, as they are mainly represented by the discovery of abandoned nests found in the winter period when the colony is uninhabited. Especially old and degraded nests could be confused with *Vespa crabro* ones, a hornet native species in Europe. Verifying whether a nest belongs to the native species or the invasive one is crucial for monitoring the presence and expansion of *V. velutina*. Luckily, in these Hymenoptera, the nest material is characterized by a blend of chemical compounds – particularly hydrocarbons – that reflects the cuticular profile of its inhabitants which could be used for species identification. In this study, we investigated the differences between the nest chemical signature of the invasive species *V. velutina* and the native species *V. crabro*. We collected 19 nests from different years (from 2014 to 2022), 9 of which belonged to *V. crabro* and the rest to *V. velutina*. Envelope and comb fragments were sampled from the nests of the two species. Hydrocarbons deposited on the nest material were extracted and analyzed by gas chromatography coupled with mass spectrometry (GC-MS). From the extracts, we identified 55 compounds. Among these, we observed 7 to be unique for *V. crabro*, and 4 unique for *V. velutina*. Moreover, some compounds seemed to be related to the envelopes or combs, although some overlap was present. We did not observe any year-specific compounds. We propose these discriminant compounds could be used to develop a diagnostic tool for the species identification of abandoned nests even in the absence of their inhabitants, especially where reports come from areas not yet invaded or on the invasion front.

KEY WORDS: Alien species, *Vespa velutina nigrithorax*, GC-MS.

POSTER



SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Biocontrol of American and European foulbrood diseases using lactic acid bacteria isolated from the gastrointestinal tract of the honeybee

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Pollinators that are essential to agriculture include the European honeybee, *Apis mellifera* L. Significant reductions in farmed and wild bee populations have been observed in many regions of the globe. The health and longevity of bees are adversely impacted by several biotic and abiotic variables, such as changes in land use and management, use of pesticides, climate change, beekeeper practices, shortage of forage, parasites and pathogens. In this regard, a healthy microbiome is essential to support honeybee health and vitality. The gut microbiota plays a crucial role in bee growth and development, immune function, and defense against pathogens. A dysbiosis of the gut microbiota can impair honeybees and contribute to Colony Collapse Disorder (CCD). Infections caused by pathogenic brood disease agents, like *Paenibacillus larvae* (White) Ash et al. emend. Genersch et al. (American foulbrood - AFB) and *Melissococcus plutonius* corrig. (ex White) Bailey & Collins (European foulbrood - EFB), occur in the bee digestive tract and can result in significant losses in beekeeping and agriculture. Currently, the eradication of symptomatic colonies and the monitoring of adjacent apiaries are the only ways to deal with the two diseases. Additionally, the use of antibiotics may leave residues in the products of the hive and result in dysbiosis in the intestine of honeybees. Prolonged therapies, as well as failing to eradicate bacterial spores, may cause or hasten the antibiotic resistance phenomenon. According to a substantial number of studies, microbiota integrity may be related to disease risk. Beneficial microbes (lactic acid bacteria – LAB) used to supplement the diet of honeybees may be a valid alternative to antibiotics. In this research eight lactic acid bacteria, four strains of *Lactiplantibacillus plantarum* (Orla-Jensen) Zheng et al. (LP 31, LP 42, LP 148, and LP 179) and four strains of *Apilactobacillus kunkeei* (Edwards et al.) Zheng et al. (ALK 181, ALK 222, ALK 268, and ALK 385), isolated from the gastrointestinal tract of honeybees, were selected for their capacity in vitro to inhibit *P. larvae* ATCC 9545 and *M. plutonius* ATCC 35311. The antimicrobial activity of the cell-free supernatant was evaluated using the agar well diffusion assay. The resulting halos were measured with a caliber and expressed as the zone of inhibition (ZOI). The recorded values of ZOI against *P. larvae* ranged from 13.7±0.2 mm (ALK 385) and 13.9±0.4 mm (LP 179) to 16.3±0.2 mm (ALK 222). In the tests against *M. plutonius*, ZOI ranged from a minimum of 12.0±0.2 mm (LP 179) to a maximum of 16.0±0.1 mm (LP 148). Further investigations are needed to assess in vivo the role that these LABs, used as probiotic supplements in the diet, can play in improving the well-being of honeybees and in biocontrol strategies against EFB and AFB diseases. The first applications of our LABs in an experimental apiary indicated an improvement of the health status and vitality of treated colonies compared to untreated ones.

KEY WORDS: *Apilactobacillus kunkeei*; *Lactiplantibacillus plantarum*; *Melissococcus plutonius*; *Paenibacillus larvae*; probiotics; antimicrobial activity.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Evaluation of the efficacy of essential oils against *Varroa destructor* and their bioactivity on *Apis mellifera*

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Varroa destructor Anderson & Trueman is an ectoparasitic mite specific to *Apis mellifera* L. The improper use of synthetic acaricides led to the development of resistant varroa populations detected worldwide. The study evaluated the biological activity of essential oils (EOs) on *V. destructor*. The EOs tested were supplied by Solimé (Cavriago, RE). Biological material was collected from an experimental apiary in Benevento province. Electroantennographic bioassays (EAG, 11 EOs) were performed on forager bees, and biological activity in cages (adult bees), in vitro (varroa adults and *A. mellifera* larvae) and in the field (efficacy on *V. destructor*). In the in vitro bioassays, 10 varroa adults and 10 *A. m. ligustica* Spinola larvae (6 replicates) were used. Six doses of EOs were administered (0.5; 1.5; 2.0; 2.5; 3.25; 5.0 µL). Checks were performed after 1, 6, 21 and 24h. Maximum mortality (100%) of mites was observed, at different concentrations, with geranium (*Geranium robertianum* L. from flowers), cajeput (*Melaleuca leucadendron* L. var. cajeputi R. from leaves) and lavender (*Lavandula officinalis* Chaix from flowers), equal to that obtained with oxalic acid, starting 21h after administration. Slightly higher, but significant, mortalities were found on the larvae compared with the control; oxalic acid induced the highest larval mortality. Twenty-five hives were used for the field trials (5 for each of the 3 EOs, 5 with sublimated oxalic acid, 5 with mineral oil). The doses administered were 3 and 4 ml of a mineral oil:EO solution (1:1). The EOs were adsorbed onto experimental dispensers (plywood tablets) and placed on the bottom of the hive. For evaluation of the efficacy of EOs in the field, mites were counted before and after the experiments; mite counts were carried out on the antivarroa bottom (before and after 6, 24, 72h, 1 week), by the powdered sugar method and in sealed cells. In the field, EOs solutions induced significantly higher mean mortality of *V. destructor* than control and comparable to that induced by oxalic acid. EAG responses revealed the higher sensitivity of *A. mellifera* to the three EOs of geranium, cajeput and lavender. The activity of these three EOs was also evaluated with toxicity bioassays on adult bees (*A. m. ligustica*, *A. m. macedonica* Rüttner), showing no mortality. EOs could be an effective and sustainable tool to control *V. destructor* by integrating them with other means of controlling *A. mellifera* adversities.

KEY WORDS: Bioassays of toxicity, LD50, EAG, Geranium, Lavender, Cajeput.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Italian distribution of genera of vespids based on data collected by pest control companies

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We examined the data collected by 109 pest control companies concerning the control interventions carried out in the years 2020, 2021 and 2022 against various species of Vespids in urban areas. The companies were divided by geographic area into Northern Italy (46), Central Italy (26), Southern Italy (27) and the Islands (10). The results represent an example of Citizen Science.

In general, the data (which refer to pest control interventions carried out at the request of citizens) can be affected by a series of variables: among these the number of the population, the people's perception of the various insects, the availability of nesting places, the annual atmospheric conditions, the increase in temperature but also the number of colonies of the different species in the various places.

In the 3 years examined (2020, 2021 and 2022 up to the month of October) there was a substantial increase in the reports of Vespids which went from 2026, in 2020, to 2818, in 2022. Hornets (which include the three species of *Vespa* present in Italy: *V. crabro*, *V. orientalis* and *V. velutina*) are, in percentage, the most represented Vespids in the three years examined, with a slight decrease in 2022. Furthermore, from 2020 to 2021, there is an increase in percentage of reports of *Vespula*, while those of *Polistes* remain more or less unchanged. As regards the total number of reported colonies, those of hornets represent the most numerous. This may depend on their visibility and the consideration that citizens have towards them compared to those of the other Vespids. A certain difference between the catches is evident. In each year, hornets and *Polistes* represent the highest percentage of species, while *Vespula* are more present in the South but are the least represented in the reports of the various years. In the South and in the Islands, the reports of *Polistes* exceed in percentage, but only slightly, those of the Hornets. In 2022 hornets accounted for about twice as many Vespids reported in the North as compared to those reported in the Central, South and Islands. It is probable that this depends also on the population density and the presence of sites suitable for nesting on human structures.

KEY WORDS: Vespids in the urban environment, Citizen science, control.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Honey bee gut microbiome from unmanage to manage

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Recent studies on the role of bacteria symbionts of the honeybee gut have pointed out their importance for host metabolism, pathogen resistance, colony health. Free leaving colonies of *Apis mellifera* L. (honey bee) show higher level of resilience and adaptation to the environmental conditions compared to managed ones. In this study we used metagenomic amplicon deep sequencing to decipher the differences in gut microbiome of managed and unmanaged honey bee colonies from the same genetic origin.

We collected foragers from free leaving honey bee colonies and from their swarm reared in a standard beekeeping hives. Total DNA was extracted from the two groups of samples and sequencing was performed on a Novaseq 6000 machine producing paired-end 250 bp reads. PCoA plot showed how the managed and unmanaged honeybee replicates clustered into two different groups. Moreover, differential abundance analyses were carried out to detect significant differences in genera abundance across the different groups. Our analyses reveal that 9 bacterial genera out of 257 detected had a significant differential abundance. Our approach allows us to identify differences in the gut microbiome between bred and feral bees to understand and clarify which and how many bacteria are involved in the bee immune response. In the future, specific breeding programs will be designed in order to combine multiple favourable gut bacteria for honey bee queen industry.

KEY WORDS: gut microbiome, honeybee management, immunity.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Lethal and sublethal effect of syntetic pesticides on honey bee (*Apis mellifera*)

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While the ecological risks associated with the use of synthetic pesticides are well known, the use of such substances in agriculture still represents one of the most common practices for protecting crops from pests. Their intense use is considered a major factor driving the recent decline of pollinating insects worldwide, a phenomenon that is bound to have drastic environmental and economic consequences on the stability and biodiversity of ecosystems and, paradoxically, agricultural productivity. As a result, in order to reduce the environmental impact and harmful effects on non-target animals, there has recently been increased interest in the development of more sustainable methods based on new synthetic and non-synthetic products (biopesticides). Unfortunately, while it is crucial that the safety of these new substances are properly evaluated, the guidelines adopted in the risk assessment of synthetic preparations are often inadequate. In particular, sub-lethal effects associated with pesticide exposure are rarely considered in risk assessment protocols. For instance, possible alterations in the normal functioning of the cognitive system of beneficial arthropods such as pollinators could have a serious impact on their survival and fitness. In this study, we evaluated the effects of two pesticides (the fungicide Sakura and the herbicide Elegant 2df), tested at field realistic doses in single and in combination, on the health and behaviour of the honey bee *Apis mellifera*. We first tested acute toxicity in caged topicated bees and showed that these chemicals additively compromised bee survival. In a second set of experiments, we used a conditioning protocol of the proboscis extension response (PER) to assess the consequence of topical exposure to these chemicals for appetitive olfactory learning and memory in forager bees. The results of this study will clarify whether those chemicals have any detrimental effects on learning abilities, which are crucial for the success of this social pollinator.

KEY WORDS: Honey bee, synthetic pesticides, pollinators.

POSTER



SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Trace heavy metal contamination elicits midgut alterations and wing aspect ratio variation, but not body size shift in honeybee workers (*Apis mellifera* L.): a preliminary analysis

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The honeybee *Apis mellifera* Linnaeus, 1758 is the most important managed crop pollinator in Europe and bees' pollination is known to increase the quality of crops. To further improve crop yields, sewage sludge can be used as soil improver. Sewage sludge are the main by-products of the wastewater treatment process plants from cities and industries and contain not only fertilizing elements but also pollutants, such as trace heavy metals. These elements transferred to the soil can bioaccumulate in plants and eventually biomagnify along the trophic network. Honeybees primary feed on nectar and pollen and may thus ingest these trace elements. Nonetheless, few studies have investigated the effects of trace heavy metals on morphology of honeybees at both external and internal (i.e., tissue) levels. Here, we studied the effects of two different soil management practises in two areas of the Parco Adda Sud (Italy): with and without the use of sewage sludge as soil improver. Specifically, we investigated the damages caused by trace heavy metals found in sludge at two different levels: at the microscopic level by studying the histology of the midgut, and at the macroscopic level by measuring the intertegular distance (proxy of body size) and wings aspect ratio (proxy of wing shape and linked with flight performance). We hypothesise that honeybees collected in contaminated areas show structural midgut alterations such as increased vacuolisation. In addition, the accumulation of heavy metals may reduce body size and alter wing aspect ratio. To test our hypothesis, we selected five sites from each area and sampled 50 honeybee workers from each site. At the histological level, in bees collected in the contaminated areas, we found increased cytoplasmic vacuolisation of epithelial cells and, the presence of thick, black spherites in these vacuoles. We think that honeybees may sequester heavy metals in these spherites of the midgut, a defensive mechanism also shown in other insects. At the macroscopic level, we did not find any significant differences in body size, though we found differences in wing aspect ratio. In bees, aspect ratio tends to increase with increasing body size. Although this occurs for bees collected in areas not contaminated, we found an opposite trend in bees collected in contaminated areas. That is, in areas where sludge is used larger bees have significantly reduced wing aspect ratio values. We think that this may be due to the possible higher accumulation of heavy metals in larger bees, resulting in an alteration of wing morphology. Reduced wing aspect ratio would then result in worse flight performance and manoeuvrability. Our preliminary study highlights possible negative consequences of trace heavy metals present in sludge used as fertiliser, underlining the importance of conducting more comprehensive studies. These should be ideally carried out by taking into account both external and internal morphological traits as well as cytotoxicity biomarkers. Further studies that aim to quantify heavy metals in the analysed bees will help to elucidate the role of specific elements underpinning the alterations found in our study.

KEY WORDS: Heavy metals, Midgut, Histological damages, Morphology, Honeybees.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Antonio Scopoli: the forgotten revealer of the millennial mystery of the fertilization of queen bees

Paolo Fontana

Edmund Mach Foundation, Italy

Giovanni Antonio Scopoli (1723-1788) was an Italian naturalist, physician and academic. Born in Val di Fiemme (Cavalese, Trento, Italy), after obtaining a degree in medicine from the University of Innsbruck he practiced the profession of doctor first in Cavalese, then in Trento and then in Venice, where he began to take an interest in natural history, collecting plants and insects in the Alps. Having become medical assistant of the imperial mines in Idrija, a small village in Carniola (Slovenia), he remained there for sixteen years. He was then called to the chair of mineralogy in Schemnitz (today in Slovakia). During his stay in Slovakia Scopoli published his main scientific works on botany and mineralogy. From 1777 he held the chair of chemistry and botany at the University of Pavia (Italy), a position he held until his death. In 1777 also, he became director of the Botanical Garden of Pavia, which under his direction became one of the most famous Italian botanical gardens. In one of his most famous works, *Entomologia Carniolica* (1763), dealing with the species *Apis mellifica* (sic), Scopoli described, first among all scholars, the phenomenon of mating in flight of queen bees with drones. A few years later he wrote a real treatise on Apoidea entitled *Dissertatio de Apibus* (1770), dedicating a large part to honey bees and beekeeping, illustrating the techniques and knowledge of Slovenian beekeepers. The part relating to the honey bee was also published in Italian a few years later (1779). Reading Scopoli's texts on bees, one can deduce his extensive knowledge of contemporary literature in this sector. It remains a mystery why Antonio Scopoli, despite being the naturalist who was the first to clearly describe how the mating between queen bees and drones takes place and despite having dealt extensively with Slovenian beekeeping and the related very advanced techniques, remained unknown to the apidological world, while being considered one of the most famous entomologists and naturalists of the 18th century. The reproduction of honey bees has always been a great mystery, from Aristotle (384-322 BCE) to the Enlightenment. The many authors who, over 20 centuries, have dealt with it, have for many centuries excluded the mating of the queen bee or have given very imaginative explanations and even descriptions of it. The modern history of scientific knowledge relating to the reproduction of honey bees has always attributed the discovery of the mating in flight of queen bees to the Slovenian Anton Janša (1734-1773) who, in his work *Hinterlassene vollständige Lehre von der Bienenzucht* (Complete manual of beekeeping), published posthumously (1775), had described in detail the mating of the queen bee in flight with several drones. The first experimental work on this phenomenon was instead due to François Huber (1750–1831) who, in his fundamental work 1792, *Nouvelles observations sur les abeilles* (1792) and in particular in the *Lettre première, Sur la fécondation de la Reine-Abeille* (First letter, On the fertilization of the queen bee), demonstrated that the mating of the queen bee can only take place outside the hive arriving to calculate, on the basis of the duration of the fertilization flights of the virgin queens, also the distance of this mating. Huber was not aware of the works of Scopoli or even of Janša, as wasn't later the noble father of scientific and technical knowledge on beekeeping, Johann Dzierzon (1811-1906), who, only after Huber's experiments, assumed the multiple mating of virgin queens in flight.

KEY WORDS: *Apis mellifera*, reproduction, history of entomology.

POSTER

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SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Beewild: the Citizen Science and the study of the wild colonies of *Apis mellifera*

Paolo Fontana, Valeria Malagnini, Daniele Andreis

Edmund Mach Foundation, Italy

The Western honey bee (*Apis mellifera* Linnaeus, 1758), the one managed by beekeepers all over the world, is a native insect in almost all of Europe, Africa and the Near East where is one of the main pollinators of the flora (both wild and cultivated). Until a few decades ago, colonies of wild (i.e., unmanaged) *A. mellifera* were largely common: in hollow trees, in holes in the ground, in cracks in rocks but also in buildings, abandoned and not. However, since the early 1980s there has been a rapid and underestimated rarefaction of the "wild" colonies due to a parasite, the fearsome *Varroa destructor* (Anderson & Trueman, 2000) mite. This parasite initially decimated, both with its direct action and due to the viruses, it transmits, both unmanaged honey bees and those managed by beekeepers, with the difference that the latter immediately understood how to protect their colonies with different techniques also including the massive use of acaricides.

The effect of the Varroa mite on unmanaged colonies was instead so strong that today in Europe most of the survived honey bees live in hives managed by beekeepers. Indeed, for many years it has even been thought that in Europe wild honey bees were disappeared or they only derive from swarms originated by those managed by beekeepers. In recent years, however, there has been a significant increase in reports of unmanaged colonies, probably due to the growing empathy of people for this insect. Unmanaged honey bee colonies are primarily important because they are a natural component of our European habitats. Unmanaged colonies of *A. mellifera* are also important for the same beekeeping because they are more directly subject to natural selection. In fact, in these colonies are more likely selected characters of resistance and/or tolerance to parasites and diseases but also of adaptation to local ecological and climatic conditions as well as to their change over time.

The unmanaged colonies are then of fundamental importance for the conservation of local subspecies and populations of *Apis mellifera*. Therefore, knowing the real distribution of the unmanaged *A. mellifera* colonies, living outside the beekeepers' hives, is very important and that is why Fondazione Edmund Mach created the BeeWild mobile application, which can be downloaded for free (and free from advertisements of any kind) both from Play Store and App Store. The BeeWild app allows citizens, through a typical Citizen Science action, to report the presence and survival over time of unmanaged colonies of *Apis mellifera*, geolocating them, also providing some simple observations relating to the environment, the type and position of the nests and allowing users to attach one or more photographs.

KEY WORDS: honey bee, varroa, Italy, protection, resilience.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Characterization of the *Apis mellifera* population on the island of Pantelleria (Sicily, Italy)

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Apis mellifera Linnaeus, 1758 has some peculiarities that make it a key organism for the conservation of biodiversity and, therefore, the global ecological balance. Unmanaged western honey bee colonies were present in the recent past everywhere and for several millenniums they have coexisted with managed colonies. In the last 35 years, unmanaged colonies have almost completely disappeared in most of Europe due to the spillover of the parasitic mite *Varroa destructor* (Anderson & Trueman, 2000) to western honey bee colonies. However, in recent years, unmanaged colonies have been apparently sharply increasing. These colonies are at the attention of the world of bee research and European beekeepers for the occurrence, in such conditions, of possible situations of resilience to changing climatic aspects and health problems, as well as for the protection of the indigenous subspecies of *Apis mellifera*. On the island of Pantelleria, where there have always been some beekeepers, managed colonies and unmanaged colonies of honey bees coexist. Many unmanaged colonies were recently recorded using the smartphone app BeeWild. Therefore, in this study, carried out mainly within the National Park of Pantelleria island, we described the morphometry and haplotype distribution of the honey bee colonies living in this isolated and very particular context, trying to point out the possible differences between managed and unmanaged colonies.

Honey bee individuals (workers) were collected from 32 colonies in the summer of 2021 and subjected to morphometric and molecular analyses. For morphometric analyses, 16 wing characters were used. A discriminant analysis, including Pantelleria honey bees, *A. m. siciliana* Dalla Torre 1896, *A. m. ligustica* Spinola 1806, *A. m. carnica* Pollmann 1879, *A. m. intermissa* Buttel-Reepen 1906, *A. m. caucasica* Pollman 1889 and *A. m. mellifera* Linnaeus 1758, was carried out. For molecular analyses different molecular markers were used (COI-COII intergenic region of mitochondrial DNA and SNIPs). As a reference *Apis mellifera mellifera* Linnaeus 1758, *A. m. ligustica* Spinola 1806, *A. m. carnica*, *A. m. jemenitica* Ruttner 1976, *A. m. siciliana* Dalla Torre 1896, *A. m. iberiensis* Engel 1999, *A. m. intermissa* Buttel-Reepen 1906, and *A. m. ruttneri* Sheppard, Arias, Grech et Meixner 1997 were used. Evolutionary lineages and haplotypes for each individual were analyzed by sequence blast on GenBank. Based on the results of morphometric analysis 68% of the colonies were similar to *A. m. siciliana*, 4% to *A. m. ligustica* and 28% were apparently hybrids (i. e. *A. m. siciliana* x *A. m. intermissa*). Furthermore, Pantelleria honey bees cluster separately from *A. m. siciliana* as well other subspecies. With the genetic analyses we found that the A evolutionary lineage is dominant on the island (71% of the samples overall), and even more so in the unmanaged colonies (93%). The most frequent A haplotype was A1, which was found in *A. m. siciliana* in the course of the reintroduction project APESLOW, followed by A4 and A2, also previously reported in *A. m. siciliana*. Furthermore, as the proportion of A lineage haplotypes was higher in the unmanaged colonies, we can hypothesise that the local population has an adaptive advantage over imported genotypes. Further analysis should be carried out to better understand the identity of Pantelleria honey bees.

KEY WORDS: Honey bee, local populations, morphometry, genetics.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

**First inventory of the Apoidea anthophila of the Val Grande National Park
(Piedmont, Italy)**

Livia Zanotelli¹, Paolo Fontana¹, Valeria Malagnini¹, Cristina Movalli²

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Pollinators and especially Bees (Apoidea Anthophila) are undergoing a worrying decline essentially linked to activities of anthropic origin which lead to the reduction of food sources and the lesser availability of sites suitable for nesting. As part of the Directive for the conservation of biodiversity with which the Ministry of Ecological Transition promotes the monitoring of pollinators, the Val Grande National Park body in collaboration with the Edmund Mach Foundation in 2021 conducted a survey which had as its focus the study of anthophilous Apoidea.

Three altitudinal transects have been identified within the Park territory: CO, BT and SC respectively in the municipalities of Premosello-Chiovenda, Cossogno and Cursolo-Orasso and Trontano and Malesco (VB, Italy). The CO transept (Alpe Piana – Colma di Premosello) was composed of 6 plots distributed starting from an altitude of 670 m a.s.l. up to 1680 m a.s.l. The BT transept (Pogallo – Bocchetta di Terza) also included 6 plots distributed at an altitude between 780 m and 1840 m a.s.l. The SC transept (Alpe la Piana – Bocchetta di Scaredi) was composed of 5 plots distributed among the 940 and 1790. Samplings were carried out in the months of June, July, August and September using pan traps of three different colours: blue, white and yellow.

The sampled specimens were all prepared by mounting them on a pin and identified on a morphological and, where necessary, molecular basis. A total of 1985 specimens belonging to the superfamily of Apoidea anthophila were examined. The results obtained highlight the presence of a complex Apoidea population both due to the abundance of individuals and the richness of species, confirming that the diversity of habitats in the investigated area ensures their conservation.

KEY WORDS: Faunistics, non-Apis bees, checklist.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Life VAIA: Valuing Afforestation of damaged woods with Innovative Agroforestry

Valeria Malagnini, Paolo Fontana, Livia Zanotelli

Edmund Mach Foundation

The LIFE VAIA project (Valuing Afforestation of damaged woods with Innovative Agroforestry) stems from the need to restore forest areas and increase the ecological, economic and social resilience of forest ecosystems damaged by extreme climate events, such as the VAIA storm that hit North East Italy in October 2018. The objectives of the project, to be pursued in 16 pilot sites (of about 2.5 hectares each) in Italy, Spain and France, are in line with the European Union's environmental strategy aimed at protecting forests from damage caused by climate change, strengthening local ecosystems and protecting biodiversity. With a duration of five years and a total budget of €6 million, of which €3 million has been allocated by the European Commission, the LIFE VAIA project aims to develop an innovative approach based on the application of "temporary" agroforestry measures (15/20 years). This strategy makes it possible to invest in the production of sustainable and low-impact products, increasing biodiversity and the sustainable use of resources. The main actions financed by LIFE VAIA concern the reproduction and cultivation of wild blueberries and other "wild" small fruits, food and medicinal plants in forest ecosystems, as well as the enhancement of beekeeping production in forest areas destroyed by storms and other extreme climate events. The forestry strategy pursued by the project will make it possible to limit the economic damage suffered by local communities and to develop innovative silviculture and value-added products.

KEY WORDS: Environmental restoration, extreme climatic events, beekeeping.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

The effect of pollen on behavioral maturation genes underpins the beneficial effects on parasitized honey bees

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Pollen is fundamental for honey bees, providing nutrients that support metabolism, growth and immunity. In a recent study, we showed that pollen can mitigate the impact of the parasitic mite *Varroa destructor*, prolonging the lifespan and limiting the associated infection with the deformed wing virus (DWV). *Varroa* parasitization accelerates the honey bees' behavioral maturation by altering the interactions between two inter-related core physiological factors, Vitellogenin and juvenile hormone.

In this study, we demonstrate that the beneficial effect of pollen on *Varroa*-infested bees is tied to the hormonal control underpinning behavioral maturation. By analyzing the expression of genes involved in this process we discovered that pollen increases the lifespan of mite-infested honey bees by reversing the accelerated maturation caused by the parasite. As expected, from the different immune competence of nurse and forager bees, the lifespan extension triggered by pollen is also correlated with a positive influence on antimicrobial peptide gene expression and DWV load, further reinforcing the beneficial effect of pollen. This study lays the groundwork for future analyses of the underlying evolutionary processes and opens new perspectives to improve bee health.

KEY WORDS: *Apis mellifera*, *Varroa destructor*, pollen, ageing.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Modelling tools for the optimisation of pollination services provision in agroecosystems

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Insect-mediated pollination is a vital ecological process that plays a crucial role in maintaining biodiversity, promoting plant reproduction, and ensuring food safety worldwide. Honey bees and wild pollinators are both important for providing pollination services and contributing to the diversity and productivity of natural and agricultural systems. However, multiple stressors, including habitat loss and fragmentation, agricultural intensification, and climate change are negatively affecting the presence, abundance, and diversity of pollinators.

In this work, we present an Individual-Based Model that simulates the foraging and pollination efficiency of interacting honey bee colonies and wild pollinator communities in a realistic landscape. The model simulates the main physiological processes of the hive, including egg-laying, development, mortality, as well as the dynamics of in-hive products such as food influx and consumption. The landscape is simulated as a mosaic of different food patches, and relevant processes such as patch phenology and attractivity, nectar and pollen production, and pollination requirements are considered based on the type of land-use. The characteristics of the wild pollinator community are also estimated considering the type of land-use.

The model represents a tool that might provide key elements for defining and implementing rational management strategies for land use and colonies deployment aimed at mitigating the potential negative impacts of competition between managed honey bees and wild pollinators, optimising the provision of pollination services, and ensuring the conservation of wild pollinators as an essential element of the ecosystems. The model presented has been developed within the SafeAgroBee project, whose main objective is to contribute to the adaptation and mitigation of the effects of climate change and other drivers negatively influencing the sustainability and resilience of the agroecosystems within the Mediterranean basin.

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KEY WORDS: *Apis mellifera*, Wild pollinators, Individual-Based Model, Pollination services, Sustainable agroecosystems.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

The role of hive constituent materials, on the welfare and productivity of domestic bees (*Apis mellifera* Linnaeus, 1758)

Erica Holzer, Sara Savoldelli, Gabriele Tarzia, Daniela Lupi

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Honey bee colonies and pollinator insect populations in general have been in steep decline in recent decades. Among the factors (mostly attributable to human activities) that severely compromise pollinator survival there are land use change and intensive cultivation, the use of agrochemicals, environmental pollution, the spread of invasive exotic species and pathogens and climate change. These factors progressively weaken the colony, altering the health status of colonies, reducing for instance the fertility of the queen, and affecting foraging capabilities of the workers. Moreover, global warming, and the extreme climatic events that follow it, influence flowering consequently compromising the availability of the nutrients required by bees for their growth and development, and can also affect colonies homeostasis with direct effects on the thermoregulatory capacities operated by bees in the different seasons. For all the above reasons, today, perhaps more than in the past, evaluating the influence of different materials on hive life and particularly on temperature fluctuations becomes important. The present study stands as a starting point for comparing the biological responses of bees in two types of Dadant Blatt hives (wooden vs. aluminium with cork insulation). The hives were placed in the Po Valley (Lombardy) starting from families of equal strength, with one-year-old queen. The 12-month monitoring involved surveying thermo-hygrometric conditions inside the hive for the duration of the study and fortnightly observation of family health and productivity. For this purpose, the following parameters were considered: honey produced, pollen collected, brood, presence/absence of queen, queen replacement, mortality in the underbasket. Analysis of the results obtained showed a different response of the two hives to temperatures. In particular, in the aluminium hive, compared to the wooden hive, temperatures were found to be lower on average in the warm periods of the year (spring-summer). Regarding the different parameters observed in the colonies, no significant differences were found for honey and brood, and significant for pollen with major collection in the wooden hive. Mortality rate was higher in the wooden hive, especially at the beginning of the season. The results obtained from these initial analyses seem to confirm the effectiveness of the aluminium and cork hive (Dadant Blatt) in terms of insulating from the external environment and are a good starting point for future studies in different climatic areas.

KEY WORDS: Colony decline.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Big cities as a model of interaction of *Apis mellifera* and wild bees

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Cities in more developed countries have a greater variability of plant and floral species, nesting sites and nesting materials, that can provide resources for both managed and wild bees. The presence of these positive aspects varies greatly between cities due to geographical, historical, social and economic factors and can be influenced differently by interactions between organisms in the environment. Furthermore there is a tendency to increase the number of managed bees in cities, rising the competition with wild species. In the present research the city of Milan has been chosen as a model for a first study in order to investigate the connection between the urban green areas its environment, plants, honeybees and wild bees. In detail More than 90 urban areas have been monitored, considering visits on flowers and mapping information over a period of 3 years (2019-2021). In every area flowered plants have been monitored for a qualitative analysis Whereas plant that covered a surface larger than 4m² were subjected to a quantitative analysis. A fillable online form created was used to collect data from the operators. When an interaction was observed, it was noted together with the food source chosen by the bee (nectar/pollen). Classification was carried out at the lowest possible taxonomic level. The work identified more than 80 species of wild bees belonging to 21 genera and 5 families in the Milan area and more than 750 associations between pollinators and the plant species monitored. The presence of *Apis mellifera* was correlated with wild bees in different areas. Mellifera showed, as hypothesised, to be the most polylectic species and to be found in all visits in all monitored areas. With regard to the spatial distribution in the monitored areas, it was found that some genera were found in almost all of the monitored areas (e.g. *Bombus* and *Anthidium*), while others found just a niche location and were only found in a punctiform manner in some urban parks (e.g. *Ceratina*, *Coelioxys*, *Colletes*). The presence of bees in cities was also related with different environmental parameters as included soil temperature, precipitations, wind, atmospheric pressure, air humidity and temperature, and pollutant concentrations. Furthermore for *Apis mellifera* also the presence and distribution of apiaries in the city was considered.

This work gives the basis to an inventory of bees in cities and their interaction with plants in gardens and of the capability of wild bee to share the same niches with *Apis mellifera*.

KEY WORDS: Urbanisation, urban beekeeping, temperature, maps.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Bibliometric analyses and systematic review on *Vespa orientalis* L.

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Vespa orientalis L., also known as the Oriental Hornet, is one of the main factors behind the reduction of the number of bees and apiaries in some areas of Sicily. Although there may seem to be a lot of information on *V. orientalis*, in reality several aspects remain to be investigated. The purpose of this work was to investigate the current state of knowledge, acquiring information regarding the different techniques applied in countries where *V. orientalis* is more prominent. To this end, two different approaches were used. The first considered the methodologies and logic of the VOSviewer software, which creates maps with keywords derived from co-occurrence analysis to reveal the main keywords used and the countries and journals which publish the most on the topic. The second followed the indications of the EFSA (European Food Safety Authority) guidelines where systematic analysis is required to evaluate the data from all the documents selected in a transparent and logical approach. The main goal was to offer a comprehensive understanding of potential control methods against *V. orientalis* as well as how future research directions should be oriented.

In total, just over 400 articles were identified, 393 of which were considered eligible for the study of the reports and selected topics. Although *V. orientalis* is widespread, above all in south-eastern Europe (including southern Italy), the Middle East, and Madagascar, most of the studies on this hornet have been carried out by Israeli research groups, followed by American, Indian, Russian, French and Egyptian researchers. The studies mainly concern issues relating to the wasp's biology, the chemical composition of its venom and its effects on humans, in particular allergic reactions following stings, as well as the venom's potential therapeutic properties. On the other hand, studies focused on comparing methods used against this hymenopteran species are poorly represented. Thus, the results highlighted the importance of continuing scientific research, in particular the exploration of conventional control methods as well as innovative solutions specific to this wasp.

KEY WORDS: Oriental hornet, control method, systematic revision, VOSviewer.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Lethal and sublethal effects of insecticides applied to control *Phenacoccus solenopsis* on the pollinator *Bombus terrestris*

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Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) is an extremely polyphagous invasive pest, native to North America that has spread widely in recent decades to Asia and the Mediterranean basin. Recently, the species was found in Italy on greenhouse tomato crops and ornamental plants in urban environment. Although several biocontrol agents have been observed feeding on this pest, synthetic pesticides are highly applied to control *P. solenopsis* in greenhouses. As part of integrated pest and pollinator management (IPPM), the lethal effects of the most effective insecticides in previous target toxicity experiments on *P. solenopsis*, acetamiprid, pyriproxyfen, sulfoxaflor and thiamethoxam, were evaluated on *Bombus terrestris* L. (Hymenoptera: Apidae), an important tomato pollinator insect in greenhouses. The products were tested at LC10 and LC90 obtained for *P. solenopsis* by conducting ingestion tests with *B. terrestris* workers, following the OECD guidelines, with some modifications and using dimethoate as a positive control. Survival analysis using the Kaplan Meier method, showed that among the four insecticides tested, pyriproxyfen proved to be the safest for *B. terrestris* with 100% survival at both concentrations tested. The survival of bumblebees exposed to acetamiprid was 80% in the case of LC90 and 96.7% in the case of LC10. Sulfoxaflor and thiamethoxam were the most toxic to *B. terrestris* at LC90 for *P. solenopsis*, respectively with 0% and 3.3% survival 72 hours after exposure. Potential anti-feeding effects were evaluated with pyriproxyfen and acetamiprid, the two insecticides that caused the lowest mortality. Anti-feeding effects were observed with acetamiprid at the highest concentration. However, further studies on these molecules are needed to evaluate other sublethal effects, such as those on locomotory activities and at the physiological level.

KEY WORDS: ecotoxicology, pollinator, risk assessment, toxicity.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Genetic and morphometric variability of Italian populations of *Vespa velutina*

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The Asian hornet, *Vespa velutina* subsp. *nigritorax*, is an invasive species that actively preys on bees in front of the hives, posing a serious threat to the beekeeping sector both due to the reduced production of hive products and the direct impact on the colony numbers. In France, losses of up to 50% of beehives have been recorded. The feeding habits of *V. velutina* involve active hunting of honeybees directly in front of the hives. The stationing in front of the colonies results in the interruption of foraging activity and the inhibition of laying by the queen. The consumption of the present stocks and the lack of generational turnover cause depopulation and the loss of the colony in a short time. From the data available in the literature it would appear that the introduction of this dangerous invasive species occurred from a single fertilized *V. velutina* queen, accidentally introduced into France from South-East Asia. Since this initial introduction, *V. velutina* has rapidly colonized the country, also spreading to neighboring states (Spain, Portugal, Italy, Belgium, Germany and the United Kingdom) with an expansion rate of up to 100 km/year.

We present here the results of a population genetics study that made it possible to quantify the genetic variability present in the populations of *V. velutina* found in Italy. The individuals analyzed come from Liguria (in particular from the provinces of Imperia, Savona and La Spezia), Piedmont (province of Cuneo) and Veneto (province of Rovigo).

After identification by antennomeres counting, the female specimens were subjected to morphometric and genetic investigations. The genetic investigation was conducted after DNA extraction from tibiae and tarsi and the use of specific SSR loci for *V. velutina*. The morphometric investigation was carried out on the distance between the nodes of the wing veins. Distance measurements were performed using ImageJ software.

Statistical analyzes of morphometric data revealed significant differences between the populations. While the statistical analyzes of the molecular data showed that the genetic variability present in Italy is considerably lower than that present in the territories of origin and this can be explained by a founder effect, confirming the hypothesis of the introduction of a single queen fertilized by several males (polyandrous behaviour). Alongside reduced genetic variability (strong reduction in the number of alleles per locus), unique alleles, also absent in Asia, were highlighted, probably for new mutants, common in SSR markers.

KEY WORDS: alien insects; microsatellites; morphometry; beekeeping.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

The Solar Bees project: integration of photovoltaic systems, beekeeping and pollinators preservation

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Honeybee (*Apis mellifera*) plays an important role in natural ecosystems through their pollinator service to crops and wild plants around the world. But factors such as the widespread use of insecticides and agrochemicals treatments in general, environmental pollution, disease occurrence, and the introduction of alien insects weigh on colony numbers' decline. Honeybees are continually subjected to stress. At the same time, the world is experiencing an energy crisis and needs to produce energy from renewable sources to reduce the global effects related to the use of non-renewable sources.

Among renewable sources, solar energy plays a leading role, with strong potential for development in a country like Italy, which can benefit from high solar irradiation for several months of the year. Solar installations that provide the most energy production include ground-mounted photovoltaic systems (PV). However, these installations, which are often large or very large, are sometimes frowned upon or opposed because of the land consumption imputed to them, also associated with high environmental and landscape impact.

In order to mitigate the effect related to soil consumption and environmental impact, an idea was developed to use part of the surface intended for the installation of PV system.

From the collaboration with Acea Solar, the idea of Solar Bees project was born, which through the enhancement of the soil on which the PV systems insist manages to combine the production of renewable energy with beekeeping. The main action consists of sowing, in the space between PV panels, a mix of nectariferous and pollen-bearing plants appropriately chosen according to their growth, potential in terms of nectar and pollen production, flowering scalarity and hardiness. Furthermore, mitigation of the outer perimeter will include the planting of trees and shrubs, also chosen according to the parameters indicated above. Subsequently, an apiary will be installed in an appropriately designated area near the photovoltaic panels. A local beekeeper will manage the hives from spring until the bees wintering. During the beekeeping season, an expert beekeeper will conduct inspections on hives to assess health status, the incidence of disease, stock presence, and populousness of the colonies.

At the same time, sampling will be carried out in some areas of the PV system aimed at evaluating the consistency and diversity of the pollinators present.

The project, which will develop over three years, will assess the potential of the PV systems from the point of view of beekeeping and the conservation and increase of pollinator biodiversity.

KEY WORDS: *Apis mellifera*; nectariferous flora; wild pollinators; environmental mitigation.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Honey bees (*Apis mellifera*, L.) as samplers of airborne Particulate Matter in urban environments

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Bees are extremely vulnerable to environmental stress and respond quickly and measurably to pollutants. In particular the honeybee species *Apis mellifera* has been used since the 1980s as a bioindicators of environmental pollution. During the wide-ranging foraging activity, the insect is exposed to pollutants, thus becoming a useful tool to trace the environmental presence of a range of contaminants including pesticides, radionuclides, heavy metals, volatile organic compounds, and airborne particulate matter (PM). Airborne PM is a heterogeneous mixture of particle pollutants, usually classified according to the aerodynamic diameter of the particle which may range from several micrometers (PM₁₀) to a few nanometers (PM_{0.1}). However, standard air quality stations only detect the mass concentration of PM mixtures with diameters $\leq 10 \mu\text{m}$ and $\leq 2.5 \mu\text{m}$, without further characterization of the particles, even if their size, morphology, and chemical composition is known to elicit adverse health effects.

Road traffic is one of the major sources of PM in urban areas, which may have both exhaust and non-exhaust sources. Though stringent regulations have ensured that exhaust emissions are under control, regulations to control the emission of PM due to non-exhaust ones are lacking. Furthermore, the actual contribution of dust emitted from non-exhaust sources and their physico-chemical characteristics remain ambiguous. This study fills this gap by using scanning electron microscopy coupled with energy dispersive X-ray spectroscopy to characterize PM collected by honeybees in the city of Milan.

The bees showed a contamination by dust originating from the erosion of neighboring soils and rock fill material as well as metallic particles, characterized by constant combinations of heavy metals. Metal PM derived from the friction of the braking system of cars and tires (non-exhaust emission sources). Bees are extremely vulnerable to environmental stress and respond quickly and measurably to pollutants. In particular the honeybee species *Apis mellifera* has been used since the 1980s as a bioindicators of environmental pollution. During the wide-ranging foraging activity, the insect is exposed to pollutants, thus becoming a useful tool to trace the environmental presence of a range of contaminants including pesticides, radionuclides, heavy metals, volatile organic compounds, and airborne particulate matter (PM). Airborne PM is a heterogeneous mixture of particle pollutants, usually classified according to the aerodynamic diameter of the particle which may range from several micrometers (PM₁₀) to a few nanometers (PM_{0.1}). However, standard air quality stations only detect the mass concentration of PM mixtures with diameters $\leq 10 \mu\text{m}$ and $\leq 2.5 \mu\text{m}$, without further characterization of the particles, even if their size, morphology, and chemical composition is known to elicit adverse health effects.

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KEY WORDS: Honey bee, particulate matter, pollutant, SEM-EDX, non-exhaust emissions.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Preliminary studies on the maintenance of *Varroa destructor* under lab conditions

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The ectoparasitic mite *Varroa destructor* is involved in the decline of *Apis mellifera* colonies. The development of an in vitro rearing method would greatly improve our chances to better understand Varroa biology, since, under natural conditions, the reproduction of the parasite takes place inside a sealed brood cell. However, a tested protocol for maintaining the mite in the absence of its host is still missing. Moreover, only few and discordant data on the longevity of Varroa on its host under lab conditions are available, meaning that even the ultimate target of an artificial rearing method is currently uncertain.

Therefore, at first we set-up an experiment to assess on which pre-imaginal honeybee stage (5th instar larvae vs pupae) Varroa would survive longer. Then, we used the stage that gave the best results to feed three groups of mites of presumably different age and assess their longevity: mites of unknown age from recently sealed honeybee worker cells, mites of unknown age after a reproductive cycle in artificial cells, newly born mites from artificial rearing cells. Concurrently, we tested Varroa survival on frozen host and on honeybee larva homogenate served through a membrane.

We found that Varroa can survive longer on larvae than on pupae (median survival: 24 vs 8.5 days, respectively). Overall, Varroa can survive up to 6 weeks under lab conditions on honeybee larvae. No differences were found in the longevity of mites according to their presumed age. Survival on frozen host is significantly longer (median survival: 3-4 days) than the negative control (mites kept without any food source) but still far away from the survival on the living host. Out of the tested membranes in the artificial feeding experiment, the 5 μ m polyethylene film proved to be perforable by Varroa, although mite survival under these conditions was still limited.

These findings give a starting point for developing an effective method for rearing Varroa in the absence of its natural host.

KEY WORDS: *Varroa destructor*, in vitro rearing, survival, host-parasite interaction, artificial feeding.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Ecological networks between *Bombus* and plants in suburban and natural areas of Eastern Sicily

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Pollinators are a decisive component of biodiversity, because they provide ecosystem services by promoting the reproduction of plants and guarantee food security deriving from the production of many crops. Among the pollinating organisms, bees (Hymenoptera, Apoidea) constitute the most important taxonomic group and, among these, the genus *Bombus* represents one of the most relevant in terms of abundance and for the marked generalism in the use of trophic resources shown by most of the species. Mediterranean areas are undoubtedly hot-spots of biodiversity for Apoidea, and the genus *Bombus* presents 43 species occurring in Italy alone. However, important ecological aspects, such as *Bombus*-plant interaction networks, have not yet been investigated in large areas of the country, particularly in the islands. Here, we have analyzed these interaction networks in an area of Eastern Sicily which is little known from an apidological point of view. The study was conducted from 2018 to 2019 in two locations: a suburban park ("La Rocca di Buticari", municipality of Nizza di Sicilia) and a natural reserve (Fiumedinisi and Monte Scuderi, in the Peloritani mountains). Through seasonal transects (April-July), specimens of *Bombus* were captured and the plants associated with the capture were identified. The bumblebees were identified with molecular techniques (DNA barcoding) and with taxonomic keys when necessary. The data analysis showed that in both studied sites there are 8 species of *Bombus*, altogether belonging to 6 subgenera, with a richness very close to that predicted by the accumulation curves. *Bombus terrestris*, *B. pascuorum* and *B. lapidarius* were overall the most abundant species. The network structures observed in both sites were highly specialized, and greater specialization (greater complementarity and less niche overlap), as well as greater modularity, have been observed in Buticari, the suburban park, which has half the floristic species than Fiumedinisi. The networks resulted not nested in both sites, i.e. there was not a strong co-occurrence of species that establish exclusive relationships and others that enter into relationships with all the elements of the community. Furthermore, an analysis of literature data revealed that environments which are richer in *Bombus* species show networks composed of more specialized species, and that Mediterranean areas show more specialized networks than continental areas. We hypothesize that typically generalist species such as those of the genus *Bombus* in the Mediterranean environment tend to partially segregate their trophic niches to limit local inter-specific competition.

KEY WORDS: *Bombus*;bees;Sicily;ecological networks;specialization.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

***Vespa orientalis*: a survey analyzing its diffusion, damage, and control strategies in Sicilian apiaries**

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In southern Italy, particularly in Sicily, the number of *Vespa orientalis* L. colonies, along with the territory it inhabits, have been increasing over the last 15 years. The causes are not yet entirely clear, though climate change may be playing a fundamental role. The fact remains that this voracious hymenopteran is a dangerous aggressor to domestic bees all over the world. Its repeated sieges during the period from July to October not only confine bees inside their hives, preventing almost all of the activities they carry out in the field, but, in the most serious cases, it causes the destruction of entire apiaries when they are not duly protected. Obtaining data on the population density and the intensity of this hymenopteran's attacks is very difficult, precisely because of its complex bio-ecology. Therefore, the starting point of this work was the creation of a questionnaire, submitted to experts, to verify the qualitative and quantitative damage that hornets cause to apiaries and to obtain further information about the defense methodologies adopted. 122 questionnaires were considered valid for the analysis. The results showed that more than 80% of Sicilian beekeepers believe that the current decline in bees is serious or even alarming. 60% of respondents said they had an anomalous loss of bee families in the previous year, mainly due to poor food availability (49%), followed by wasp attacks (39%). *V. orientalis* is particularly widespread in the Palermo area, with very serious attacks; the situation is less pressing in the Messina, Catania, and Ragusa areas, though the distribution area is expanding. Furthermore, there does not seem to be any correlation between *V. orientalis* attacks and the type of apiary management employed, nor with the bee subspecies or hybrids that are bred. Moreover, the methodologies for controlling this hymenopteran are very heterogeneous and not particularly effective. In conclusion, the need for further in-depth studies of the oriental hornet's biology, distribution, and possible containment methods is evident, as is the need for an assessment of the damage it causes to apiaries.

KEY WORDS: *Vespa orientalis*, apiary die-off, damage, questionnaire.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

**Monitoring wild bees in the National Park of Asinara island (Sardinia, Italy):
comparison between two sampling methods in relation to flowering plants**

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Wild bees contribute substantially to pollination ecosystem services. The well-documented decline of wild bees is a serious threat to food safety and security as well as biodiversity conservation. There is an increasing need to acquire information on native bee communities in natural areas because anthropogenic pressure can alter ecosystem functioning and terrestrial community structure. In this study, wild bees and flora were monitored in the Asinara Island National Park (Sardinia, Italy). This minor island, which covers 51.22 km², is not permanently inhabited and is mainly devoted to tourism and nature conservation.

The present work aimed to compare two sampling methods commonly used for monitoring pollinating insects, i.e., transect and pan traps, in relation to flowering plants.

The surveys were conducted monthly, from February to November 2021. In two transects (2 m x 200 m) characterised by comparable floristic communities, all wild bees present on flowers or inflorescences were captured. Three pan traps stations were also placed along the same paths, each consisting of three plastic bowls (ca. 15 cm diameter and 7 cm height) painted with three different fluorescent colours (blue, yellow and white). All captured bees were identified at species level. While bees were monitored, the species and coverage of all angiosperms visited or visitable were recorded along the same transects.

During the whole observation period, 144 specimens of wild bees were captured in the transects and 656 in the pan traps. However, the number of bee species detected was higher in the transect method than in the pan traps (36 vs. 24). A total of 46 bee species were recorded. The catches made along the transects were significantly correlated with the main flowering period (spring period). On the contrary, catches taken with pan traps were higher in the summer-autumn period, i.e., when the number of flowering species was lower or close to zero. A total of 134 plant species were detected, but only 20 species, all of high pollen and/or nectar value, were visited by the sampled bees.

KEY WORDS: Pollination, Ecosystem, Biodiversity, Pan traps, Transect.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Seasonality and landscape heterogeneity affect pollen collection and the development of *Apis mellifera* colonies in Mediterranean agro-sylvo-pastoral systems

Alberto Satta¹, Matteo Lezzeri¹, Giuseppe Brundu¹, Ignazio Floris¹, Nicola Palmierii¹, Roberto Antonio Pantaleoni¹, Panagiotis Theodorou², Michelina Pusceddu¹

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The development, productivity, and health of *Apis mellifera* colonies are strictly dependent on the availability of floral resources from which they obtain pollen, the main source of proteins and other essential nutrients. In this study, we aimed to explore how seasonality and landscape composition affect the abundance and/or richness, diversity and composition of pollen collected by honeybees in an area of Northern Sardinia (Italy) where agro-sylvo-pastoral systems are largely widespread. These lands, common in the Mediterranean environment, are more stable and show a higher species richness compared to conventional agricultural systems, due to a higher resource diversity and a lower degree of specialisation. In this context, four livestock farms were selected. One was characterised by a predominance of arable crops and another by a predominance of seminatural habitats, whereas the other two with an intermediate condition but different between them. In each locality, four colonies of *Apis mellifera* were placed, and pollen samples were collected and analysed from February to October in 2021. Since pollen collection affects the development of bee colonies, observations on the brood extension was made over time.

We found that season and sampling site significantly influenced the amount of pollen collected. However, the differences observed among study sites were not associated with habitat diversity. This was likely because foragers have selected one or a few main pollen sources from which to obtain their supply. Instead, habitats diversity significantly affected the richness and diversity of pollen samples in addition to the season. However, considering only the main pollen sources, i.e., those detected with the highest relative abundances, we observed a large overlap among all 4 sites regardless of time. In fact, the permutational multivariate analysis of variance, confirmed that pollen composition was not significantly affected by the study sites. Finally, our study also showed a positive effect of the amount, richness and diversity of pollen collected on brood rearing. Because the highest amounts of brood were observed in the sites with a greater presence of semi-natural habitats, where bees visited mainly spontaneous flora, we believe that the quantity and quality of pollen available and thus collected by the honeybees determined the amount of brood reared by the colonies.

KEY WORDS: Honey bee, land use, pollen taxa, sealed brood.

ORAL PRESENTATION



SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Use of honey bees and mason bees as bioindicators of pesticide risk in agroecosystems

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Many cultivated plants rely on insect pollination services to produce fruits and seeds. Together with wildflowers present in agroecosystems, the flowers of these crops provide essential pollen and nectar resources for bees and other pollinators. At the same time, agricultural environment may be contaminated with pesticides that are hazardous to pollinators. Honey bees have often been used to monitor pesticide loads. Yet, it is unclear to what extent the information obtained with the use of honey bees is representative of the contamination risk of other bee species. In this study we used honey bees and mason bees of the genus *Osmia* as bioindicators of environmental quality, in terms of pesticide risk and plant diversity, in 34 Italian farms. Our results show that honey bees and mason bees interact with the agricultural landscape in complementary ways, together providing a more complete picture of pesticide contamination in the agroecosystems. Analyses of food provisions of the two species revealed that honey bees and mason bees intercepted different compounds. In agreement with their shorter foraging range, mason bees intercepted mostly pesticides used on the target crop, and their overall pesticide risk levels were not influenced by plant diversity. Conversely, honey bees, with a longer foraging range, visited a wider array of floral resources, and their overall pesticide risk decreased with increasing plant diversity. Our findings confirm that diversifying flowering resources in agroecosystems can enhance honey bee health and mitigate pesticide exposure, but this strategy may not be as effective for mason bees and other solitary bees.

KEY WORDS: Bees, Pesticides, Biomonitoring.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Modelling tools supporting the design of sustainable and resilient agroecosystems

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Reaching a balance between agricultural production intensification while preserving biodiversity and fundamental ecosystem services and ensuring the income for farmers and beekeepers is one of the main challenges to address in the near future. Here we present the results of two modelling tools, an Individual-Based Model (IBM) and a Health Status Index (HSI) for honey bees to support the implementation of actions towards more sustainable and resilient agroecosystems developed within the SafeAgroBee project. The main objective of SafeAgroBee is to contribute to the adaptation and mitigation of the effects of climate change and other drivers negatively influencing the sustainability and the resilience of the agroecosystems within the Mediterranean basin, ensuring the income for farmers and food security.

The IBM simulates the population dynamics of a honey bee colony considering the major role of environmental drivers and land use. The model is composed by several modules simulating i) the biology and physiology of individual bees (e.g., egg-laying, development, mortality, food consumption), ii) the dynamics of food influx and consumption, iii) the dynamics of the cells within the hive, iv) the foraging process and pollination services provision, and v) the characteristics of the landscape (e.g., flowering periods, attractivity, nectar and pollen production, and pollination requirements of a food patch). The model also simulates both the presence and influence of wild pollinator communities in consuming food sources and in providing pollination services, as well as the management actions of the beekeeper. The model allows identifying the number of honey bee colonies that can be economically viable to place in a given area, balancing the needs of both farmers and beekeepers while ensuring the presence and the diversity of wild pollinators communities and the delivery of pollination services.

The HSI is a multi-dimensional indicator that quantifies the health status of a honey bee colony considering the influence of biotic (e.g., pests and diseases) and abiotic (e.g., weather and land-use) drivers. The HSI is based on structural equation modelling approach, which is a widely used methodology for analysing latent variables (e.g., honey bee health), and the assessment of the relation between the latent variable and its indicators to summarise them into a composite index. The HSI allows evaluating the health and productivity of a honey bee colony, identify the relative role of the main driving variables, and comparatively assess the outcomes of different farm management practices or land management strategies.

KEY WORDS: *Apis mellifera*, Individual-based model, Population dynamics, Honey bee health, Wild pollinators health.

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

From biomonitoring to laboratory: exposure, toxicity, and risk assessment to disentangle the complex effects of multiple stressors in bees

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Bees are essential for both food production and biodiversity. Therefore, their global health decline, caused by multiple stressors, carries vast environmental and social implications. Among the main adverse factors to bee health, pesticides and malnutrition play a primary role.

This work integrates various activities aimed at assessing the risk caused by stress, in particular pesticides and malnutrition, on pollinating insects of major environmental and economic importance. To date, environmental risk assessment on pollinators focuses only on single stresses with acute lethal effects on honeybees (*Apis mellifera* L.). However, the real world exposes bees to much more complex and detrimental scenarios.

The main goal of our work is to describe and quantify the realistic effects of multiple stresses on pollinating insects with different ecological traits. We show new approaches for a more accurate exposure assessment that includes sublethal and chronic effects of single and multiple pesticides on both social (*A. mellifera*) and solitary (*Osmia bicornis* L.) bees. With both local and international biomonitoring activities (Piedmont, Italy, and USA) with bees we have demonstrated that multiple pesticides vastly contaminate bees and the environment across space and time (more than two active ingredients per sample on average, with a maximum of 28 per sample). Using laboratory set ups, we have focused on the lethal and sublethal, acute and chronic toxicity of the single and multiple pesticides. We demonstrate that pollinating insects are exposed to sublethal and chronic levels of pesticides and that such effects can cause amplified damages on their survival and behavior (e.g., altered coordination and feeding abilities) especially in the long term and when combined with chemical and nutritional stresses. It is complex to achieve an accurate risk assessment because of the complexity of the environment where bees live and of the effects caused by multiple realistic stresses. An integrative approach, which allows to provide more information on how environmental stresses affect the health of social and solitary bees is therefore essential to adequately protect them. To do this, we show that scientific research and risk assessment activities can be better aligned to complex real-world conditions. This work finally proposes new methodologies and initiatives of environmental biomonitoring and risk assessment aimed at better harmonizing scientific and risk assessment results for a better protection of bees.

KEY WORDS: bees, pollinators, risk assessment, environment, pesticides, nutrition, biodiversity, pollination.

ORAL PRESENTATION

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Biomonitoring and bee health: Efficacy of artificial brood break and related virus evolution in honey bees (*Apis mellifera* L.)

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The health of pollinating insects is declining worldwide, together with their abundance and diversity. A major pollinator is the honey bee (*Apis mellifera* L.), but its health is influenced by a variety of biotic and abiotic stressors. One of the major sources of stress for honey bees is the Varroa mite (*Varroa destructor*) which transmits various viruses and weakens the immune system of their host. Since Varroa mite reproduces and feeds in the brood cells of the honey bee colonies, its population decreases the winter period, when they have no brood. The absence of brood allows beekeepers to further reduce the Varroa population through treatments, which target the Varroa in the phoretic phase (i.e., when the Varroa lives on the body of adult honey bees). However, due to climate change, winter temperatures are becoming warmer and often, the honey bee colonies do not have a natural winter brood break anymore. Moreover, winter treatments against Varroa are less efficient when the honey bee brood is present. This consequently reduces the ability of the colonies and beekeepers to control the incidence of the parasite. To address this problem, beekeepers have developed a forced artificial brood break in winter through the caging of the queen.

Our study aims at assessing the effectiveness of the artificial brood break to reduce the Varroa mite populations in the colonies, reduce honey bee susceptibility to five major bee viruses, and strengthen honey bee populations. Focusing specifically on the quantification of the beehive viral load, we compare three different treatments: i) hives with natural brood break, ii) hives without natural or artificial brood break, and iii) hives without natural brood break but with artificial brood break.

To do this, we sampled honey bees from 240 French and Italian hives for two years (autumn 2021-autumn 2023), three times a year (autumn, spring and summer). We extracted RNA from a pool of 80 honey bees per colony. We then reverse transcribed the RNA into cDNA for quantification by Real Time-PCR. Quantitative Real Time-PCR allowed to quantify five of the most important viruses for bees: Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Chronic bee paralysis virus (CBPV), Deformed wing virus (DWV) and Sacbrood virus (SBV). The first results on the beehive viral load depending on the Varroa control treatments, allow us to describe the evolution of the viruses and to evaluate the efficacy of the beekeeping practice on artificial brood break in winter. Furthermore, the study may show interregional differences in the sustainable management of beekeeping. Consequently, this can help to strengthen interregional cooperation in the beekeeping sector.

KEY WORDS: Biomonitoring, Virus, *Apis mellifera*, Varroa, Brood break, Viral load.

POSTER

SESSION XII

SOCIAL INSECTS AND APIDOLOGY

Honey as representation of specific territories and the importance of formal analysis supporting its characterization: the case of the honey produced in Migliarino–San Rossore–Massaciuccoli Regional Park

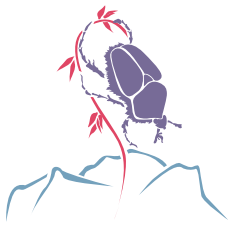
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Honey is a complex matrix, very variegated depending on the floral origin, and can become an important product to valorise specific territories. Migliarino–San Rossore–Massaciuccoli Regional Park (Tuscany) hosts one of the largest sand dunes systems in Italy, where also few beekeeping activities are carried out. The aim of this study was to characterize the honey produced, monitoring the production of an apiary in the park for two years. The production was stable in 2021 and 2022, both for the floristic origin (defined through melisso-palynological analysis), the phytochemical characterization (assessed through gas and liquid chromatography coupled with mass spectrometry), and the sensory and colour analysis. Honey was defined as polyfloral, without dominant pollens and many pollens of the dune habitat plants in the category of minor/sporadic pollens. The allochthonous *Amorpha fruticosa* L. and the ruderal *Rubus fruticosus* L. pollens in the category of the secondary pollens testify the alteration of the expected vegetation, as well reported for Migliarino–San Rossore–Massaciuccoli park. The phytochemical profile of this honey was however very rich in polyphenols and other interesting compounds were coumarine derivatives (4-hydroxycoumarine and 7-hydroxycoumarine), already found in propolis and very likely attributable to resin-laden plants as rockroses (Cistaceae), long chain hydroxyacids typical of royal jelly and nicotinic acid and its analogues (2-hydroxynicotinic acid and 2-hydroxyquinoline). We can reasonably conclude that the features of honey produced in Migliarino–San Rossore–Massaciuccoli Regional Park is not mainly created by *Helichrysum* spp. as described in the technical sheet of this honey and very often on the labels, and the definition of “foreshore” honey in Tuscany PAT list (Traditional Agroalimentary Products list) is not totally descriptive of the honey produced in the area. All the flora composing the ecosystem of the territory, included the allochthonous and ruderal, contribute to the features of this honey, and the analytical support was important to well-describing it, integrating the information of beekeepers and land and agriculture managers.

KEY WORDS: honey floral origin, melisso-palynology, honey phytochemistry, conformity of agro-food products.

POSTER



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P A L E R M O

XXVII Congresso Nazionale
Italiano di Entomologia

12-16 June 2023

Session XIII

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Aleurocanthus spiniferus* (Hemiptera: Aleyrodidae): a possible threat to floriculture and preliminary biological control strategy**

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Aleurocanthus spiniferus (Quaintance), the orange spiny whitefly (OSW), is an invasive pest native to Asia and widespread in Australia, Africa, and the Pacific zone. The first official report in Europe occurred in Apulia (Italy) in 2008. Since then, *A. spiniferus* has been found in different Italian regions, especially in the Centre and the South, and irregularly in some northern zones. Meantime, other OSW populations were intercepted in Greece, Croatia, and Montenegro. *Aleurocanthus spiniferus* is a highly polyphagous pest with a relevant preference for plants belonging to the genus *Citrus*, but it can develop also on more than 90 host plants such as *Vitis* spp., *Malus* spp., *Pyrus* spp., and on different ornamental crops including *Prunus* spp., *Hedera* spp., and *Rosa* spp. OSW-infested plants suffer a severe reduction of photosynthetic activities and show a progressive weakening due to the sup-sucking activity of nymphal stages. In addition, the nymphs also produce large amounts of honeydew promoting the development of sooty mould that can completely cover the plant leaves and fruits. Due to its high adaptability to typical European climatic conditions, *A. spiniferus* is included in the EPPO A2 List as a quarantine pest and it is also classified among harmful organisms listed in the Regulation (EU) 2016/2072. The real chance that OSW could rapidly spread represents a threat to all the European countries where greenhouse floriculture is economically important. Moreover, the currently recommended active ingredients don't have a valid efficacy in the control of this pest. So, investigating ornamental plant species potentially suitable for OSW attack and studying the options for eco-sustainable control of this pest is essential. For this aim, individual leaves of *Poinsettia* sp., *Gerbera* sp., *Chrysanthemum* sp., *Fragaria* sp., and *Mandevilla* sp. were exposed inside isolators to *A. spiniferus* adults collected on infested plants of *Citrus* sp. (females:males 7:3 ratio). In parallel, plants of the same species were placed in infested sites and monitored weekly. Furthermore, preliminary biological control tests were carried out using the predatory mite *Amblyseius swirskii* Athias-Henriot (Parasitiformes: Phytoseiidae). *Aleurocanthus spiniferus* eggs and first instar nymphs were exposed to *A. swirskii* adult females on *Hedera* sp. leaf disc arenas. Then, the number of predated eggs and nymphs was evaluated. The preliminary results suggest that *Poinsettia* sp. could represent a suitable host for *A. spiniferus* rather than the other ornamental crops tested, on which OSW did not seem to be able to complete its life cycle. Moreover, the first observations on the predation rate of the predatory mite show that *A. swirskii* females can predate the 24 hours old OSW eggs, while 6- and 9-days old eggs proved to be unsuitable. The mite *A. swirskii* can also predate the first instar nymphs of *A. spiniferus*, with an apparent preference for those still mobile and with not yet completely sclerotized exoskeletons. Further tests are currently underway to confirm/discard the susceptibility of different ornamental plants to OSW attack and optimize the use of *A. swirskii* in the biological control of this highly invasive pest.

KEY WORDS: natural enemies, orange spiny whitefly (OSW), ornamental crops, predatory mite.

POSTER

XXVII ITALIAN NATIONAL CONGRESS OF ENTOMOLOGY

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SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Evaluation of the possible impact of entomopathogenic fungi and nematodes against soil fauna

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Popillia japonica Newman is a polyphagous invasive alien insect present in the Azores Islands since 1970, in Northern Italy since 2014, and in Switzerland since 2017. Entomopathogenic nematodes (EPNs) and entomopathogenic fungi (EPF) hold a prominent place among biological control strategies. In order to test their efficacy in-field, native EPNs and EPF strains of the Piedmont region were selected, namely *Heterorhabditis bacteriophora* strain POP16 and *Metarhizium robertsii* strain 17/T02. The strains were selected based on their efficacy against *P. japonica* larvae in laboratory tests.

Since the application of such biocontrol agents could affect also other microarthropods present in the treated area we investigated such aspects in the frame of the research project H2020 "IPM-Popillia".

The experimental field was selected in the province of Vercelli where nine plots were treated with EPNs, nine plots with EPF, and nine plots were addressed as control group. Indicators such as *P. japonica* larvae abundance, other arthropod abundance, number of taxa, and biological quality of soil BSQ-ar, were evaluated before and after the treatments.

The data obtained, three months after treatments, highlighted a significant decrease in *P. japonica* larvae in EPNs treated plots, while no significant variation was found in EPF treated plots. No negative effect on non-target edaphic arthropods has been reported in EPF and EPNs treated plots. Interestingly, some taxa showed an increase in individual abundance. Our data suggest that the selected strain of *H. bacteriophora* POP16 could be considered as a good candidate for *P. japonica* integrated pest management with no impact on non-target species.

KEY WORDS: alien invasive species; entomopathogenic fungi, entomopathogenic nematodes; non-target species; *Popillia japonica*.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Aganaspis daci*, parasitoid of *Ceratitis capitata*: welcome to Italy!**

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It is known that the expansion of the distribution area of a biotic entity is a dynamic process in which human involvement may be remarkable. However, following to the introduction of allochthonous organisms, the objective to prevent variations in the biocenotic structure and associated interrelationships in natural and/or artificial habitats appears unrealistic. For this purpose, in the last decades, a regulation invoked by environmentalists, and not only, has been approved and applied in European countries. This regulation requires the risk assessment of the introduction of allochthonous species imported for the biological control of invasive species. The preparation of the risk assessment is time-consuming and expensive, also for overcoming bureaucratic barriers; consequently, the authorization to release the biocontrol agents is obtained after several years. Furthermore, data acquired by laboratory tests may be surely not reliable in the open field. An emblematic example is the biological control program of the brown marmorated stink bug *Halyomorpha halys*, native to Asia and recorded for the first time in Italy in 2012. The authorization for the first release of its own exotic parasitoid *Trissolcus japonicus* has been obtained in 2020! In the era of “organic” and “eco-sustainable” agriculture, it is really paradoxical that the classic biological control of insect pests finds strong barriers that favor the use of pesticides. This critical situation is lessened by the fact that rules and controls cannot prevent the accidental introduction of predators and parasitoids of harmful species in new environments. These events, with the exclusion of scientific literature, are completely overlooked. In this context, we report on the presence in Campania, for the first time in Italy, of *Aganaspis daci*, a parasitoid of several tephritids. From the introduction attempt to France in 70s, *A. daci* was recorded in Greece, the first European country, in 2003, then in Spain in 2009.

In Italy, the species has been obtained from wild puparia of *Ceratitis capitata* reared from larvae developed in infested fruits (*Citrus* spp. and *Prunus persica*) collected in fields in Naples (Sant’Agnello, Palma Campania) and Avellino (Quindici) in autumn 2022 and early winter 2023. The adult of *A. daci*, 2-3 mm in length, is recognizable for having a black, compressed body, yellow ochraceous legs, female antenna 13-segmented, clava with 9 subglobular segments, male antenna 15-segmented, longer than wide, with 3rd segment slightly bent, 2.5× as long as 4th. The species is known as a larval-pupal, solitary, multivoltine and polyphagous endoparasitoid. Recorded hosts of *A. daci* are several tephritids, but the preferred host in open-fields is *C. capitata*. In Campania, monitoring surveys are in progress on the parasitoid associated with *C. capitata* and potentially with *Bactrocera dorsalis*, with the purpose to collect data on its distribution, phenology, and parasitic activity..

KEY WORDS: Accidental introduction, Campania, larval-pupal parasitoid, medfly.

POSTER



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Repellent and toxic effects of essential oils and hydrosols against aphid vectors for the control of cucurbit viruses

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In the last decades, natural compounds have gained attention in life sciences due to their wide-range biological properties. They are used in the pharmaceutical and food industry and are widely investigated for their potential use in agriculture. Indeed, the rising concern and increasing regulations on synthetic pesticides, which cause drug resistance and affect human health and the environment, has fostered the urgent need for sustainable alternatives to be integrated into pest management programs. Among natural compounds, essential oils and their associated hydrosols are being widely screened to control plant pests, exerting antiviral, antimycotic and antibacterial actions. Also, the toxic, repellent and antifeedant activity of essential oils and hydrosols has been successfully tested against arthropods, and environmental protection agencies have started to register their use.

This study investigated the potential use of essential oils and related hydrosols obtained from *Foeniculum vulgare* Mill. and *Mentha suaveolens* Ehrh. against zucchini yellow mosaic virus (ZYMV, Potyviridae) and its aphid vector *Aphis gossypii* Glover (Hemiptera: Aphididae). The chemical characterization of extracts by GC-MS indicated that *F. vulgare* and *M. suaveolens* hydrosols were mainly constituted of fenchone and decanenitrile, respectively, while essential oils displayed a more complex composition. These extracts proved to have anti-viral activity on ZYMV-infected *Cucurbita pepo* L. plants in vivo, by decreasing virus titre and stimulating plant defence response. They were further tested for their potential repellent and toxic effects against the aphid vector in laboratory bioassays. The repellence was assessed by performing choice tests, and all the compounds showed to efficiently reduce the settling of *A. gossypii* on treated zucchini leaves compared to the untreated control. Essential oil from *M. suaveolens* was the most efficient, repelling around 90% of the tested adults for at least 24h after treatment. This repellent effect can potentially contribute to delaying the aphid feeding activity and thus reduce the chance of virus acquisition and transmission. The toxicity was evaluated as mortality and parthenogenetic reproduction rates of *A. gossypii* adults on treated vs untreated zucchini leaves. Significant toxic effects were only shown by *M. suaveolens* hydrosol that halved the aphid population in just one day and significantly reduced the offspring production of the remaining adult specimens.

The direct antiviral activity, as well as the effects observed on *A. gossypii* candidate essential oils and related hydrosols obtained from *F. vulgare* and *M. suaveolens* to be included in an integrated pest management of cucurbit crops, to potentially combine the control of the virus and the vector within the same treatment.

KEY WORDS: *Foeniculum vulgare*, *Mentha suaveolens*, *Aphis gossypii*, potyvirus.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Cold storage of *Ganaspis brasiliensis* immature stages for optimized classical biological control of *Drosophila suzukii*

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The importation, rearing and release of the Asian parasitoid *Ganaspis brasiliensis* (Ihering) (Hymenoptera: Figitidae) is ongoing in the major countries threatened by its host *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). Despite wasp mass rearing and release planning are expensive and time-consuming procedures, cold storage technique could allow to optimize parasitoid production and the host-parasitoid synchronization during field releases. In this study, third instar larvae, pupae and pharate adults of *G. brasiliensis* were exposed to 10 or 15 °C for 2, 4, 6 or 8 weeks to assess the emergence rate and reproductive output of emerged adult females. Developmental stage and storage duration were the main factors affecting the emergence of parasitoid. Larval stage had the lowest survival and emergences were recorded only following exposure at 10 and 15 °C for two weeks. Survival of stored pupae was significantly lower than the control for both temperatures and adults emerged within six and four weeks of storage when exposed to 10°C and 15°C, respectively. On the contrary, parasitoids emerged from pharate adult stage within 2 weeks of exposure regardless temperature and with emergence rates significantly similar to the control treatment. Fertility and sex-ratio of progeny significantly decreased following pupal exposure at 10°C for six and four and six weeks of storage, respectively. However, not enough emergences allowed such assessment on the larval stage. Results suggest that a short storage of *G. brasiliensis* as pharate adult at low temperatures would allow to successfully store the parasitoid prior of releases delaying their emergence and ensuring fitness of emerged wasps.

KEY WORDS: invasive species, low temperatures, parasitoid, mass rearing, spotted wing drosophila.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Optimization of the female removal technique for the *Cydia pomonella* (Lepidoptera: Tortricidae) management in apple orchards

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Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is an insect pest of apple, pear, quince, and walnut trees. Widespread in all continents, its management is necessary to limit crop losses and the repeated use of insecticide is nowadays crucial to control the infestations. There is a need to develop new alternative solutions to support organic and integrated management of this pest, also considering the recent “farm to fork” strategy within the “European Green Deal” set by the European Community, which requires a 50% decrease in pesticide use and a 25% increase in organically managed areas within 2030. In recent years, through international collaboration, we developed an innovative attractant based on volatile organic compounds effective on *C. pomonella* females. It consists of a non-pheromone blend of kairomonal substances already known to be mediators of plant-insect interactions: ethyl (2E,4Z)-deca-2,4-dienoate (pear ester), (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), 6-ethenyl-2,2,6-trimethyloxan-3-ol (pyranoid linalool oxide, LOX) and acetic acid. This 4 component attractant has renewed the possibility of implementing control techniques based on female moth mass trapping. In 2022, an extensive field study aimed to evaluate the feasibility of this new technique has been started in Trentino-Alto Adige region (Italy), considering six trials of 1-hectare adjacent paired apple plots managed with the same grower spray program of both organic and integrated farming. The treatment consisted of 60 non-saturating bucket traps per hectare lured with the 4-component lure. Each plot treated with mass trapping was compared with a control plot (without traps) in terms of *C. pomonella* damage recording the fruit injury across the season. Total *C. pomonella* captures, the sex ratio and the mating status of the trapped females were recorded to evaluate the performance of the female removal technique. A high trapping efficacy has been demonstrated with an average of 13.15 ± 7.83 (mean \pm SD) captures per trap over the 15-week period, removing an average of 826.25 ± 487.27 *C. pomonella* adults (sex ratio) per hectare. Little or no non-target species were recorded within traps. Further studies, planned for season 2023, will better understand the relationship between moth mass trapping and apple damage reduction using the female removal technique.

KEY WORDS: mass trapping, organic pest management, acetic acid, codling moth, kairomones, linalool oxide, codling moth, pear ester.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Surveys on the distribution and parasitisation rate of *Verrallia aucta* (Diptera: Pipunculidae) on *Xylella fastidiosa* vectors in Apulia

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Search for effective parasitoids of spittlebugs is one of the main objective of the research programs tackling the management of the epidemic of *Xylella fastidiosa*. Spittlebugs represent the main group of insect vectors in the outbreaks reported in the Mediterranean countries, with *Philaenus spumarius* (L.) being predominant in the epidemic of *X. fastidiosa* in olive trees in Apulia region (southern Italy). Current management of the vector populations include primarily weed management (tillage, mulching, application herbicides, pyroweeding) to reduce the juveniles' stages, and secondly chemical applications for adults. Although in Europe, during the past few years, surveys for natural enemies of spittlebugs have been intensified, very limited is the current knowledge on the potential biological control agents. Recent studies reported parasitization of field-collected spittlebugs with the endoparasitoid *Verrallia aucta* Fallén in Northern Italy. In this work, we carried out surveys for the presence of *V. aucta* in different spittlebug adults collected in olive groves in the region of Apulia. From 2020 to 2022, a total of 43 locations covering the whole provinces, were monitored. A total of 2750 adults spittlebugs [*P. spumarius* (n. 1284); *N.eophilaenus campestris* (Fallén) (n. 1404); *Philaenus italosignus* Drosopoulos et Remane (n. 62)] were collected by sweep net and dissected under a stereomicroscope, to verify the presence of larvae of the pipunculide. The percentage of spittlebugs found to be parasitized was extremely variable among the three years and the species. None adults of *P. spumarius* collected in 2022 was parasitized, and very low-rate parasitisation (<5.5%) was recorded in 2020 and 2021. Conversely, *N. campestris* resulted more frequently parasitized, with parasitized specimens ranging from 4.8% (2021) to 23.5% (2022). Indeed, two larvae of *V. aucta* were found in adults of *P. italosignus*. DNA from seven representative fly larvae was amplified and sequenced using COI barcoding primers. All recovered sequences were compared with the sequences available in the databases GeneBank and BoldSystems, confirming the specificity with *V. aucta*. These data provide the first evidence on the presence of *V. aucta* in Apulia, suggesting that further investigations to explore the potential role of this parasitoid in the framework of sustainable control strategy for vector populations of *X. fastidiosa* in olive grove. This work was carried out in the framework of the European projects XF-ACTORS (727987) and BeXyl "Beyond *Xylella*, Integrated Management Strategies for Mitigating *Xylella fastidiosa* impact in Europe".

KEY WORDS: natural enemies, parasitoid, spittlebug.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Sublethal effects of a neonicotinoid insecticide on *Trissolcus japonicus*, a biocontrol agent of *Halyomorpha halys*

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In agroecosystems, parasitoids are exposed to lethal doses of insecticides that may have a detrimental effect on their survival, but also to lower doses that could negatively affect their foraging behaviour. *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is a key parasitoid of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in its native area, with high level of parasitism. Here, we tested the effect of a low dose (LD20) of a commercial product of Acetamiprid, a neonicotinoid commonly used against *H. halys*, on *T. japonicus* ability to exploit cues from two stinkbugs, i.e., *H. halys* and the predatory species, *Arma custos* (Fabr.) (Hemiptera: Pentatomidae). In addition, we tested the effect of the neonicotinoid on the learning ability of the parasitoid. We conducted open arena behavioural bioassays with treated versus non-treated female parasitoids to test their response to kairomones left by both pentatomid host species. Both naïve and experienced (i.e., with oviposition experience) females were tested to uncover the effect on their learning ability. Results revealed that the parasitoid successfully exploited cues from both species, although with a small preference towards *H. halys* compared to *A. custos*. Exposure to low doses of the neonicotinoid insecticide altered the foraging duration, although no direct effect on learning was revealed. In conclusion, our study provides evidence that the behaviour of *T. japonicus* can be impaired by exposure to sublethal doses of a neonicotinoid insecticide. More investigations are needed to develop sustainable but effective control strategies against *H. halys*, in particular in those areas where biocontrol with *T. japonicus* is in place.

KEY WORDS: associative learning, acetamiprid, parasitoid.

POSTER

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Current status of the spread of *Aleurocanthus spiniferus* and its natural enemies in Sicilian citrus groves

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In 2020, the thorny citrus fly, *Aleurocanthus spiniferus* Quaintance (Hemiptera, Aleyrodidae) was recovered for the first time in Sicily, in the urban areas of Catania, Syracuse, Palermo, and later in the citrus groves of Caltagirone and Grammichele (Province of Catania). After being reported in Puglia in 2008, the species has spread to many Italian regions and other Mediterranean countries (Croatia, Montenegro, Greece and Albania). Its presence has been reported on wild and ornamental plants, and infestation in Sicilian citrus groves has been reported for the first time. The species is included in the list of quarantine pests EPPO A2 and therefore the Italian phytosanitary services are applying phytosanitary containment measures to mitigate its further spread.

In 2021, a monitoring activity was initiated to follow the infestation trend and diffusion to study the biology of *A. spiniferus* and to verify the presence of natural enemies of the whitefly. Monitoring took place every two weeks through direct observation and sampling of the plants and with the use of yellow chromotropic traps.

Results on the monitoring of *A. spiniferus* and the presence of its natural enemies are reported; the role of some predators as potential biocontrol agents of citrus spiny whitefly in Italian citrus groves is also discussed.

KEY WORDS: Whiteflies, citrus, biology; predators, biological control.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Validation of the PESTonFARM model for the management of Mediterranean fruit fly infestations

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The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera, Tephritidae), is considered one of the most damaging pests in the World, as it is able to infest the fruit of more than 300 species of plants including many commercially important ones; it is also able to adapt to a wide range of climatic zones. In those countries where it is not present, the pest is subject to very strict phytosanitary regulations that impose barriers and controls on imported fruit to prevent its introduction and outbreaks of infestation. Traditionally, the management of *C. capitata* infestation has been done with the use of synthetic organophosphate compounds and pyrethroids. Other control methods include the addition of protein baits to insecticides, mass trapping, attract and kill, biological control, and application of the sterile insect technique.

In this paper, we report results obtained in 2022 in the management of *C. capitata* infestations in three Italian fruit farms using scenarios proposed by PESTonFARM model. The PESTonFARM approach uses a agent-based simulation of behaviour of individual *C. capitata* females based on the combined effects of farm structure, fruit phenology, spatiotemporal distribution and population density of the pest, type and cost of control techniques, and local weather patterns. The simulations obtained in 2022 were based on data collected in three Italian farms, characterized as mixed orchards, relatively isolated and with IPM management based mainly on insecticide treatments. Specifically, the following surveys were carried out: evaluation of host fruit phenology, identification of *C. capitata* overwintering resources, monitoring of the adult population, estimation of the level of fruit damage, and collection of basic socioeconomic information of the experimental farms.

The best scenarios proposed by the PESTonFARM model for the year 2022 were: Farm 1 - use of the attract and kill method on all orchards without any insecticide treatment; Farm 2 - application of the attract and kill technique on late cultivars and optimization of insecticide treatments. In Farm 3, no management changes were made from previous years and served as the control.

The scenarios created by PESTonFARM were found to be adherent to the data collected in the field, showing that the model can be used as a tool to improve the integrated pest management of *C. capitata*. In particular, its application in 2022 allowed a reduction in the use of insecticides, by 66% in Farm 1 and by 33% in Farm 2 in comparison with previous years, in favour of more eco-friendly control techniques and improved the economic balance of the two experimental farms where the suggested management scenarios were applied.

KEY WORDS: *Ceratitis capitata*, monitoring, integrated pest management, predictive models.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Evaluation of the efficacy of some biological insecticides on pomegranate for control of *Aphis punicae* Passerini

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Pomegranate (*Punica granatum* L.) cultivation is clearly expanding especially in southern Italy and throughout the Mediterranean area. The increase of cultivation area leads to several phytosanitary problems including a high presence of the Pomegranate Aphid (*Aphis punicae* Passerini). The eco-nomic impact of this pest on the pomegranate crops is extremely high and its control requires a large number of insecticide applications. However, due to the scarce availability of active ingredients authorized on the crop, corroborants are frequently used even if their efficacy, application methods, and correct positioning in the control strategy are little investigated. To contribute to the knowledge on the insecticidal activity against aphids of some of the most frequently used corroborants, field efficacy trials were carried out in two consecutive years (2021-2022). This is in line with the new European provisions on the sustainable use of plant protection products, which encourage the use of new techniques and new molecules with a low environmental impact. Trials were carried out in Nardò (Apulia Region, Italy) on four-years old plants of *Punica granatum* cv. Ako. The screening involved some of the active ingredients on the market with potential insecticide effect against *A. punicae*. In particular, an extract of plant origin (*Urtica dioica* L.), an inert dust (zeolite of Cuban origin) and some formulations of potassium salts of fatty acids C14 - C20 (soft soaps) were tested. All formulations were applied twice, at seven days intervals after detecting the presence of colony-forming aphids on the shoots. The trials were designed in randomized blocks with four replicates and four plants per plot, in compliance with the principles of good experimental practice (GEP), as defined by directive 91/414/EEC and according to the guidelines EPPO PP 1/135 (4), 1/152(4), 1/181(4), 1/225(2), 1/239(2) and 1/72(2). The efficacy was evaluated three and seven days after each of the two applications on 20 previously pre-marked shoots per parcel. The data analysis was conducted through the use of the ARM software by applying the Henderson-Tilton statistical processing method. Specifically, after the second application, the commercial formulation Flipper, based on potassium salts of fatty acids, showed efficacy levels of 99.3% (2021) and 97.9% (2022) which were significantly higher than those of other soft soaps tested. This difference was probably due to the type of fatty acids present in each formulation. The zeolite of Cuban origin, characterized by a high presence of clinoptilolite, has reached efficacy levels of 89.1% (2021) and 90.7% (2022), statistically similar to those of Flipper. The efficacy of *O. dioica* extract ranging between 75.1% (2021) and 81.8% (2022) was statistically lower than that of Flipper. Overall, the results of this study showed a satisfactory insecticidal activity against the pomegranate aphid of products with direct action on the cuticle, both chemical (potassium salts of fatty acids) and mechanical (zeolite), assisted by the dehydrating action of high temperatures. The timely application of these products together with an accurate distribution over the entire vegetation can make a significant contribution to the implementation of integrated control strategies for pomegranate aphids.

KEY WORDS: Pomegranate, Bio-control, Aphids, IPM, corroborants.

POSTER

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Investigation of the *Drosophila* spp. parasitoids community in the Veneto region

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Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is an invasive fruit fly native to Southeast Asia. Since its accidental introduction in Italy, in 2008, it has become the major threat to soft-skinned fruits like cherries and small fruits. Furthermore, the pest exploitation of wild host plants in semi-natural habitats contributes to raising infestation in fruit crops making Integrated Pest Management (IPM) control strategies difficult to apply. Despite the indigenous parasitoid community adapted to *D. suzukii*, its action can't be considered sufficient to maintain the pest population under the economic damage threshold.

The Veneto region, since 2021, has been funding a classical biocontrol program based on the introduction of the coevolved exotic larval parasitoid *Ganaspis brasiliensis* Ihering (Hymenoptera: Figitidae) in cherry and small fruits production areas within the regional territory. In this context, an extensive sampling activity was carried out in 2022 on which the present work is inserted, which aims to analyze the natural *Drosophila* parasitoids community composition in the 38 sites where the exotic parasitoid has been released.

The monitoring program has been based on collecting infested fruit samples, both of cultivated or wild species, collected directly from plants or fallen to the ground. From all the samples, the largest possible amount of drosophilids pupae has been removed and stored singularly to assess whether the different parasitoid species emerged from *D. suzukii* or other drosophilids pupae.

In total, 215 fruit samples (188 collected from plants and 27 from the ground) of 23 different host plant species resulted infested by drosophilids. From the total amount of samples developed around 12.700 drosophilids and 2.550 associated parasitoids. The latest emerged from samples, represented by 17 host species, collected in 26 sites. The naturally introduced exotic larval parasitoid *Leptopilina japonica* Novković & Kimura (Hymenoptera: Figitidae) revealed to be the most abundant and frequent parasitoid species. Indeed, it has been detected in 25 sites and 16 host plant species. *L. japonica* specimens represented almost 99% and about 20% of the parasitoids that emerged respectively from the isolated pupae of *D. suzukii* and other drosophilids. While *Leptopilina boulardi* Borbotin (Hymenoptera: Figitidae) resulted to be highly linked to non-suzukii drosophilids, especially the ones infesting ground fallen fruits.

Our research revealed the presence and widespread distribution of *L. japonica* in the Veneto region and underlined its role in the biocontrol of *D. suzukii*. Moreover, this work represents the baseline to assess how, in a variegated scenario as the Veneto agricultural landscapes, the *D. suzukii* parasitoids community will change in the following years due to the introduction of *G. brasiliensis*.

KEY WORDS: *Drosophila suzukii*, *Leptopilina japonica*, *Ganaspis brasiliensis*, exotic parasitoids, fruit sampling.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Effects of the biopesticide *Beauveria bassiana* on *Apis mellifera* and *Polistes dominula*

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Biopesticides are considered a sustainable alternative to synthetic pesticides whose massive use and negative impact on human health and biodiversity is jeopardizing the sustainability of agricultural production. In recent years, however, it has been demonstrated that these products can cause a vast array of adverse effects on non-target insects as well. These effects can affect the physiology and behavior of individuals, with potential repercussions on the maintenance of the colony integrity and survival in the case of social species. In the present work, we investigated the potential detrimental effects of a common biopesticide, the entomopathogenic fungus *Beauveria bassiana*, on the physiology and behavior of the honey bee, *Apis mellifera*, and the paper wasp *Polistes dominula*. These insect species provide important ecosystem services, such as pollination by honey bees, or predation of pest arthropods, in the case of paper wasps. Through experiments carried out under laboratory conditions, following topical exposure of individuals to biopesticide concentrations comparable to those applied in the field, we investigated the impact of *B. bassiana* on crucial individual traits, such as survival, activity, feeding rate and immune ability. Our results show how this agent of control, which use is considered mostly safe with respect to non-target insects, can instead cause a number of undesirable effects which, depending on the species, range from the reduction of individual survival and feeding rate, to physiological alterations in terms of immune response.

KEY WORDS: biopesticide, entomopathogenic fungi, biocontrol, non-target insects, sublethal effects.

POSTER



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Resistance induction in tomato plants reduces the damage caused by the zoophytophagous predatory mirid *Nesidiocoris tenuis*

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Although widely used as biological control agent on tomatoes, the predatory mirid *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) can cause damage to host plants. Recent studies have shown that tomato plants attacked by sucking insects can trigger a series of responses, as the emission of volatile organic compound that, acting as elicitors, make them more resistant to herbivores, more attractive to natural enemies, and less susceptible to pathogens. In this work, it was hypothesized that the damage caused by *N. tenuis* might be reduced by eliciting plant defenses. In order to induce a defensive response, tomato plants were exposed to (Z)-3-hexenyl propanoate (Z3HP), a volatile organic compound that was applied through polymeric dispensers releasing a constant rate. Individuals of *N. tenuis* were released onto these plants and onto control plants not exposed to the volatile. In both cases, *N. tenuis* established on the plants, but surprisingly, the number of necrotic rings, which are the characteristic damage caused by *N. tenuis* on tomato, were significantly lower in the Z3HP-exposed plants despite the higher number of individuals. To confirm that the plants exposed to the volatile had activated defenses and to compare it with the response induced by the trophic action of the mirid bugs, the gene expression of markers related to the metabolic pathways of abscisic, jasmonic, and salicylic acids was evaluated. Plants activated by exposition to Z3HP, plants exposed to *N. tenuis* for 24 hours, plants continuously exposed to *N. tenuis* and control plants were compared. The results showed significant differences among treatments in gene expression, particularly for those genes related to salicylic acid. The achieved results pave the way for an improved management of the important natural enemy *N. tenuis* through enhancing plant defenses.

KEY WORDS: (Z)-3-hexenyl propanoate, necrotic rings, gene expression, elicitor, volatile.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Two potential biological control agents for *Toumeyella parvicornis* (Hemiptera: Coccidae): *Exochomus quadripustulatus* and *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae)

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Toumeyella parvicornis (Cockerell, 1897), commonly known as pine tortoise scale, is an alien pest for the European territories affecting the genus *Pinus*. In recent years the species is rapidly spreading, causing several diebacks of the stone pines (*Pinus pinea* L.), its main host plants. The infestations are extensive, since the stone pine is largely distributed in the Italian Peninsula, particularly in urban areas where plants are considered as a landscape symbol. Despite local authorities are controlling the pest through endotherapeutic treatments, it is necessary to investigate an alternative control strategy which may also be applied in natural systems such as the pinewood forest, a forest type widely distributed along the Italian coastal areas. We tested the activity of two predators belonging to the Coccinellidae family, in controlling *T. parvicornis*. More precisely, we focused on two species: *Exochomus quadripustulatus* (L.) and *Cryptolaemus montrouzieri* Mulsant. Laboratory tests were conducted to examine two biological traits of the predators' adult females: i) survival tendency, and ii) attraction response. Semi-field experimentations were assessed by applying net sleeves on infested stone pine twigs and considering three treatments: i) sleeves with *E. quadripustulatus*, ii) sleeves with *C. montrouzieri*, and iii) control sleeves containing only the pest population. *E. quadripustulatus* showed a quicker response to the offered prey than *C. montrouzieri*, with respect to the laboratory condition test. *C. montrouzieri* showed a more consistent predation effect on the prey than *E. quadripustulatus* during the semi-field condition test. Findings showed a promising response of the two species in controlling *T. parvicornis* and endorses further exploration of this potential control strategy.

KEY WORDS: Biological control, tortoise scale, stone pine, urban landscape, natural enemies.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Entomopathogenic potential of *Trichoderma* spp. fungal isolates against *Philaenus spumarius*, the main vector of the quarantine bacterium *Xylella fastidiosa*

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Philaenus spumarius L. (Hemiptera, Aphrophoridae), the meadow spittlebug, is a highly polyphagous species widespread in the Holarctic Region. Nymphs and adults are “xylem-feeders” on nearly all the above-ground parts of many plants. This insect, due to the capability of acquiring and inoculating the bacterium *Xylella fastidiosa* Wells et al. subspecies *paucis*, is considered to be the main responsible for the spread, in Apulia region, of the “Olive Quick Decline Syndrome”. The control of *P. spumarius* is therefore a key element in slowing the spread of the disease. In the frame of eco-sustainable control strategies, an innovative approach is based on the use of bioactive compounds of microbial origin; in particular, entomopathogenic fungi are a source of several bioactive metabolites, many of which are involved in the complex interactions fungi-insect-plant. With the aim to identify bioactive compounds towards *P. spumarius*, entomopathogenicity laboratory tests were conducted using 5 isolates of the genus *Trichoderma* in form of powdered cultures: *T. citrinoviride* Bissett ITEM 4484, *T. atrobrunneum* Rocha et al. ITEM 908 and ITEM 908-5, *T. minutisporum* Bissett DAOM 167069, *T. chlorosporum* Chaverri & Samuels GJS 91-150. The isolates were tested either by injecting a fungal suspension into the spittle secreted by nymphs or by dipping adults in the suspension for ten seconds. None of the powdered fungal cultures tested was bioactive when injected into nymph spittle. Conversely, dipping test showed that *T. chlorosporum* GJS 91-150 exhibits, both as fungal suspension and cell-free culture supernatant, a remarkable lethal effect on adults (97% and 87% mortality, respectively) already within 24h, and that the effect is dose- and time-dependent. Since the lethal effect does not correlate to the presence and growth of mycelium on the cuticle, it can be assumed that the mortality is partly due to the presence of toxic metabolites produced in the culture medium and persisting in the powdered fungal culture. It is relevant to note that immediately after treating adult insects, either with the powdered culture of *T. chlorosporum* GJS 91-150, or the supernatant, some insects exhibited a state of apparent death, preceded by tremors, from which they recovered after a few hours. The observations conducted on such phenomenon, revealed that the percentage of apparently dead insects is concentration-dependent and it decreased within the first hour of exposure, suggesting a temporary paralysis. The use of entomopathogenic fungi and their metabolites with entomotoxic activity may be an effective, low-impact strategy for the control of *P. spumarius*; results obtained encourage further investigation, to be conducted by appropriate bioassays in both confined and field environments.

KEY WORDS: Entomopathogenic fungi, *T. chlorosporum*, Meadow spittlebug, Olive Quick Decline Syndrome, Biocontrol.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Entomopathogenic potential of *Trichoderma* spp. fungal isolates against *Philaenus spumarius*, the main vector of the quarantine bacterium *Xylella fastidiosa*

Sonia Ganassi¹, Carmela Di Domenico¹, Claudio Altomare², Gary J. Samuels³, Pasqualina Grazioso⁴, Dalila Di Criscio¹, Pardo Di Cillo⁵, Laura Pietrantonio⁵, Antonio De Cristofaro¹

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Philaenus spumarius L. (Hemiptera, Aphrophoridae), the meadow spittlebug, is a highly polyphagous species widespread in the Holarctic Region. Nymphs and adults are “xylem-feeders” on nearly all the above-ground parts of many plants. This insect, due to the capability of acquiring and inoculating the bacterium *Xylella fastidiosa* Wells et al. subspecies *pauca*, is considered to be the main responsible for the spread, in Apulia region, of the “Olive Quick Decline Syndrome”. The control of *P. spumarius* is therefore a key element in slowing the spread of the disease. In the frame of eco-sustainable control strategies, an innovative approach is based on the use of bioactive compounds of microbial origin; in particular, entomopathogenic fungi are a source of several bioactive metabolites, many of which are involved in the complex interactions fungi-insect-plant. With the aim to identify bioactive compounds towards *P. spumarius*, entomopathogenicity laboratory tests were conducted using 5 isolates of the genus *Trichoderma* in form of powdered cultures: *T. citrinoviride* Bissett ITEM 4484, *T. atrobrunneum* Rocha et al. ITEM 908 and ITEM 908-5, *T. minutisporum* Bissett DAOM 167069, *T. chlorosporum* Chaverri & Samuels GJS 91-150. The isolates were tested either by injecting a fungal suspension into the spittle secreted by nymphs or by dipping adults in the suspension for ten seconds. None of the powdered fungal cultures tested was bioactive when injected into nymph spittle. Conversely, dipping test showed that *T. chlorosporum* GJS 91-150 exhibits, both as fungal suspension and cell-free culture supernatant, a remarkable lethal effect on adults (97% and 87% mortality, respectively) already within 24h, and that the effect is dose- and time-dependent. Since the lethal effect does not correlate to the presence and growth of mycelium on the cuticle, it can be assumed that the mortality is partly due to the presence of toxic metabolites produced in the culture medium and persisting in the powdered fungal culture. It is relevant to note that immediately after treating adult insects, either with the powdered culture of *T. chlorosporum* GJS 91-150, or the supernatant, some insects exhibited a state of apparent death, preceded by tremors, from which they recovered after a few hours. The observations conducted on such phenomenon, revealed that the percentage of apparently dead insects is concentration-dependent and it decreased within the first hour of exposure, suggesting a temporary paralysis. The use of entomopathogenic fungi and their metabolites with entomotoxic activity may be an effective, low-impact strategy for the control of *P. spumarius*; results obtained encourage further investigation, to be conducted by appropriate bioassays in both confined and field environments.

KEY WORDS: Entomopathogenic fungi, *T. chlorosporum*, Meadow spittlebug, Olive Quick Decline Syndrome, Biocontrol.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Native European ants can discourage host colonization and reduce reproductive success of the invasive ambrosia beetle *Xylosandrus compactus* (Curculionidae: Scolytinae)

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Xylosandrus compactus (Eichhoff) (Coleoptera: Curculionidae: Scolytinae) is an invasive fungus-farming beetle species native to Asia and able to cause serious damage to a broad range of natural and cultivated plants worldwide, exerting significant ecological and economic costs. Its biology makes conventional control strategies often ineffective, while little is known about its natural enemies. In the present study, we evaluated the predatory interactions between *X. compactus* and four species of native Euro-Mediterranean ants that usually forage on plants: *Crematogaster scutellaris* (Olivier), *Tapinoma magnum* Mayr, *Temnothorax affinis* (Mayr), and *Temnothorax mediterraneus* Ward, Brady, Fisher & Schultz. Results indicate that ants are able to kill the beetle foundresses and reduce their offspring. Moreover *X. compactus* females avoid nesting in twigs previously visited by ants. At least *T. mediterraneus* can fully invade *X. compactus* nests, killing larvae, pupae and adults. These results encourage to explore possible applications of ants in the biological control of *X. compactus* and the ecological implications of these interactions in the field.

KEY WORDS: Biological control, ambrosia beetle, Hymenoptera, Formicidae, Invasive pest, Predators.

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Biological activity of garlic essential oil-based bio-insecticides against *Spodoptera littoralis* Boisduval

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The eco-toxicological, environmental and social consequences of the widespread and often indiscriminate use of synthetic insecticides in agriculture have led researchers to find more sustainable alternatives to conventional insecticides. Among the alternative solutions, botanical extracts, and in particular essential oils (EOs), are receiving significant attention from researchers and consumers. EOs, due to their wide distribution across the globe, relative affordability, rapid degradation, and proven biocidal activity, seem to be the perfect candidates for the development of innovative and environmentally sustainable insecticide formulations. The objective of the trial was the development of an EO-based insecticide formulation of garlic (*Allium sativum* L.) and its evaluation against the lepidoptera pest *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae). Experimental activities included the development of a nano-formulation based on garlic EO and the evaluation of its biological activity against *S. littoralis* larvae. The development of the nano-insecticide was carried out using the spontaneous emulsification process combined with sonication. The physicochemical characteristics of the developed formulation (Particle size, polydispersion index and surface charge) were analyzed by dynamic light scattering (DLS) apparatus. The evaluation of larvicidal activity was carried out by testing the efficacy of 8 concentrations (3-0.25%) of EO and 1 control treatment (water only). Each treatment was replicated 6 times using, for each replication, 5 second-instar larvae. For each concentration tested sweet pepper leaf discs were treated by immersion and, after air-drying, they were placed in arenas (5.5 cm in diameter) together with the larvae. Mortality was observed daily, and was recorded 24, 48 and 72h after treatment. The developed nano-emulsion had an average particle size of 141.0 ± 1.375 nm, Pdl values of 0.146 ± 0.009 and a surface charge of -27.4 ± 1.91 mV; no phase separation was highlighted during the experiments. The biocidal activity of the developed formulation against larvae showed a dose-dependent mortality pattern that fits the Probit model. The data obtained from the experiments highlight the potential of the use of garlic EO in the formulation of botanical insecticides that, suitably formulated, could be evaluated for the inclusion in biological and/or integrated control programs against *S. littoralis* larvae.

KEY WORDS: Key-words. Botanical extracts, Cotton leafworm, sweet pepper, Bio-pesticides.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

In-field evaluation of symbiotic control against *Halyomorpha halys* in hazelnut groves

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Halyomorpha halys (Hemiptera, Pentatomidae) is a phytophagous insect native to Eastern-Asia, now widespread in several regions of the Northern hemisphere. This species causes severe damages to many cultivated plants and is particularly detrimental for hazelnuts. Chemical control strategies used so far are mainly based on broad-spectrum insecticides, enhancing the risk of threatening beneficial insects and resulting harmful for the environment. Among the sustainable control strategies, symbiotic control stands out as a promising approach. This strategy is based on treating the egg masses with antimicrobial substances for preventing the newly hatched insects from acquiring the primary endosymbiont '*Candidatus Pantoea carbekii*'. The use of a commercial biocomplex, Dentamet®, to treat the egg masses under laboratory conditions inhibits symbiont acquisition, but field evidence is still limited. The objective of this work is to measure the real efficacy of symbiotic control in Piedmontese hazelnut orchards. An experimental hazelnut grove was selected to develop a symbiotic control plan, by applying subsequent treatments with Dentamet® after the beginning of the oviposition period of *H. halys*. Sentinel egg masses glued in the centre of a cardboard tag were placed in different position all throughout the plant canopy immediately before the treatments. Water sensitive paper was attached to the boards to assess the wetting percentage. After each treatment, egg masses were collected and reared in controlled conditions. Nymphal mortality was checked daily; at the end of the trials, insect samples were collected to measure symbiont infection. The egg mass position on plants and in the field was correlated to the wetting percentage resulting from treatment. Nymphal mortality within second instar was correlated with elimination or lower acquisition of primary endosymbiont. Tag wetting percentage resulted significantly correlated with the position in field and on canopy. Mortality was significantly higher in nymphs hatched from treated egg masses compared to nymphs coming from untreated ones. Symbiont acquisition was not totally prevented in field conditions, but a reduced symbiont concentration was observed in dead nymphs. Taken together, our preliminary results confirmed that field treatments with symbiont-targeting substances successfully reach the egg surface and result in increased nymphal mortality after emergence. Finally, after field treatment *P. carbekii* is not completely eliminated, but the observed reduction still causes a significant increment of mortality. The persistence of a low symbiotic density prevents from the selection of insect populations that can tolerate the absence of *P. carbekii*.

POSTER

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Role of strategies targeting insect microbial symbioses in integrated pest management plans

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The implementation of low-impact pest control is currently considered a priority in global policies; among the alternative techniques that have been recently proposed as an alternative to the use of insecticides, symbiotic control is of particular interest. This strategy involves the manipulation of symbioses between insects and microorganisms, such as by disrupting obligate associations with bacteria, in order to contain pest populations or pathogen transmission. A shifted control target from the insect to symbiotic bacteria can be guaranteed using low-impact substances, offering a potential contribution to the reduction of chemical input in agriculture. However, evaluation of the compatibility between this technique and other strategies currently used for pest management is necessary to develop and apply sustainable control plans. Specifically, recent findings will be reported on the possibility to perform multi-target treatments, as well as on the impact of anti-symbiont treatments on non-target organisms, including natural enemies employed in biological control programs. Application areas in which symbiotic control can build synergy with other techniques will also be presented, taking advantage of the nonlethal harm observed for some insects, which can promote the antagonistic effect of other stressors. In particular, knowledge will be reviewed about two families that include pest species of extreme economic importance, namely Pentatomidae in the order Hemiptera and Tephritidae in the order Diptera. Finally, operational limitations that may arise in the application of symbiotic control will be listed. Particular attention will be addressed to the absence of specific label directions for use against insects, the need to increase the number of applications for multi-target treatments, and possible effects that are still not fully known on the different components of the agroecosystem in which the applications are planned. These critical issues highlight the need to conduct further studies aimed at evaluating the efficacy of other active substances or biocontrol agents in altering insect microbial symbioses, investigating more target species and considering the effects on non-target species. Increased knowledge is also a key factor to promote the development of regulatory policies for products targeting insect symbionts to make these strategies readily available as part of low-impact crop protection plans.

KEY WORDS: Biological control, Integrated Pest Management, Pentatomidae, Symbiotic control, Tephritidae.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Effect of Alfalfa saponins in the control of the Japanese beetle *Popillia japonica*

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Popillia japonica Newman (Scarabaeidae: Rutelinae) is an invasive pest, native to Japan and established in 28 US countries and several provinces of Canada. In Europe it is established in the Azores, Italy, and Switzerland. Given the high reproductive rate, the dispersal ability, and the polyphagous diet, *P. japonica* could elicit important economic, environmental, and social impacts, being classified as a quarantine pest of priority interest for the EU (EU Reg. 2019/1702). Sustainable and effective containment strategies, alternative to chemical methods, are essential to limit its spread in newly infested areas with favourable climatic and environmental conditions. Saponins are biologically active molecules widely distributed in plants, displaying a well-known repellent activity combined with a mortality effect against insects. Saponins are particularly abundant in Fabaceae and especially in the genus *Medicago*. *Medicago sativa* L. is the most cultivated forage crop in the world and represents an important source for the extraction of these bioactive molecules. Although the effect of saponins on insect survival and feeding strategies is known, information regarding their direct effect on *P. japonica* are lacking. In this work, we aimed to investigate alfalfa saponins regarding their possible effects as repellent, feeding deterrent, and/or biocidal capability against *P. japonica* adults. Different concentrations of alfalfa saponins have been applied to the leaves of two of the most susceptible host plants: hazelnut (*Corylus avellana* L.) and grapevine (*Vitis vinifera* L.). Under laboratory conditions, a food deterrence effect and a significant mortality rate were observed using *C. avellana* leaves treated at increasing saponin concentrations, ranging from 1% to 5% w/v. Semi-field condition experiment supported the food deterrence effect, as a significant food preference was observed for untreated plants of *V. vinifera* compared to treated plants. The promising results obtained suggest that alfalfa saponins could represent a potential eco-friendly approach for Japanese beetle control.

KEY WORDS: invasive alien species, biopesticides, Integrated Pest Management, *Medicago sativa*.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Surveys on egg parasitoids of stink bugs (Hemiptera Pentatomidae) in the Sardinia Island

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Among the potential biological control agents (BCAs) of the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera Pentatomidae), numerous studies have focused on egg parasitoids because they seem to play an important role in containing the pest. The use of egg parasitoids as BCAs in classical biological control programs requires special attention in assessing their impact on indigenous biocoenoses. In Sardinia, where the introduction of *Trissolcus japonicus* (Ashmead) (Hymenoptera Scelionidae) is part of the National Biological Control Program for the control of the spread of *H. halys*, preliminary surveys have been carried out on egg parasitoids of Pentatomoidea, particularly of the family Pentatomidae.

Since 2020, sentinel egg masses of pentatomids, obtained from laboratory-reared adults of *Ancyrosoma leucogrammes* (Gmelin), *Dyroderes umbraculatus* (F.), *Graphosoma lineatum* (L.), *H. halys* and *Nezara viridula* (L.), were exposed in three sites in the Northwest of the Island. Egg masses were exposed on fruit, vegetable, wild, and ornamental plants in both agricultural and urban area. After 72 hours, the egg masses were collected and reared in the laboratory under controlled conditions waiting for the emergence of parasitoids or nymphs.

A total of 198 egg masses were exposed of which 48 were found to be parasitized by Encyrtidae, Eupelmidae and Scelionidae (Hymenoptera). *Ooencyrtus* sp., *Telenomus* sp. and *Trissolcus* sp1 emerged from *A. leucogrammes* egg masses. *Anastatus bifasciatus* (Geoffroy) and *Trissolcus* sp2 emerged from eggs of *D. umbraculatus*. *Telenomus* sp., *Trissolcus* sp1 and *Trissolcus* sp2 emerged from *G. lineatum* egg masses. *Trissolcus basalis* (Wollaston) emerged from *H. halys* egg masses. *A. bifasciatus*, *Ooencyrtus* sp., *Trissolcus basalis* and *Trissolcus* sp2 emerged from egg masses of *N. viridula*. Some of the parasitoid species collected, although their identification is still waiting for morphological confirmation, are reported for the first time in Sardinia.

Information on the community of egg parasitoids related to the indigenous pentatomid complex present on the island represents the essential preliminary knowledge for evaluating the biocenotic effects of any classical biological control strategy.

KEY WORDS: Scelionidae; *Anastatus bifasciatus*; sentinel egg masses; first record.

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Sown flowering areas sustain multiple functional taxa in wheat agro-ecosystems of Northern Italy.

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The intensification of agriculture has led to fragmentation of agricultural landscape with negative consequences for biodiversity. The need to use low environmental impact strategies is becoming increasingly urgent, as also highlighted by the European Commission in the recent common agricultural policy. In the wake of this change, a number of food companies are planning transformations in their value chains to improve sustainability from field to market.

This four-year project (2018-2021) was aimed at investigating the effectiveness of flowering areas implementation in soft wheat fields (*Triticum aestivum* L.) and, as far we know, represent the biggest large-scale study carried out in Italy until now. The action is a part of a multidisciplinary initiative promoted by an important food company and aimed at developing a document for the sustainable production of soft wheat. Specific aims were to study the effects of flowering areas on: i) the abundance and species richness of the insect pollinators, including wild bees (Hymenoptera, Apidae), hoverflies (Diptera, Syrphidae), and butterflies (Lepidoptera, Papilionoidea and Esperidae); ii) wheat aphid (Rhynchota, Aphidoidea) and bug infestations (Rhynchota, Scutelleridae and Pentatomidae) and iii) the assemblages of biological control agents of aphids (coccinellids (Coleoptera, Coccinellidae) and hymenopteran parasitoids (Hymenoptera, Braconidae)). Insect surveys were carried out in 61 farms where soft wheat was grown located in Northern Italy. In all the farms, insect pollinators were monitored, whereas wheat aphid and wheat bug infestations, and their biological control agents were assessed only in a sub-sample of 23 farms.

Results indicated a general positive impact of flowering areas on multiple taxa involved in pollination and biological control agents, in comparison with spontaneous field margins. More specifically, the effect of flowering areas on the biological control agents varied among the different taxa and was affected by the distance from the field border; in some case, an interaction between the distance and the investigated management strategies (flowering areas vs control) was evinced.

In conclusion, sown flowering areas can be an important tool for promoting beneficial taxa providing multiple ecosystem services in agro-ecosystems. The successful establishment of this ecological infrastructures for both natural enemies and pollinator taxa relies on careful selection of flowering plant species, appropriate management practices, and on the motivation of farmers in the application of sustainable production strategies.

KEY WORDS: flower areas, functional biodiversity, *Triticum aestivum* L., cereal aphids, wheat bugs, biological control agents, insect pollinators, sustainable agricultural production.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Physiological and behavioral bioassays to evaluate the potential use of the Sterile Insect Technique to control *Bagrada hilaris* (Hemiptera: Pentatomidae)

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The most recent widespread invasive polyphagous Pentatomidae, *Bagrada hilaris* (Burmeister, 1835), bagrada bug, is native to Africa, Central Asia and the Middle East (Colazza et al., 2004). It has rapidly spread outside its native range in recent years: now it has an almost worldwide distribution and has recently become a serious concern on Brassicaceae crops. *Bagrada hilaris* was identified for the first time in Europe on the Pantelleria island (Italy) in the late 70s and since then, it has mainly established on caper (Colazza et al. 2004). The control of bagrada bug populations is generally achieved by conventional chemical insecticides. One of the possible alternative methods is the application of the Sterile Insect Technique (SIT), as part of an Area-Wide Integrated Pest Management (AW-IPM) approach. There are no cases of the SIT technique being applied on Hemiptera, due to the feeding activity of sterile released adults (Klassen et al., 2021). However, irradiation of *B. hilaris* adults was initially taken into consideration as a control strategy in a recent study (Cristofaro et al., 2022). The results showed that irradiating males at 64 Gy achieved 90% sterility, and that 100 Gy is the lowest dose to approach total sterility. The application of SIT technique needs laboratory bioassays on the physiological and behavioural responses of the insect to sterilization. The main objectives of this work were:

a) To understand the physiological mechanisms of selection of the sperm by the female.

Since *Bagrada hilaris* is a polyandrous species, it is important to understand whether the female has the skills to make a cryptic choice of the sperm, because if the sperm of the healthy male is preferred for fertilization, the potential of the technique may be nullified.

b) To evaluate if the irradiated males are sexually competitive respect to non-irradiated individuals both in no-choice and choice conditions

c) To evaluate the feeding impact of the irradiated adults, alone or during the mating.

Two types of experiments were carried out, confining bagrada adults according to the following protocol:

1. No choice: female + irradiated male (control, 60, 80, 100 Gy)

2. Choice: female + irradiated male (control, 60, 80, 100 Gy) + non-irradiated male.

Observations of 18-hour pairs of individuals composed of healthy females and healthy or irradiated males were carried out. Mating time and frequency and feeding activity were assessed.

KEY WORDS: Sterile Insect Technique, *Bagrada hilaris*, irradiation, sterility, biological control, pentatomids.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Identification of host plants and new parasitoids species of *Aleurocanthus spiniferus* (Quaintance, 1903) in Emilia Romagna

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Aleurocanthus spiniferus (Quaintance 1903) (Hemiptera: Aleyrodidae), native to China, South and Southeast Asia, was recorded for the first time in southern Italy in 2008 and is currently reported in seven Italian regions. Considered the most destructive whitefly affecting citrus crops, *A. spiniferus* is included in the A2 list of the European and Mediterranean Plant Protection Organization as a quarantine pest. It is a highly polyphagous pest, which attacks more than 90 plant species belonging to 38 families, including many important crops such as *Vitis* sp., *Pyrus* sp, *Malus* sp., *Prunus* sp. Chemical control has proved ineffective against *A. spiniferus*. Biological control mainly relies on hymenopteran parasitoids such as the Chalcidoidea *Encarsia ablerus*, *Eretmocerus* sp. and the Platygastroidea *Amitus* sp, which have been shown to be successful in suppressing *A. spiniferus* in different countries. A survey was conducted in a botanical reserve in Emilia Romagna region to evaluate the preferred host plants and the potential natural antagonists of this pest in the early stages of its invasion in northern Italy. During the summer season, the level of infestation by *A. spiniferus* was evaluated on several plant species of agricultural interest. The presence of natural enemies and their activities were recorded through direct observation and field samples. The results show the *Vitis vinifera* was preferred over different cultivars of *Malus* sp and *Pyrus* sp. Egg predation by beetles of the genus *Delfastus* sp. has been recorded. Furthermore, a new species of parasitoid has been detected and its molecular identification is currently underway. This study lays the foundations for future investigations on *A. spiniferus*, in particular on the presence and implementation of its natural enemies for biological control purposes.

KEY WORDS: Invasive species, Aleyrodidae, quarantine pest, natural antagonists, grapevine.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

The HALY. ID project: an innovative field data acquisition system based on drones and machine learning to monitor *Halyomorpha halys*

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HALY.ID (<https://www.halyid.eu>) is a European project focused on the implementation of technological systems to improve the field detection of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae), known also as a brown marmorated stink bug. *Halyomorpha halys* is a highly polyphagous species native to Asia that has rapidly become a cosmopolite invasive species in the last few years. First officially detected in Italy in 2012, it spread rapidly and quickly became the key pest of fruit orchards in the northern regions, with damage estimated at €588 million in 2019. Monitoring is currently carried out with active methods which are expensive in terms of time and energy of the personnel or with traps baited with aggregation pheromones which do not provide reliable estimates of the pest population and cause a significant increase in damage to the plants surrounding the traps. The HALY.ID project aimed to develop an application for using a drone inside an orchard to timely detect and monitor *H. halys* on pear trees. First, the effects of a drone flying at different heights on the behaviour of *H. halys* were evaluated, quantifying the flight and freezing responses. The results allowed the development of a specific autonomous flight protocol to monitor the presence of *H. halys* in a pear orchard by acquiring a mosaic of images. The implementation of a machine learning data analysis to recognize *H. halys* associated with a specific app allows real-time quantification of the number of pests in the orchard. The whole system permits to obtain a reliable monitoring of the pest with a consistent reduction of time and energies by the operators and can be applied in the future for the monitoring of other pests in other agroecosystems..

KEY WORDS: invasive insects, brown marmorated stink bug, behaviour, pest monitoring, drones, machine learning.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

The genus *Sclerodermus*: biodiversity to support the control of xylophagous beetles

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In the biocontrol of damaging xylophagous beetle species, the genus *Sclerodermus* Latreille (1809) (Hymenoptera: Bethyridae), appears as a good candidate, as its characteristic morphology allows it to search for victims in their feeding tunnels and to move over short or long distances. The genus comprises around 80 species but in many countries there have been no recent revisions. Records, however, go back more than a century. In order to compile knowledge about the genus, 248 articles from 126 journals and 3 books were surveyed, using 25 keywords. The genus is confirmed as cosmopolitan as its presence is reported worldwide. Despite longstanding and wide interest in the genus, the species studied biologically are only 30% of the total described. Studies on *S. guani*, *S. domesticus*, *S. sichuanensis* and *S. pupariae* account for 31%, 12%, 9% and 6% of all studies. In 13% of the sources analysed, the organisms are only referred to as *Sclerodermus* spp. There are many medical-sanitary studies (mostly referring to *S. domesticus*) (n=29, 12%) but the majority deal with the study of behaviour (22%), biological control (19%) and general biology (10%). These contributions have revealed common features in all the species treated in terms of biology and sex ratio. The latter is extremely female biased, with the few males in each brood emerging earliest (protandry). There has been an increase in behavioural studies over the last few decades, mainly due to growing interest in the exhibited quasi-sociality, whereby adult females (foundresses) sharing a host and care for their offspring communally until their maturity. Several studies have shown that social relationships are influenced by the degree of kinship between foundresses. In terms of biological pest control, *Sclerodermus* species exhibit good propensity to be reared in laboratory, an important factor to implement augmentative or inundative control. However, it is evident that the biology of many species is unexplored and that the lack of updated information on their geographical spread and current presence is a major knowledge gap. More information on the regional species richness and its interaction with the xylophagous beetles present would be of great value for a better management of the invasive species.

KEY WORDS: Review, Parasitoid, Biological control, Bioethology.

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Long-Lasting Insecticide-treated Nets: a new strategy to control the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae)

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Popillia japonica Newman is a scarab beetle native to Japan that in the last century has spread into the US, Canada, The Azores, Italy and Switzerland. Due to its potential harmfulness to agricultural crops as well as its ability to spread, this insect has been included within the EU priority pest list since 2019.

In the recent past, chemical insecticides were often used to control this pest. However, growing attention to environmental protection by the competent authorities has encouraged the search for biological control agents and with low environmental impact that limit the spread of chemicals in the field. In this context, a promising technique for the control of *Popillia japonica* with low environmental impact has been developed in recent years and consists in the use of attract-and-kill (A&K) devices. These devices are made up of long-lasting insecticidal nets (LLINs) and an attractant specific to the target pest. In this way, adults of *P. japonica*, attracted by the pheromone, walk across the LLINs for a few dozen seconds. About twenty minutes after contact with the LLIN, the insect paralyzes and eventually dies, depending on the concentration of the insecticide and the contact time between the insect and the LLIN.

As part of the Horizon 2020 project IPM-Popillia, the effectiveness of two LLINs (Storanet[®], BASF[™], active ingredient: α -cypermethrin 1.57 mg/g fiber and ZeroFly[®] Vestergaard[™], active ingredient deltamethrin 3, 85 mg/g fiber) was evaluated based on the number of dead and paralyzed beetles as a function of exposure time to the LLIN. After the laboratory results, the duration of effectiveness of the A&K devices was measured in the field throughout the flight period. Finally, it was experimentally evaluated which was the best A&K ratio per hectare. The results of the laboratory tests showed that both LLINs were effective in inducing beetle mortality, although with some differences in effectiveness. Field tests showed that the efficacy of A&K decreases by about 30% after the first month of application. In subsequent months, efficacy continues to decline, thus pointing out the need to replace LLIN exposed in the field with a new one after about 40 days. Finally, field trials found that application of one A&K device per hectare effectively reduces the population of *P. japonica* in the affected area.

In conclusion, our data provide a practical guidance on the duration, efficacy, and deployment methods of these devices and suggest that the use of attract-and-kill devices in integrated control programs could be suitable to eradicate minor outbreaks, protect valuable crops, or slow down the range of expansion of the pest.

KEY WORDS: Japanese Beetle, *Popillia japonica*, integrated pest management, LLINs, Attract and Kill.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Aculus taihangensis* (Acari: Eriophyidae), a tiny arthropod for the management of Tree of heaven (*Ailanthus altissima*): recent achievements and state of research**

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Tree of heaven, *Ailanthus altissima* (Mill.) Swingle) is a fast-growing deciduous tree native to China, considered a serious invasive species worldwide (Europe, Asia, South Africa, Australia, North, and South America). It competes with autochthonous flora, forming dense stands that displace native vegetation and cause several ecological and socio-economic impacts. In addition, Tree of heaven is also a primary host for two insect pests: the brown marmorated stink bug [(*Halyomorpha halys* (Stål))] and the spotted lanternfly (*Lycorma delicatula* White), both of which impact a diversity of crops, including grapes and tree fruits, and a range of native plant species. While spotted lanternfly is not yet present in Italy, the occurrence of Tree of heaven can increase the risk of spread of this pest into Italy and across Europe. Conventional agronomic control strategies have limited efficacy on Tree of heaven and an environmentally friendly alternate management would be beneficial. *Aculus taihangensis* (Hong & Xue) is an eriophyid mite species occurring on Tree of heaven, recorded in 17 European countries, which could offer a suitable option for its control. In fact, eriophyid mites are obligate plant feeders with high host specificity, efficacy, and long-lasting effects on their hosts and *A. taihangensis* does not seem to be outdone. Preliminary host range tests with 18 nontarget species, 13 of which are relevant in the European context, pointed out that this mite seems to have a narrow host range. Histological observations and impact tests showed that the feeding activity of this mite damages Tree of heaven leaves, reducing the fitness of new sprouts and severely damaging seedlings, reducing their biomass by about 80%. Based on these data, investigations on the use of *A. taihangensis* in an integrated management plan, such as the felling of large trees followed by the inoculation of the mites on re-sprouting plants, are underway. We believe that the combination of specificity, strong impact on the target, and the ability to increase its population to high levels in a relatively short amount of time, make *A. taihangensis* a very promising candidate biological control agent which does not require to be imported, but would rather be redistributed and/or used in an augmentative approach for the control of Tree of heaven in Italy, as well as in Europe, and potentially in other regions with similar climate.

KEY WORDS: *Ailanthus altissima*, *Aculus taihangensis*, biological control, invasive species, eriophyid mite.

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Egg parasitoids of *Halyomorpha halys* in Emilia-Romagna (Northern Italy): impact of *Trissolcus japonicus* releases and influence of landscape structure

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Egg parasitoids are considered the most promising biological control agents for the brown marmorated stink bug (BMSB) - *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) - an invasive pest which has been causing severe damage to orchards in the USA and Southern Europe. Approximately 140,000 individuals of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) were released between 2020 – 2022 in 300 different sites across the Emilia-Romagna region (Northern Italy) within the framework of one of the largest classical biological control projects ever attempted in Italy. The aims of this study were to explore: i) the abundance and distribution pattern of BMSB egg parasitoids across years; ii) the impact of *T. japonicus* releases on BMSB and non-target species; iii) the influence of landscape composition and configuration on parasitoid assemblages. Field monitoring at both release and no-release sites was performed to collect egg masses naturally laid by BMSB and other stinkbugs. Only sites in which at least 7 BMSB egg masses had been collected (71, 68 and 58 in 2020, 2021 and 2022, respectively) were considered for data analyses. A significant increase in parasitism rate by *T. japonicus* was found across years and distribution maps showed a progressive range expansion, thus demonstrating that this species successfully established and overwintered. Although with decreasing impacts across years, the European native parasitoid *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) was found in most of the sites and exerted the overall highest parasitism rate on BMSB egg masses. The exotic species *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae), whose adventive populations had been detected in Emilia-Romagna since 2019, showed a decline especially at sites where *T. japonicus* was released, possibly indicating interspecific competition in the field. Preliminary landscape analyses pointed out that, especially for *T. japonicus*, connectivity of ecological corridors around the sampling sites was a greater driver of parasitization than landscape composition (percentage of seminatural areas, orchards, buildings and arable crops). Although the impact of egg parasitoids on BMSB populations is still not enough to reduce yield losses, the establishment and the spread of the exotic species could become a valuable factor of pest suppression in the next years.

KEY WORDS: classical biological control, brown marmorated stink bug, natural enemies, *Trissolcus mitsukurii*, *Anastatus bifasciatus*.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Comparative study of different garlic essential oil nano-emulsions against the phytomize *Planococcus citri* Risso

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The huge use of conventional pesticides and the eco-toxicological consequences that these molecules have on both the environment and the human health, stimulated the different stakeholder to demand new and eco-friendly pests control tools. Among the green solutions, essential oils (EO), because of some interesting characteristics (insecticidal efficacy, low level human toxicity, etc), seem the ideal candidates to develop new eco-friendly pesticides. Despite these promising potentials, these phytocomplexes are affected by some limitations (degradability, poor solubility in water, phytotoxicity, flammability, volatility) that make difficult the use of the EOs as such in real operating conditions. The encapsulation of EOs inside nanostructures (nanoparticles and nano-emulsions) represent a promising strategy to overcome all the afore mentioned limits.

In this study four different nano-emulsions charged with high amount of garlic (*Allium sativum* L.) EO (15%) were developed. Different production methods (spontaneous homogenization, sonication, assisted emulsification, high pressure, homogenization and their combination) were developed. Insecticidal efficacy of the developed formulations was tested against the second and third instar of the citrus mealybug *Planococcus citri* Risso (Hemiptera: Pseudococcidae).

Garlic EO was chemically characterised (GC-MS) and the developed formulations was analysed to measure the particle sizes, polydispersity index (PDI), surface charge (ζ potential) and the stability over time. Insecticidal activity of the developed insecticide formulations was evaluated through residual toxicity tests. The mortality was evaluated 24 and 48 hours after the treatments.

The developed formulations showed variable particle sizes depending on the production method, and the stability over time was influenced by particle size and ζ potential values.

The results demonstrated that the particle sizes of the different nano-insecticides play a key role on both the insecticidal efficacy and the stability over time. In addition, the results highlighted the potential use of these insecticidal formulations while the production methodologies developed can be further implemented to obtain new and effective tools for the control of harmful insects.

KEY WORDS: *Allium sativum*, citrus mealybug, nanotechnologies, biopesticides.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Establishment, distribution and impact of *Trissolcus japonicus* and *Trissolcus mitsukurii* in NW Italy

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For over a decade now, the brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae) has been a threat to many crops in North Italy. More recently, in the same area, the egg parasitoids *Trissolcus japonicus* and *Trissolcus mitsukurii* (Hymenoptera: Scelionidae) have also been found, which play an effective role in containing populations of *H. halys* in the native area. After surveys carried out in Piedmont, NW Italy, from 2016 to 2018 to assess the presence of native egg parasitoids able to adapt to the exotic host, which led to the detection of *T. japonicus* in 2018, research continued to evaluate the establishment, distribution and impact of the egg parasitoids. Therefore, from 2019 to 2021, three surveys per year (June, July and August) were carried out in 28 sites distributed throughout the region by observing and collecting *H. halys* eggs. In 2020 and 2021, at the site where *T. japonicus* was first detected, weekly surveys were also carried out in the same way from May to October to verify the seasonal abundance of the parasitoid. Since the national biological control program against *H. halys* through the multiplication and release of *T. japonicus* was started in 2020, adults of this species that emerged from eggs collected in sites within 5 km from the release points were considered to belong to the released population.

Overall, 4,717 egg masses (i.e., 123,231 eggs) of *H. halys* were collected, during the 3-year survey. *Trissolcus japonicus* was found in 2019 at 6 sites, in 2020 at 21 sites (including 12 away from the release points) and in 2021 at 19 sites (including 10 away from the release points), with an overall parasitism rate increasing from 4.9% to 13.1%, and a maximum of 37% at one site with release in 2021. *Trissolcus mitsukurii* was first recorded in 2019 at 4 sites, in 2020 at 14 sites and in 2021 at 19 sites, with an overall parasitism rate increasing from 0.2% to 4.7%, and a maximum of 19% at one site in 2021. Through weekly egg collection, *T. japonicus* proved to be synchronized with the exotic host with which it co-evolved, similarly to what was observed in the native range. Therefore, both exotic parasitoids confirmed that they were established in the Piedmont area, also showing an increasing trend in terms of both spread and overall impact on *H. halys* eggs, reaching annual values similar to those found in China. In conclusion, *T. japonicus* and *T. mitsukurii* appear to be promising biological control agents under the current environmental conditions of NW Italy. It will be necessary to monitor in the future how any changes in environmental and climatic conditions will affect their populations, and how the two species will interact in the sites where they are both established.

KEY WORDS: biological control, egg parasitoids, *Halyomorpha halys*, Scelionidae.

POSTER

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Symbiotic control has no adverse effect on the parasitism of *Halyomorpha halys* by egg parasitoids

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The brown marmorated stink bug *Halyomorpha halys* (Stål) is a polyphagous insect, which has a harmful impact on agricultural production in many countries. The lethal manipulation of symbiont vertical transmission, through surface-sterilization of stink bug eggs, has been recently regarded as a promising way for the implementation of control programs against this insect. In an integrated perspective, a major advantage of this strategy is the compatibility with biological control, since it allows an insecticide-free approach. The general aim of this research was to improve the knowledge on the effect of symbiotic control on non-target insects, and to ameliorate the outcomes related to the introduction of this strategy in pest management. In our work, we evaluated the impact on parasitism by the native egg parasitoids *Anastatus bifasciatus* (Geoffroy), *Ooencyrtus telenomicida* (Vassiliev), and *Trissolcus kozlovi* Rjachovskije, as well as by the exotic *Trissolcus japonicus* (Ashmead) and *Trissolcus mitsukurii* (Ashmead) after treatment with the biocomplex Dentamet[®], which shows antibacterial activity. The native wasp species were tested in no-choice bioassays, showing that treatment of the egg masses did not affect emergence percentages, but the non-reproductive effects were often reduced by the biocomplex. The exotic species were used in no-choice and paired choice bioassays. No-choice tests indicated the highest successful parasitoid emergence on biocomplex-treated egg masses for *T. japonicus*, and no preference in the paired comparison with treated with water and untreated eggs. In contrast, *T. mitsukurii* displayed the lowest parasitism after Dentamet[®] treatment in no-choice tests, and preferred egg masses treated with water or untreated in paired choice tests. In addition, no evident negative impact on the fitness of these wasp species was found in short-term, since the progeny number was not affected. Moreover, the wasp fitness was not altered by dysbiotic effects caused by treatments, since no horizontal symbiont acquisition was recorded. Our observations confirmed that treatment of the egg masses did not affect emergence rates and parasitism activity of the tested parasitoid species; moreover, the results confirmed the absence of direct harmful effects on parasitoid females resulting from their contact with egg masses treated with Dentamet[®]. Taken together, our results support the combination of symbiotic control and biological control, especially when performed by exotic egg parasitoids, for the containment of productive losses caused by *H. halys*. Nevertheless, further work is needed to understand the potential long-term impact of the biocomplex on egg parasitoid activity under field conditions.

KEY WORDS: Symbiont-targeted control, Biological control, Brown marmorated stink bug, *Trissolcus japonicus*, *Trissolcus mitsukurii*, Integrated pest management.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Trichoderma* spp. treatments for Asian chestnut gall wasp control: preliminary results**

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Soil harbors numerous microorganisms that can interact in various ways with plants and animals. Some of them live within plants as endophytes, strongly affecting plant activities and vital functions. For example, fungi of the genus *Trichoderma*, which live in the soil and within plant tissues, are considered beneficial to their hosts. It was demonstrated that they promote plant growth and tolerance to abiotic stresses, besides being capable of stimulating defence responses against parasites. Properties of this fungus, demonstrated in various agricultural contexts, are employed in several commercial products that successfully deploy biological control against plant pests. Various methods of applying *Trichoderma* inoculum have been tested. For example, it can be applied to seeds or propagation material, distributed with irrigation water, or injected into the trunk through endotherapy. Knowledge on this topic largely concerns the efficacy of this biocontrol agent on herbaceous plants or young trees against phytopathogenic fungi. Studies on its efficacy on adult trees and against insect pests are less numerous and more recent.

Among insect pests, *Dryocosmus kuriphilus*, the Asian chestnut gall wasp (ACGW), deserves particular attention because of the high damage it has caused to chestnut cultivation, at least until its main natural enemy, *Torymus sinensis*, succeeded in containing its populations. However, in particular years or situations, attacks by the insect can still be severe, compromising chestnut production.

In this study, to measure its efficacy against ACGW, a suspension of *Trichoderma* spp. conidia was injected, through BITE[®] tool, inside adult trees from two chestnut groves in June. Of the selected plants, some were treated while others were left as controls. The inoculum included three *Trichoderma* species (*T. viride*, *T. atroviride* and *T. harzianum*) with a concentration of 1×10^7 conidia/mL. To verify the effectiveness of the treatment against the ACGW, samples of chestnut branches were collected from treated and untreated trees. In the laboratory, ACGW galls were counted and dissected for the presence and health status of ACGW and its parasitoids (*T. sinensis* in particular). Preliminary results were quite encouraging, as although the treatment did not reduce the mean number of galls and cells per shoot, the number of live ACGW specimens decreased significantly in treated trees compared to untreated ones. In addition, the treatment had no adverse effects on *T. sinensis*, which on the contrary seemed to be favoured on treated chestnuts; in fact, parasitization rates significantly increased on treated trees.

KEY WORDS: biocontrol agents, endotherapy, *Dryocosmus kuriphilus*.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

The effect of the sterile insect technique on vibrational communication: the case of *Bagrada hilaris* (Hemiptera:Pentatomidae)

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The painted bug *Bagrada hilaris* is a stink bug native to East Africa, South Asia and the Middle East. In addition to being an agricultural pest in its original areas, it has recently been recorded as an invasive species in the southwestern part of the US, Chile, Mexico and two islands in the Mediterranean basin. Its polyphagous diet allowed it to cause severe damage to many economically important crops, mainly from the Brassicaceae family. Control is primarily pursued through synthetic pesticides that are often expensive, ineffective and harmful to the ecosystem. Recent physiological bioassays to evaluate its potential control through the sterile insect technique (SIT) showed that irradiating newly emerged males at doses of 64 and 100 Gy achieved respectively 90% and 100% of sterility. Moreover, the successful application of the SIT requires that the reared sterilized males can successfully compete and mate with their wild counterparts and be competent in their ability to communicate with females as receiver and/or sender of signals. In this work, the mating abilities of virgin males irradiated at 60 and 100 Gy with untreated virgin females are measured through the study of short-range courtship mediated by vibrational communication. The results indicate that males irradiated at 100 Gy emit signals with lower peak frequencies and mate significantly less than untreated healthy males, not going beyond the early stages of courtship. Conversely, males treated at 60 Gy present vibrational signal peak frequencies analogous to control and mating success comparable to it. These findings suggest that, because of the relationship between sterility and mating success, *B. hilaris* individuals irradiated at 60 Gy are good candidates for the control of this species through an area-wide integrated pest management program that incorporates the sterile insect technique.

KEY WORDS: sterile insect technique, biotremology, biological control, behavioral bioassays, sexual selection.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Evaluation of lethal effects of natural products on *Trissolcus japonicus*

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The brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive pest, native from East Asia, that has spread to many countries in Europe and America. This herbivore has a wide host range, around 300 cultivated plant species, and causes significant economic loss to agriculture globally. Since its first detection in Italy, *H. halys* has caused severe damage to fruit trees and other crops. Chemical insecticides have been used as primary protection means against the spread of *H. halys*. However, in addition to their low sustainability, these insecticides revealed to be largely ineffective. More sustainable methods have been proposed, such as the use of natural insecticides and the release of biocontrol agents. Specifically, the coevolved egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) has been released in several Italian regions to implement classical biological control of *H. halys*. The aim of this research was to evaluate the lethal non-target effects of natural insecticides and particle films on *T. japonicus*. Mortality of adult *T. japonicus* was evaluated using the Munger cell method. Parasitoids were exposed to field dosages of natural and commercial products for a 7-day period, and survival was observed at different time intervals. Tested products included zeolite, diatomaceous earth, kaolin, basalt dust, sulphur, potassium salts of fatty acids, azadirachtin, orange oil, and calcium polysulphide. Sulphur and calcium polysulphide were tested in powder form, while the other products were dissolved in distilled water. Among these products, sulphur, zeolite and calcium polysulphide caused high mortality rates (between 80% and 100%) on *T. japonicus*. Our results will be useful in selecting products that are most suitable for use against *H. halys* in those areas where biological control with *T. japonicus* is in place.

KEY WORDS: egg parasitoid, biological control, integrated pest management.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Novius (=Rodolia) cardinalis* as a biocontrol agent in natural ecosystems:
protection of Sicilian native species on Mount Etna**

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Novius (=Rodolia) cardinalis (Muls.) is an important historical example of classical biological control. This specialist coccinellid predator was introduced from Australia to control the invasive cottony cushion scale *Icerya purchasi* (Mask.) in California, USA in 1888 and the success of this introduction launched the practice of classical biological control. The beetle was introduced to Sicily in 1910 and continues to control the scale in citrus orchards there to this day. *I. purchasi* is, however, polyphagous, and in Sicily it colonizes a number of native species, including *Spartium junceum* L. (Spanish Broom) and *Genista aetnensis* (Biv.) DC.) (Mt. Etna Broom). These are important pioneer plants that colonize the volcanic habitats of Mt. Etna, playing an important role in the reforestation process. Our goal is to investigate whether the biological control agent controls *I. purchasi* on these plants, and if so what level of protection the plants derive from this control. We found both *I. purchasi* and *N. cardinalis* on Spanish and Mt. Etna Brooms in natural habitats on the slopes of Mt. Etna, and our observations suggest strong suppression activity of the scale on *S. junceum*. To investigate the impact of the beetle we covered *I. purchasi* on some branches with predator-exclusion sleeves and are comparing population growth of *I. purchasi* within these sleeves with no-exclusion controls. We are also using laboratory experiments to determine the impact of the scale on three brooms species and the development cycle of the scale on these host plants.

KEY WORDS: biological control, Coccinellidae, *Icerya purchasi*, *Novius cardinalis*, *Genista aetnensis*, *Spartium junceum*.

POSTER

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Effect of temperature on the biology and behaviour of *Trissolcus japonicus* and *Trissolcus mitsukurii*

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Trissolcus japonicus (Ashmead) (Hymenoptera: Scelionidae) and *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae) are Asian egg parasitoids of the invasive brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Biological control using egg parasitoids is considered the most promising long-term solution for *H. halys* management, with *T. japonicus* and *T. mitsukurii* as the best candidates. Information on temperature-driven biology and behaviour is fundamental for understanding the impact of these natural enemies on pest suppression. Laboratory experiments were performed to evaluate the life table parameters of these two egg parasitoids under 3 constant temperatures (21, 26, and 31°C) and the behavioural response along a temperature gradient from 5 to 25°C. Results showed that the net reproductive rate of *T. japonicus* is higher than *T. mitsukurii* at 26 and 31°C but with differences in their sex ratio. The intrinsic and the finite rate of increase at 26 and 31°C were similar between the two species, but at 21°C, these parameters were higher for *T. mitsukurii* than *T. japonicus*. Behavioural response showed that the activity of *T. mitsukurii* starts at a lower temperature than *T. japonicus*. The results obtained here suggest that *T. mitsukurii* is more adapted to lower temperatures than *T. japonicus* that can show high performances under warmer conditions.

KEY WORDS: Biological control, invasive species, egg parasitoids, Scelionidae, *Halyomorpha halys*.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Lobesia botrana* mating disruption with aerosol dispensers in Italian vineyards:
does application density matter?**

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Over the last decade, *Lobesia botrana* mating disruption (MD) programs in European wine-growing areas relied essentially on the use of reservoir dispensers, continuously and passively releasing a species-specific blend of major pheromone components in the agro-ecosystem. Synthetic pheromone constituents are also of interest in other techniques such as autoconfusion, lure-and-kill, as well as in the development of novel devices for MD programs, such as aerosol emitters, nanofibers, and microencapsulated formulations. To the best of our knowledge, the optimal application density of aerosol emitters in Italian vineyards has been scarcely investigated. In the present study, a novel experimental aerosol emitter, i.e., MISTER[®] L, was evaluated for two years at three different densities (i.e., 2, 3 and 4 units/ha), in three Italian regions (Apulia, Emilia-Romagna and Tuscany). The efficacy of MISTER[®] L was assessed comparing it with an untreated control and with two MD commercial formulations, i.e., the passive dispenser Isonet[®] L TT and the active aerosol emitter Checkmate[®] Puffer LB. The tested device MISTER[®] L led to no male catches in pheromone traps as well as to a significant reduction in the number of infested clusters/bunches and nests per flower cluster/bunch. Overall, the effectiveness of this novel aerosol was comparable to that of the two devices used as reference standards, allowing – at the same time – a nocturnal pheromone emission pattern more tailored on the *L. botrana* male flight. According to our data, MISTER[®] L can be considered for effective MD approaches against *L. botrana* at the minimum installation density of 3 units/ha, reducing the overall installation cost of MD programs.

KEY WORDS: European grapevine moth, grape pest, Lepidoptera, sex pheromones, Tortricidae.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Phanerotoma leucobasis* Kriechbaumer, 1894, a potential biological control agent of *Cryptoblabes gnidiella* (Millière, 1867) (Lepidoptera, Pyralidae)**

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The honeydew moth, *Cryptoblabes gnidiella* (Millière, 1867) is native of the Mediterranean basin, but only recently it became a primary pest, especially in vineyards located along the Italian, French and Spanish coastal areas. It is an extremely polyphagous species, feeding and developing on more than 60 plant species belonging to 30 different botanical families, including *Daphne gnidium* (Thymelaeaceae), considered its most common wild host plant.

C. gnidiella females lay eggs mainly on the green parts of the cluster, such as racemes, pedicels and only seldom on the berries. The eggs and the newly hatched larvae, occurring in the innermost parts of the bunches, are difficult to detect, hampering a proper definition of intervention thresholds and reducing the effectiveness of insecticide strategies. Although this pest starts flying in the vineyards in April-May, finding eggs, larvae, or damage on the bunches in May-June is quite rare. The larvae mainly feed on the green parts of the ripening bunches, preferring the late compact-bunch varieties. The progressive decay and dryness of the bunch related to the larval trophic activity is further increased, in the presence of high humidity, by the proliferation of mold and the presence of saprophagous insects.

Despite the increasing relevance of this pest, the studies performed until now failed to identify a reliable strategy for its management and still little information is available on the natural enemies associated to this moth pest in the different areas where it occurs. In this study we have listed the parasitoids associated to *C. gnidiella*, detected in recent studies performed in vineyards and natural areas in Tuscany and Apulia. Of note was the detection and identification of *Phanerotoma leucobasis* Kriechbaumer, 1894, an Hymenoptera Braconidae of subfamily Cheloniinae displaying high parasitisation rates both in the open field as well as in the laboratory. This species, newly recorded in Italy, may be eligible to become a biological control agent of this growing threat for the Mediterranean viticulture.

KEY WORDS: Hymenoptera, Braconidae, vine, biological control, honeydew moth, parasitoid.

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Simultaneous mating disruption of *Lobesia botrana* and *Cryptoblabes gnidiella*

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The European grapevine moth (EGVM), *Lobesia botrana* (Lepidoptera: Tortricidae) and the honeydew moth (HM), *Cryptoblabes gnidiella* (Lepidoptera: Pyralidae) are important pests of grapevine in the Mediterranean region. In the last 20 years, pheromone-based mating disruption (MD) has been successfully employed to manage EGVM populations, allowing a reduction in the use of chemical insecticides in the vineyards, thus safeguarding human health and the environment. On the other hand, no well-established strategy is available for HM management. In the present study, a biodegradable double capillary dispenser (Isonet® LCG BIOX235), which simultaneously releases the synthetic pheromones of EGVM and HM, was evaluated for MD purposes. This experimental device was tested in two study sites (i.e., Apulia and Tuscany) at three different application densities (i.e., 300, 400 and 500 dispensers/ha). The trials were conducted monitoring EGVM and HM populations in MD vineyards, by insecticide treatments (grower's standard) or without treatments (untreated control). For both pest species, simultaneous MD led to a significant reduction of male catches, as well as to a reduction in the number of infested clusters, if compared to the untreated control. As a general trend, no significant differences were found between the three MD dispenser densities tested in our study. Overall, our results showed that the use of Isonet® LCG BIOX235 against EGVM and HM can contribute to the sustainable management of both pests, albeit with more difficulty against HM when highly abundant populations are present. Further field experiments are ongoing to assess the effectiveness of this MD approach.

KEY WORDS: European grapevine moth, Honeydew moth, Pyralidae, Phycitinae, Integrated Pest Management, pheromone.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

**Target and non-target effects of synthetic and botanical insecticides on
Phenacoccus solenopsis and one of its predators**

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Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) is a polyphagous invasive pest, native to North America that has invaded several Mediterranean countries in recent years, becoming a potential threat also for horticultural cropping systems such as greenhouse tomatoes. Recently, *P. solenopsis* has been reported in Italy on solanaceous horticultural plants and ornamental plants, so the development of strategies to control it is of paramount importance. Synthetic chemicals are still widely used for controlling *P. solenopsis* worldwide, but botanicals, namely essential oils (EOs) (e.g. *Allium sativum* and *Mentha x piperita*), may represent a sustainable control tool for its management. On the other hand, entomophagous generalist predators such as the mealybug destroyer *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae) can be exploited for controlling *P. solenopsis* through augmentative and/or conservative strategies. In this context, the assessment of chemical toxicity towards the mealybug and its predator is required when Integrated Pest Management (IPM) programs are adopted.

We first determined the baseline toxicity of different chemicals on *P. solenopsis*. Then, the survival and the orientation behavior of *C. montrouzieri* after 3 days exposure to plants and mealybugs sprayed with insecticidal substances were assessed in the laboratory. For this, we applied the chemicals at different lethal concentrations, previously estimated for the target organism. Among the tested insecticides, systemic ones (e.g. acetamiprid) were the most effective in controlling the mealybug. The results showed that the survival and orientation ability of *C. montrouzieri* significantly differed between the chemicals and concentrations tested, compared to the control. In general, the behavior of *C. montrouzieri* was negatively influenced by synthetic insecticides at higher concentrations, whereas EOs presented a safer ecotoxicological profile. The results showed that EOs can be a valid tool for the sustainable control of *P. solenopsis* compared to synthetic molecules, however, it is important to carefully evaluate the potential side effects of insecticides for their inclusion in IPM programs, to exploit the combined use of natural enemies and selective biopesticides.

KEY WORDS: biological control , IPM, invasive species, tomato, side effects.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Prospects and constrains in the implementation of *Drosophila suzukii* classical biological control

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In the last decade, the invasive fruit fly *Drosophila suzukii* (Matsumura) has become a major pest to the soft-skinned fruit production worldwide. Current integrated pest management exploits different control tools, including conservative or augmentative biocontrol, but relies mainly on insecticides. Classical biological control (CBC) is an approach that is expected to reduce long-term management costs of *D. suzukii* and to increase economic and environmental sustainability of farms. Following foreign explorations, quarantine risk assessment and release approval, a voluntary partnership of researchers from nine Italian regions/provinces set up a three-year CBC program using a Japanese G1-lineage population of the larval endoparasitoid *Ganaspis brasiliensis* (Ihering). Two years after the initial release of *G. brasiliensis*, we discuss the constraints encountered throughout the different work stages, from the importation procedure of the biocontrol agent to its mass-rearing, up to the large sampling effort undertaken in the post-release phase to characterize the biological control and nontarget impacts. Lastly, we provide prospects of *D. suzukii* biocontrol in Italy based on the results of the first two release seasons.

KEY WORDS: Invasive alien species, *Ganaspis*, Figitidae, propagative biocontrol.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Updates on the Italian national classical biological control program against the spotted-wing drosophila, *Drosophila suzukii*.

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Here we present the activities of the Italian *Drosophila suzukii* Working Group coordinating the control efforts against the spotted-wing drosophila, *Drosophila suzukii* Matsumura. The Working Group was formally established in January 2021 on the recommendation of the National Phytosanitary Committee, to define and implement a three-year classic biological control program against the invasive pest using the exotic parasitoid *Ganaspis brasiliensis* Ihering. The first release campaign of the parasitoid, which started in August 2021 following the authorization granted by the former Ministry for Ecological Transition (now Ministry of the Environment and Energy Security), involved seven regions (Campania, Emilia-Romagna, Piedmont, Puglia, Sicily, Valle d'Aosta and Veneto) and two autonomous provinces (Trento and Bolzano). In 2023, the Lombardy and Tuscany regions will also join the release program. The results of the monitoring of the first two seasons, carried out on 100% of the release sites between May and November of each year, suggest that *G. brasiliensis* is establishing in several areas and confirm its specificity towards *D. suzukii*, as it was already observed in laboratory studies.

KEY WORDS: propagative biocontrol, invasive alien species, *Ganaspis brasiliensis*, Figitidae, parasitoid.

POSTER



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

The use of contour maps as a decision-making tool for the control of the brown marmorated stink bug, *Halyomorpha halys*

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Halyomorpha halys (Stål, 1855) (Hemiptera: Pentatomidae) or brown marmorated stink bug (BMSB) is a phytophagous insect native to Asia and present in Italy since 2012. The control methods against BMSB are particularly difficult because the species is highly polyphagous and has a high reproductive rate. The project “CONTR-HALYS - Synergistic approach for the sustainable protection of fruit and vegetable crops against the brown marmorated stink bug (*Halyomorpha halys*)” funded by PSR 2014-2020 Emilia Romagna Region, aims at i) improving the monitoring system to study the degree of infestation of the crop and ii) use this information as a decision-making tool for the control of BMSB. We monitored two crops, tomato and pear trees, located in three agricultural farms within Parma and Piacenza municipalities, respectively. The BMSB monitoring was carried out by using sticky traps lured with aggregation pheromones. The traps were located at the edge of the crop fields and in the proximity of shelter sites (e.g., barn, depot, house, etc.) or microhabitats which may be attractive for the pest (e.g., for the presence of feeding/host plants). At the edge of the crop fields, we seeded an attractive cover cropping made of fava bean and soy (*Vicia faba minor* and *Glycine max*). With the monitoring data we created contour maps that were then used to choose the proper timing to carry out an insecticide treatment with Acetamiprid or pyrethrum on the cover cropping. Our results suggest that contour maps are a good tool to identify hotspots of BMSB infestation, and to evaluate when the target treatment has to be carried out on the cover cropping avoiding contact of insecticide with the principal crop.

KEY WORDS: BMSB, monitoring, pest control, contour map.

POSTER



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Field efficacy and persistence of entomopathogenic nematodes and entomopathogenic fungi in biological control programs against *Popillia japonica*

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Popillia japonica Newman (Coleoptera, Scarabaeidae) is an invasive alien insect first reported in Northern Italy in 2014 and classified as a priority pest (EU Reg. 2019/1702). Since *P. japonica* lives just below the soil surface at the larval stage, entomopathogenic nematodes and fungi (EPNs and EPF) play an important role in the realization of biological control programs.

Previous virulence laboratory assays carried out with EPNs and EPF isolated from soil samples collected in the area infested by *P. japonica* in Piedmont, allowed the identification of the indigenous strains *Heterhorabditis bacteriophora* (POP 16) and *Metarhizium robertsii* (17/T02).

In the frame of the H2020 project "IPM-Popillia", one of the aims is to test, in the short and long term, the effectiveness and the persistence of indigenous strains and commercial products of EPNs and EPF. Regarding *M. robertsii* (17/T02), two different formulations were tested: encapsulated in sodium alginate pellets (SAPs) and colonized barley kernels (FCBKs). The treatment was carried out in the Piedmont region, in three fields with different irrigation management, following a preliminary assessment of the level of larval infestation and the possible presence of indigenous EPNs and EPF.

The analysis of the soil samples, collected at different times after treatment, showed a significant reduction in the number of *P. japonica* larvae in plots treated with EPNs. Instead, the larval decrease was less pronounced in plots treated with EPF. As regards persistence, the EPNs and EPF strains used were also isolated after the winter season and after one year, with differences depending on the formulation used.

In conclusion, our results suggest a good persistence of EPNs and EPF, but a higher efficacy of EPNs in the containment of *P. japonica* larvae.

KEY WORDS: *Popillia japonica*; entomopathogenic nematodes; entomopathogenic fungi; biological control.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

A 4.0 approach to automate monitoring and decision making for the control of *Bactrocera oleae* and *Ceratitis capitata*: the Fruitflynet II project

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Decision Support Systems (DSS) for Integrated Pest Management (IPM) require the processing of information collected continuously in the field, such as weather data, crop phenological stages, spatio-temporal dynamics of pest populations and related damage, to provide agricultural producers and other stakeholders with practical prescriptions to make critical decisions on how, when and where to carry out pest control interventions. New systems for the collection and management of information, developed with the tools of Agriculture 4.0, make it possible to automate the processes of receiving, transmitting and processing monitoring data, allowing time and cost optimization in the use of DSS.

The objective of the Fruitflynet II European project, funded by ENI CBC Med program, is to develop an automated prototype for optimizing the monitoring and management of infestations of two tephritid fruit flies: *Bactrocera oleae* Rossi and *Ceratitis capitata* Wiedemann.

The prototype, which is geo-referenced and connected to the cloud, consists of an automated trap (e-trap) activated with specific attractant and a set of integrated information technology services.

The e-trap is equipped with a camera to collect images of the sticky panel inside the trap itself and send them to a remote server, from which identification and counting of captured fly adults can be carried out through an algorithm, in automatic mode, or through visual observation of the image, in semi-automatic mode.

The electronic part of the device consists of two complementary units: the logic unit, to which the camera is linked, which connects to the cloud; and the power unit, which provides the energy to the system and is powered by a photovoltaic panel.

Among the information technology services being optimized are: digitization of agronomic and landscape information regarding the experimental farm, including the location of trees in the orchards; automatic or semi-automatic counting of flies from the images collected by the e-traps; the decision-making module based on the risk calculated by a specific DSS for each of the two phytophagous insects, which returns prescription maps for the insecticide treatments to be carried out; a module to guide the operator in the field during treatments using a GPS system; and a web application that allows the various inputs and outputs to be visualized and managed.

In the present work, we report the results regarding field verification tests of the various components of the prototype for the two investigated phytophagous insects. It is intended to increase the productive sustainability of the concerned crops, both economically and environmentally, through the rationalization of pest control treatments, in terms of active ingredient used, number of treatments carried out and area covered.

KEY WORDS: tephritids, IPM, agriculture 4.0, DSS..

POSTER



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

**Effect of two *Fusarium* mycotoxins in the *Triticum aestivum*-*Fusarium* spp.-
Sitobion avenae system**

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The English grain aphid *Sitobion avenae* (Fabricius) is a serious pest of both spontaneous and cultivated Poaceae, which may cause plant damage during feeding as well as because of the release of abundant honeydew and transmission of viruses. The damage can be very high when attacks occur on the forming ear, as even a low infestation level can lead to severe yield losses. During wheat anthesis, ears can also be infected by various *Fusarium* species, causing *Fusarium* Head Blight. In Italy, the most abundant *Fusarium* species are *Fusarium graminearum* and *Fusarium avenaceum*, which during plant infection biosynthesise several secondary metabolites, including deoxynivalenol (DON) and enniatins (ENNs). These compounds (particularly DON) show a strong toxic activity towards both humans and animals, while their effects (in particular ENNs and combination of DON+ENNs) on other fungal species (including those belonging to the genus *Fusarium*), some animal tissues, the wheat plant and insects are less known. In this study, the toxic effect of DON and enniatin B (ENB, which is one of the most common ENNs occurring in wheat grains in Italy) towards *S. avenae* was investigated. In particular, the contact effect of the two mycotoxins alone (at different concentrations) and in combination was studied. The experiments revealed DON contact toxicity starting from the concentration of 25 mg kg⁻¹ (ppm) to 1st instar neanids of *S. avenae*, while ENB did not show any activity even at 100 ppm. In addition, the combined effects of the two mycotoxins at 100 ppm concentration showed a slightly reduced effect when compared to DON alone. This study is part of a multidisciplinary research project aimed at understanding the effects of these mycotoxins on phytotoxicity, toxicity on human cell lines, and their possible effects on the development of *F. graminearum* (ENNs) and *F. avenaceum* (DON).

KEY WORDS: Deoxynivalenol, enniatins, toxicity, aphids, *Fusarium* head blight.

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Do ants' scents deter *Philaenus spumarius* trophic activity on olive trees?

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The meadow spittlebug, *Philaenus spumarius*, is the major vector of the bacterium *Xylella fastidiosa*, the agent of a severe vascular disease that is leading to remarkable economic losses in olive production in Southern Italy. Here we tested for the first time, using 40 binary-choice experiments, the potential deterrent effect of ants' scent on *P. spumarius* visits and activity on olive twigs. We found evidence that the scent released by freely-walking *Crematogaster scutellaris* ants significantly reduces the visiting probability by meadow spittlebugs with respect to controls. Our results contribute to open new avenues to manage *X. fastidiosa* vectors and need to be corroborated by further chemical, electrophysiological and behavioural studies to identify the main constituents of *C. scutellaris* scent, responsible of this trait-mediated indirect effect.

KEY WORDS: *Crematogaster scutellaris*, meadow spittlebug, *Xylella fastidiosa*, trait-mediated interactions, ant pheromone, olive twigs.

ORAL PRESENTATION



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Impact and seasonal abundance of *Aphelinus mali* parasitoid of *Eriosoma lanigerum* in apple orchards in NW Italy

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The woolly apple aphid (WAA), *Eriosoma lanigerum* (Hemiptera: Aphididae), is one of the main harmful apple pests, which has recently become an increasing threat also as a result of the withdrawal of use of several active ingredients, including neonicotinoids and organophosphates. Native to North America, in Europe WAA carries out its entire cycle on apple trees, on which it colonizes roots, trunks and branches. Although several natural enemies are present, biological control of this aphid relies mainly on the action of the coevolved specialized endoparasitoid *Aphelinus mali* (Hymenoptera: Aphelinidae), which was introduced as biological control agent in one of the most successful cases of classic biological control. *Aphelinus mali* accomplishes several generations per year on the host, overwintering as a pupa or larva in the body of a parasitized individual, and reaching high parasitism levels at the end of the season. To evaluate the role of the parasitoid in containing WAA infestations, two-year surveys were performed in seven apple orchards (five organic and two IPM) located in northwestern Italy in 2021-2022. Through field surveys and laboratory analysis, the following were ascertained: I) the presence of *A. mali* in the orchards using yellow sticky traps; II) the parasitism trend by observation of WAA colonies on 10 shoots per orchard, identified and marked at the beginning of the season; III) the parasitism index by sampling 10 randomly selected shoots per orchard and observation of WAA colonies in the laboratory. In 2022, climatic data were also recorded in two apple orchards to correlate the population levels of *A. mali* with temperatures. As regards the presence of *A. mali*, trap captures were higher in organic apple orchards in both years. The first adults of *A. mali* were caught with traps between weeks 20 and 21 in 2021, and later from week 24 in 2022. In both years, on the 10 shoots marked at the beginning of the season, parasitization started in late May and was completed in late June in all surveyed apple orchards. The parasitism index was significantly higher in organic orchards and peaked from week 22 to week 25 in both years. In the two apple orchards where temperature was recorded, a good response of *A. mali* to temperature increase was detected, while spring temperature drops had negative repercussions. The results obtained in this study can provide a starting point for setting effective and environmentally sustainable control strategies. It will be necessary, in the coming years, to continue research to further investigate the biology and behaviour of *A. mali*, with the aim of preserving and promoting its control activity against WAA.

KEY WORDS: woolly apple aphid, Hemiptera Aphididae, Hymenoptera Aphelinidae, biological control, parasitism rate.

POSTER

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

**Impiego di funghi entomopatogeni e miridi predatori per il controllo di
*Phthorimaea absoluta***

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Native to South America, the tomato pinworm *Phthorimaea absoluta* (= *Tuta absoluta*) (Lepidoptera: Gelechiidae) is considered an invasive exotic species now widespread in tomato crops worldwide. Containing its populations is particularly difficult due to its cryptic habits, short life cycle and high reproductive capacity. The current management of *P. absoluta* is mainly based on the intensive application of insecticides, although these promote the selection of resistant populations and damage natural enemies and pollinators. Integrated pest management is preferred because it allows a sustainable approach to crop protection that reduces the use of pesticides in favour of other means, including biological control agents. The predator *Macrolophus pygmaeus* (Hemiptera: Miridae) is widely used in augmentative biological control of insect pests, including *P. absoluta*, in greenhouse tomato in Europe. Entomopathogenic fungi are also used as endophytes because they can regulate insect populations; for example, *Beauveria bassiana* and *Lecanicillium lecanii* can play a role in pest control. The present research aimed at testing the effects of these two fungi on both *P. absoluta* and *M. pygmaeus*. Specifically, the following were evaluated: i) direct toxicity by exposing pest larvae and predator nymphs to fungus-treated or untreated tomato leaflets; ii) preference of pest and predator adults for fungus-inoculated or non-inoculated tomato plants; iii) development rate of pest larvae and predator nymphs on fungus-inoculated or non-inoculated tomato plants. In trials to test direct toxicity, significantly higher mortality was caused by *B. bassiana* and *L. lecanii* on *M. pygmaeus* nymphs and *P. absoluta* larvae, respectively. In olfactometer, *M. pygmaeus* showed a significant preference for both non-inoculated plants and inoculated with *B. bassiana* in comparison to plants inoculated with *L. lecanii*. Development rate was significantly lower on plants inoculated with *L. lecanii* for *P. absoluta*, whereas no significant differences were found between fungus-inoculated and non-inoculated plants for *M. pygmaeus*. Based on the results, the use of *L. lecanii* appears promising as it proved to be more effective against *P. absoluta* and, at the same time, the plants inoculated with this fungus were less attractive to *M. pygmaeus*. However, these results should be validated in the field to implement the best control strategy.

KEY WORDS: tomato, *Beauveria bassiana*, *Lecanicillium lecanii*, *Macrolophus pygmaeus*, laboratory trials.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Field trials with *Beauveria bassiana* (strain ATCC 74040) against woolly apple aphid

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The apple cultivation is often threatened by various aphid species characterized by a peculiar biology and a different harmfulness level. However, the rosy apple aphid and the woolly apple aphid represent the species generating a major concern among apple producers and it needed ever to guarantee a satisfactory protection measure. In recent years it was observed a widespread recrudescence of *Eriosoma lanigerum* outbreaks. It is a complex phenomenon attributable to a series of concurrent causes appeared at same time at once, e.g. law restrictions about the use of some pesticide groups (organophosphate) and the higher frequency of mild and drought winter events as advantaging factor of the insects wintering. Other important aspect to consider it was the documented side effect of insecticides on the natural pest enemies, into specific the parasitoid *Aphelinus mali*. The aphid overwintering as neanid stage preferably in the trunk cracks and branches and on the roots under the soil. During spring it occurred the development of the season first apterous virginopars followed by 18-20 parthenogenetic generations.

In the years 2020 and 2021, field trials was carried out in Trentino to evaluate a microbiological insecticide (Naturalis®) based on *Beauveria bassiana* in the containment of woolly apple aphid infestations. The entomopathogenic fungus has proven to be an important biological control agent (BCA) against various pests and on apple trees it was authorized to use it against mites and psyllids.

Starting from some published laboratory study evidence, we decided to setup an open field experiment. Our study aimed to verify the effectiveness of the *B. bassiana* ATCC 74040 strain as commercial product, already on the market and admitted in organic farming, in containment of the overwintering aphid nymphs outbreak registered during spring migration phase. The trials in both years were carried out in different point within a commercial Granny Smith apple orchard, with an experimental design in randomized blocks and four replicates.

The study protocol compared five different treatments, three based on *B. bassiana* used alone at different timing and rate. Two treatment strategies scheduled number of 5/6 applications of *B. bassiana* at 1 and 2 l/ha respectively (treatment 2 and 3), as alternative it was 3 applications at 2 l/ha (treatment 4). A local standard chemical strategy (treatment n.5) and untreated trees (treatment 1) were included in the study design. In both field trials the WAA crawlers migration ended at early June and it was when registered the percentage of infested shoots. In the plots untreated the shoots attacked was around 85% in 2020 and 60% in 2021.

In this worst-case experimental context starting from 2020 the action of Naturalis® proved to be very interesting.

Naturalis when applied 5 times at 1 or 2 L/ha registered infested shoots values of 43% and 31% respectively similar to chemical standard result (26%). Considering the period of applications, particular attention should be paid to the compatibility between the microorganism and the fungicides normally used to control scab.

KEY WORDS: woolly apple aphid, apple, *Beauveria bassiana*.

POSTER



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

***Anthonomus rubi* on strawberry in Trentino: phenology, ecology and evaluation of integrated management techniques**

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For about a decade, a new type of damage caused by *Anthonomus rubi* Herbst (Coleoptera: Curculionidae) has been observed in Trentino. In fact, in addition to the typical damage caused by the severing of the flower buds after the egg laying, the pest damages the strawberry fruit. Damage is reported from the first stages of fruit enlargement up to its complete ripening, with consequent loss and commercial depreciation of the fruit product and a significant increase in collection and sorting times. Since 2019, experiments have been conducted to increase the knowledge of pest biology and ecology, as well as to identify possible integrated pest management strategies. From the appearance of the first flower stems (beginning of May) until the end of the crop cycle (end of October), damages are observed on ever-bearer strawberry varieties grown in soilless soil. Injuries show different trends over time between severed buds and fruit damage. Over the years, an increase in the population density of the phytophagous has been observed, and a consequent increase in economic damage. Both are increasingly earlier during the season. The adults of Strawberry blossom weevil overwinter (also) inside the strawberry orchard. At the end of February, the adults are present inside the turf under the strawberry cultivation system and in other more sheltered areas. In April, their presence is abundant on the dandelion flowers present in the grassing inside the cultivation tunnels. They colonize the strawberry young plants following transplantation and cause damage throughout the production season. In Autumn, adults are found again in abundance on dandelion flowers, and there is an increase in flowers with damaged petals. In the four-year period of the study, considering the arising ecological aspects, various techniques of integrated pest management were tested (such as periodic removal of severed buds, mass trapping, application of insecticide, etc.). Some of these techniques have provided interesting results and are therefore recommended to farmers working in the area.

KEY WORDS: Strawberry blossom weevil, dandelion, mass trapping, new type of damage, insecticides.



SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

New insights on the egg parasitoids of *Gonocerus acuteangulatus* in Sicilian hazelnut orchards

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Gonocerus acuteangulatus (Goeze) (Hemiptera: Coreidae), responsible for traumatic abortion of hazelnut seeds and for bugged hazelnuts, is considered the key pest of Sicilian hazelnuts. The control of its infestations is mainly relying on chemical control tools with negative toxicological and ecological consequence. The evaluation of alternative and sustainable control strategies for the management of the hazelnut bug becomes necessary in Integrated Pest Management (IPM) context. In this perspective, the egg parasitoids, able to actively search the host and prevent the nymph's birth, constitute a group of natural enemies potentially able to control the damage caused by the coreid. In this context, it is relevant to study and evaluate the impact of these natural enemies against *G. acuteangulatus*.

In order to assess the impact of egg parasitoids in one of the most representative areas of the Sicilian hazelnuts, a trial was started to estimate the biocontrol of these natural enemies in relation to the coreid infestations.

Surveys were carried out every two weeks on 15 plants in five hazelnut farms located at different altitude using the shaking method (frappage) and conducting direct observations of the canopy.

On each identified plant, the number of eggs (alive or parasitized) and different instars of *G. acuteangulatus* were recorded and collected for further laboratory observations. In addition, eggs of other species of Heteroptera, both phytophagous and predators, were also sampled and observed. A sample of 100 hazelnuts per site was also taken to assess the level of damage at harvest.

The results showed different levels of *G. acuteangulatus* infestation in relation to altitude. Among the egg parasitoids of *G. acuteangulatus*, *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) was the most widespread attacking 77% of the collected eggs, followed by *Hadronotus* spp. (Hymenoptera: Scelionidae) attacking 19% overall. Finally, the average value of hazelnut damaged was recorded from 20 to 30%.

Further investigations on the phenology of the egg parasitoids in relation to the availability of coreid eggs are needed and their potential for mass rearing in augmentative biological control programs in Sicilian hazelnut crops need to be evaluated.

KEY WORDS: augmentative biological control, monitoring activity, bugged hazelnuts, biodiversity, *Anastatus bifasciatus*, *Hadronotus* spp.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

The parasitoid complex of drosophilids in northwestern Italy

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The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an invasive insect that has spread across Europe, North and South America in less than a decade. Due to the high economic losses, especially on cherries and soft fruits, several strategies for controlling the populations of this exotic drosophilids, including biological control, have been developed and evaluated. The only survey on the complex of drosophilid parasitoids in North-West Italy was carried out in 2014 with field exposure of previously infested fruit. Therefore, in the years 2020, 2021 and 2022 field surveys were conducted in 10, 14 and 20 sites distributed in north-western Italy, respectively, to update previous data on the parasitoid species that can attack drosophilids, pending the authorisation of the release of the exotic parasitoid *Ganaspis brasiliensis* (Ihering). Specifically, objectives of the work were to investigate i) the species of parasitoids currently widespread in the territory, ii) their preference in relation to the plant species infested by drosophilids, and iii) their seasonal abundance in four sites monitored on a regular basis. Fruit samples were collected from both wild and cultivated plants, and transferred to the laboratory where they were reared for drosophilid and parasitoid emergence. Overall, 3,447 parasitoid individuals emerged from the infested fruits during the three-year period. The identified species were three larval parasitoids, *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault), *L. heterotoma* (Thomson), *L. japonica* Novkovic & Kimura (Hymenoptera: Figitidae), and two pupal parasitoids, *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae), and *Trichopria* cf. *drosophilae* (Perkins) (Hymenoptera: Diapriidae). *Leptopilina japonica* was detected for the first time in north-western Italy in 2020 (5 individuals), and was the most abundant parasitoid emerged in the following two years (2,407 individuals). The complex of drosophilid parasitoids obtained from the field-collected fruits is presented and discussed, with information on their seasonal abundance and their preference towards the infested fruit species.

KEY WORDS: spotted wing drosophila, field survey, soft fruits, cherries, *Leptopilina japonica*.

ORAL PRESENTATION

SESSION XIII

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Investigation on egg parasitoids of *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) in Northwestern Italy

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The meadow spittlebug, *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), is the main vector of the plant pathogen *Xylella fastidiosa* Wells in Europe. This bacterium is the causal agent of the severe disease of Olive Quick Decline Syndrome (OQDS) in Apulia. The vector adults play a key role in the spread of the bacterium in olive agroecosystems, making its control essential to limit *X. fastidiosa* spread. Current vector containment strategies, based on tillage in April to target the juvenile stages and insecticide applications against the adults, have not successfully contained the epidemic.

Biological control could represent a further tool to enhance the effectiveness of the control strategies of *P. spumarius*, which is abundant and ubiquitous in olive agroecosystems. Egg parasitoids are promising candidates as biocontrol agents of spittlebugs, as they kill the host at the egg stage, before it could have any epidemiological role in spreading the pathogen.

Few information is currently available on egg parasitoid communities associated to *P. spumarius*. However, *Ooetonus vulgatus* Haliday (Hymenoptera: Mymaridae) was first reported as an egg parasitoid of the spittlebug in Corsica in 2020, showing high parasitization rates (up to 69%), making it an interesting biocontrol agent of *P. spumarius*.

The aim of this work was to monitor the presence of egg parasitoids of *P. spumarius* in Northwestern Italy, by exposing sentinel egg masses in five locations of Piedmont and Liguria, known to host high populations of *P. spumarius*. Sentinel eggs were obtained both from i) *P. spumarius* rearings under controlled conditions and ii) spittlebug females engaged in the field with proper substrates for oviposition (straw and cardboard). The sentinel eggs were exposed during two periods – from November to January and from January to February – to assess the flight periods of egg parasitoids. A total of about 1200 eggs and 29 spittlebug females were placed in 24 replicates (3 – 6 per site) over the two exposition periods. About 700 eggs were retrieved after exposition, transferred in petri dishes with moist filter paper and maintained in climatic chamber (18–20 °C, 14:10 L:D). The eggs were checked daily, until the emergence of parasitoids or spittlebug nymphs. Egg parasitoids emerged from two sites and all were morphologically identified as *O. vulgatus*.

This preliminary study i) highlights the presence of *O. vulgatus* as a parasitoid of *P. spumarius* also in Northwestern Italy, ii) provide some biological information on this species and iii) tests the effectiveness of few setups for sentinel eggs exposition. Further studies should be carried out to investigate the biology and parasitization efficiency of *O. vulgatus*, as well as its presence and prevalence in the olive agroecosystems in Apulia and its possible implementation in biological control programmes targeting *P. spumarius* in Italy and Europe.

KEY WORDS: *Xylella*, spittlebug, biological control, egg parasitoids, *Ooetonus vulgatus*, sentinel eggs.

POSTER



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XXVII Congresso Nazionale
Italiano di Entomologia

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Awards

OSELLA AWARD

The role of mimicry in the diversification of the western Palearctic ants: a multidisciplinary approach to the genus *Colobopsis*

Enrico Schifani¹, Daniele Giannetti¹, Sándor Csősz², Filippo Castellucci³, Andrea Luchetti³, Cristina Castracani¹, Fiorenza Spotti¹, Alessandra Mori¹, Donato A. Grasso¹

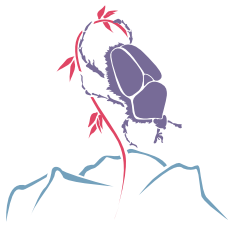
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For a long time, in the western Palearctic region, the ant genus *Colobopsis* was considered to be represented by a single species. However, our field observation and the analysis of scattered literature accounts suggested the existence of peculiar chromatic and behavioral differences within its range, potentially linked to the mimicry of different model species. We studied the meaning of these differences through integrative taxonomy: by relying on a multidisciplinary approach, based on the combined use of ecological, ethological, biogeographical, genetic, and morphological data, we discovered that the *Colobopsis* populations in the region belong to two distinct species. These are allopatrically distributed in a way that matches biogeographic patterns already known in other ants. They are distinguished by significant but very subtle morphometric differences and show a polyphyletic mtCOI pattern. These two elements suggest a recent isolation of the two species. At the same time, chromatic and behavioral differences appear to be strong. *Colobopsis truncata* and the newly described *Co. imitans* show chromatic patterns that closely resemble two more abundant and well-armed species that often nest in the same trees: respectively *Dolichoderus quadripunctatus* and *Crematogaster scutellaris*. Moreover, *Co. imitans* workers frequently infiltrate the large foraging trails of *Cr. scutellaris*, evading their attacks when occasionally intercepted. The strong divergence of these traits in sister species of recent isolation suggests for the first time an important role of mimicry as a diversification driver in ants. Further investigations are necessary to identify the predators that may be responsible of the selective pressures favoring these adaptations.

PAROLE CHIAVE: Formicidae, Batesian mimicry, dilution effect, allopatric speciation, evolutionary adaptations

ORAL PRESENTATION



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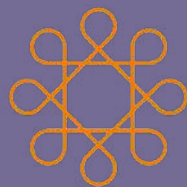


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