



SCIENTIFIC PROGRAMME &

BOOK OF ABSTRACTS

15-18 November 2017

EUROPEAN PhD NETWORK "INSECT SCIENCE"

VIII Annual Meeting
15-17 November 2017

GIORNATE CULTURALI DI ENTOMOLOGIA

Accademia Nazionale Italiana di Entomologia Società Entomologica Italiana 17 November 2017

ACCADEMIA NAZIONALE ITALIANA DI ENTOMOLOGIA

General Assembly and Public Session 18 Novembre 2017

FEDERICO II Convention Centre

Via Partenope 36, Napoli – Italy



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Wednesday 15 November 2017

EUROPEAN PhD NETWORK "INSECT SCIENCE" - VIII Annual Meeting

09:00 - 09:40

An insect pheromone primes plant defenses with community wide effects <u>John F. Tooker</u>, The Pennsylvania State University - Department of Entomology, University Park, PA USA

09:40 - 10:00

Multiple symbiont acquisition and host shifts in a termite-protist mutualistic association

<u>C. Michaud</u>, Institut de Recherche sur la Biologie de l'Insecte, CNRS - University of Tours, France

10:00 - 10:20

Electrophysiological and behavioral studies in the evaluation of semiochemical attractants for *Stegobium paniceum* L. (Coleoptera: Anobiidae)

<u>S. Guarino</u>, University of Palermo - Dipartimento di Scienze Agrarie, Alimentari e Forestali, Italy

10:20 - 10:40

The larval midgut of *Hermetia illucens* is characterized by a highly complex structural organization

<u>D. Bruno</u>, University of Insubria - Department of Biotechnology and Life Sciences, Varese. Italy

10:40 - 11:20

Coffee Break

11:20 - 11:40

Molecular and functional characterization of *Hermetia illucens* larval midgut <u>M. Bonelli</u>, University of Milan - Department of Biosciences, Italy

11:40 - 12:00

Immunological properties of engineered *Asaia* symbionts: implications for the control of mosquito-borne diseases

I. Varotto Boccazzi, University of Milan - Department of Biosciences, Italy

12:00 - 12:20

ABC-transporter gene silencing inhibits insecticide detoxification in Anopheline malaria vector

<u>A. Negri</u>, University of Rome "La Sapienza" - Department of Environmental biology, Italy



12:20 - 12:40

Impact of Halyomorpha halys (Stål) on grapevine in Veneto region

D. Scaccini, DAFNAE – Entomology, University of Padova, Italy

12:40 - 13:00

New acquisition about the role of *Colomerus vitis* in the transmission of Grapevine Pinot gris virus

<u>D. Valenzano</u>, Università degli Studi di Bari "Aldo Moro" - Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Italy

13:00 - 14:00

Lunch

14:00 - 15:00

Poster exhibition and discussion

15:00 - 15:40

The non-target effects of modern insecticides on natural enemies of pests <u>A. Biondi</u>, University of Catania – Department of Agriculture, Food and Environment, Italy

15:40 - 16:00

RNA interference of *Varroa destructor* salivary genes: a tool to disclose honeybee-mite immune interactions

<u>A. Becchimanzi</u>, University of Napoli "Federico II" – Department of Agricultural Sciences, Italy

16:00 - 16:20

Bacteria expressing dsRNA as a delivery tool in RNAi-based pest control technologies

<u>F. Astarita</u>, University of Napoli "Federico II" – Department of Agricultural Sciences, Italy

16:20 - 16:40

Development of an e-trap for the monitoring of the olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae)

<u>P. Calabrese</u>, University of Molise – Dipartimento Agricoltura, Ambiente, Alimenti, Campobasso, Italy

16:40 - 17:00

Human based genetic tools to refine genetic populations structure of honey bees (*Apis mellifera* ssp L.) (Hymenoptera: Apoidea: Apidae) colonies at regional scale

<u>G. Noël, Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Belgium</u>



Thursday 16 November 2017

EUROPEAN PhD NETWORK "INSECT SCIENCE" - VIII Annual Meeting

09:00 - 09:40

Forest insect invasions - the role of global change

A. Battisti, DAFNAE – Entomology, University of Padova, Italy

09:40 - 10:00

Modelling the shifts in the timing and spatial distribution of *Vitis vinifera*, *Lobesia botrana* and *Trichogramma* sp. phenology in a warming climate <u>C. Victorine</u>, University of Geneva - Institute of Environmental Sciences, Switzerland

10:00 - 10:20

The role of cuticular proteins located at the surface of the acrostyle, an organ at the tip of aphid stylets, in plant-aphid interactions

M. Deshoux, INRA Montpellier - UMR 385 BGPI, France

10:20 - 10:40

Molecular and functional characterization of an immune gene of *Spodoptera littoralis*

<u>I. Di Lelio</u>, University of Napoli "Federico II" – Department of Agricultural Sciences, Italy

10:40 - 11:00

NF-kB signaling is impaired by neonicotinoid insecticides

<u>G. Di Prisco</u>, University of Napoli "Federico II" – Department of Agricultural Sciences, Italy

11:00 - 11:40

Coffee Break

11:40 - 12:00

Archaeoentomological studies provide information about the biodiversity of the Sardinian entomofauna in the last centuries

<u>G. Giordani</u>, University of Huddersfield – Department of Biological Sciences, School of Applied Sciences, UK

12:00 - 12:20

First records of *Synthesiomyia nudiseta* (Diptera: Muscidae) from forensic cases in Italy

<u>F. Tuccia</u>, University of Huddersfield – Department of Biological Sciences, School of Applied Sciences, UK

12:20 - 12:40

Drosophila suzukii spillover from natural habitats to vineyards and cherry orchards

L. Tonina, DAFNAE – Entomology, University of Padova, Italy



12:40 - 13:00

High synchronization of *Drosophila suzukii* activity along elevational gradients

G. Santoiemma, DAFNAE – Entomology, University of Padova, Italy

13:00 - 14:00

Lunch

14:00 - 14:40

Poster exhibition and discussion

14:40 - 15:20

Gendercide symbionts in arthropods, and other stories of sex, symbiosis, and survival

C. Bandi, University of Milan - Department of Biosciences, Italy

15:20 - 15:40

Role of VOCs from *Brassica oleracea* in host location processes of *Bagrada hilaris*: electrophysiological and behavioral studies

<u>M. A. Arif</u>, University of Palermo - Dipartimento di Scienze Agrarie, Alimentari e Forestali, Italy

15:40 - 16:00

Inter-specific relationship among stored-product Coleoptera: role of VOC emissions

<u>G. Giunti</u>, Università Mediterranea di Reggio Calabria - Dipartimento di Agraria, Italy

16:00 - 16:20

Influence of cover crop management techniques on soil ecosystem services <u>S. Magagnoli</u>, Alma Mater Studiorum University of Bologna - Dipartimento di Scienze Agrarie, Italy

16:20 - 16:40

Influence of landscape complexity and vineyard management on leafhoppers abundance in North-Italian vineyards

G. Zanettin, DAFNAE – Entomology, University of Padova, Italy

16:40 - 16:50

An innovative approach for the management of spotted wing drosophila (*Drosophila suzukii*): improve crop protection using an environmentally friendly formulation

<u>I. Castellan</u>, Free University of Bozen - Bolzano, Faculty of Science and Technology, Italy

16:50 - 17:00

New strategies in organic pest insect control by the characterization of bioactive plant volatile organic compounds of agricultural crops

<u>M. Preti,</u> Free University of Bozen - Bolzano, Faculty of Science and Technology, Italy



Friday 17 November 2017

GIORNATE CULTURALI DI ENTOMOLOGIA

9:30 - 9:35

Romano Dallai, President of "Accademia Nazionale Italiana di Entomologia"

I session: Insect-Plant Interactions

9:35

Introduction - Coordinators AMR Gatehouse, MC Digilio

9:40 - 10:15

Plant Response to Stress: Can this be exploited for Crop Protection? Angharad MR Gatehouse. University of Newcastle. UK

10:15 - 10:40

Host tree-insect interactions in northern forest ecosystems in a changing climate

Deepa Pureswaran, Laurentian Forestry Centre, Québec, Canada

10:40 - 11:05

Insect and microorganisms interactions and the induction of plant defence *Maria Cristina Digilio*, *University of Napoli Federico II*, *Italy*

11:05 - 11:30

Coffee break

11:30 - 11:55

Smart plants or naive insects?

Salvatore Cozzolino, University of Napoli Federico II, Italy

11:55 - 12:20

How plants react to the insect challenge

Rosa Rao, University of Napoli Federico II, Italy

12:20 - 12:45

From leaf-miners to gall-inducers: a continuum of plant manipulators?

<u>David Giron</u>, Institut de Recherche sur la Biologie de l'Insecte, CNRS - University of Tours, France

12:45 - 13:10

Ecology of egg parasitoids in crop protection

<u>Stefano Colazza</u>, University of Palermo - Dipartimento di Scienze Agrarie, Alimentari e Forestali, Italy

13:10 - 13:30

General discussion

13:30 - 14:30

Lunch



Il session: Insects for the Court, Insects for the History: a Journey through Forensic Entomology and Funerary Archaeoentomology

14:30 - 14:35

Introduction - Stefano Vanin, University of Huddersfield, UK

14:35 - 15:05

PMI estimation a challenge for forensic pathologists

Carlo Campobasso, University of Campania Luigi Vanvitelli, Napoli, Italy

15:05 - 15:35

Hidden information within mummies and other burials

Gino Fornaciari, University of Pisa, Italy

15:35 - 16:05

Forensic Entomology and Funerary Archaeoentomology: a source of answers <u>Stefano Vanin</u>, University of Huddersfield, UK

16:05 - 16:30

General discussion

16:30 - 17:00

Presentation of the book: "Il piacere delle api" - Paolo Fontana

17:00 - 18.00

ECE 2018 Organizing Committee Meeting

Saturday 18 November 2017

PUBLIC SESSION AND GENERAL MEETING OF THE "ACCADEMIA NAZIONALE ITALIANA DI ENTOMOLOGIA"

10:00 - 10:30

Welcome coffee

10:30

General Assembly

11:30

Public Session

"Attuali ricerche su specie di *Asphondylia* (Diptera: Cecidomyiidae) causanti galle fiorali su Lamiaceae"

Gennaro Viggiani, University of Napoli Federico II, Italy

13:30

Lunch



ORAL PRESENTATIONS

EUROPEAN PhD NETWORK
"INSECT SCIENCE"



ABSTRACTS



Role of VOCs from *Brassica oleracea* in host location processes of *Bagrada hilaris*: electrophysiological and behavioral studies

Mokhtar Abdulsattar Arif¹, Salvatore Guarino¹, Ezio Peri¹, Stefano Colazza¹

The Painted bug, Bagrada hilaris Burmeister is an herbivorous insect native of Asia and Africa, invasive in Southern Europe and North America where it causes major damage on various vegetable crops mainly belonging to Brassicaceae family. Young plants at cotyledon stage of B. oleracea are particularly attractant and susceptible to B. hilaris feeding activity. To elucidate the main volatile organic compounds (VOCs) involved in the attraction of *B. hilaris* individuals toward the host plant *B. oleracea*, laboratory experiments were carried out using vertical Y-shaped olfactometer and electroantennographic techniques (EAG). Both adults and late nymphal instars were used in these experiments. Olfactometer experiments were done with young seedlings (one week old) and their VOCs headspace extract collected by porapak Q and eluted in hexane. The VOCs elution was tested and afterward fractionated by silica gel column in order to assess the active fraction. Result indicated attraction of adults and nymphs toward volatiles of living seedlings and their VOCs extract. Furthermore, the non-polar fraction elicited attraction while no response was determined by polar fractions. These results were confirmed by the EAG experiment: only the whole extract and non-polar fraction elicited significant response. These data suggest that the non-polar compounds emitted from young seedlings of B. oleracea can play an important role in B. hilaris host location processes.



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Bacteria expressing dsRNA as a delivery tool in RNAi-based pest control technologies

F. Astarita, E. Barra, I. Di Lelio, S. Caccia, F. Pennacchio

Department of Agricutural Sciences - Division of Agricultural Sciences, University of Naples "Federico II", Via Università 100-80055, Portici (Napoli), Italy

The need to reduce the use of chemical pesticides in agriculture and the decrease in number of available molecules has promoted intensive research to identify new bioinsecticides of natural origin and effective delivery strategies.

A promising pest management strategy is the so called "RNAi mediated crop protection". The targeted gene silencing action of tailored dsRNAs confer to these novel tools for pest control a high degree of selectivity, that make them ideal candidates to be used in sustainable IPM plans. However, the major threat for the exploitation of these molecules is the development of specific delivery strategies in order to allow these molecules to resist the harsh physicochemical environment of the insect gut and to overcome the lining epithelial layer. The present work aims at investigating new delivery strategies for oligonucleotidic molecules to control phytophagous insect pests. To promote the feasibility of RNAi technology for pest control, we developed a new delivery strategy of dsRNAs, here used to target host immune genes, in order to induce an immunosuppressive status enhancing the impact of entomopathogens. Two immune genes of the noctuid moth Spodoptera littoralis have been considered (SI 102 and SI gasmin), one of which (SI gasmin) has been isolated and characterized, showing its potent role as opsonizing factor mediating phagocytosis. Recombinant vectors for the expression in bacteria of SI 102 and SI gasmin dsRNA have been designed and recombinant bacteria producing SI 102 dsRNA have been orally delivered to S. littoralis larvae. This treatment produced high level of gene silencing along with the immunosuppressive phenotype induced. The results obtained with dsRNA-expressing bacteria are very promising and contribute to inspire new ideas for the delivery of insecticidal molecules silencing host immune genes, in order to enhance insect sensitivity to natural antagonists.



Gendercide symbionts in arthropods, and other stories of sex, symbiosis, and survival

Claudio Bandi

Dipartimento di Bioscienze – Università di Milano

Symbiosis is a widespread biological phenomenon. High levels of integration are frequently observed between the partners of symbiotic associations, at the physiological, metabolic and even genetic and genomic levels. In insects and other arthropods, intracellular microbial symbionts are normally transmitted to the progeny of the host, through the female oocytes/reproductive apparatus. This uniparental type of transmission implies a conflict between the interests of the symbionts (whose reproductive success is linked to the success of females, or even to the success of those females that transmit them), and the interests of the host population as a whole (where males, or even uninfected females, are not useful to the symbiotic microorganisms). In these form of symbiosis, characteristics that would be apparently ununderstandable (e.g. the generation of sterilized females, or the death of male embryos) can found an explanation if we focus our attention on the interests of the symbionts, rather than on the interest of the hosts.



Forest insect invasions - the role of global change

Andrea Battisti

DAFNAE - Entomology, University of Padova, Italy

In recent decades much has been said about global change and increasing threats from insect pests to forestry. Such claims are based on the effects of trade and weather, in particular temperature, on insect spread and survival. However, a fair amount of these claims lack scientific support. It should be remembered that almost all insect populations are part of organism communities where trophic interactions play a crucial role. This means that extrapolating from these effects on insect individuals, or populations in isolation, to real insect populations in complex food webs must be viewed with caution. Accidental introductions of non-native organisms do not always lead to them being invasive, i.e., becoming established as economically important pests. In cases where alien species do expand their distribution range from the point of introduction their successful establishment is assumed to be favored by changes in climate. There are good reasons to suspect that climate change has contributed to insect species becoming invasive, although there is no unequivocal support for this suggestion. It will be interesting to see if alien species already established may colonize the colder parts of the potential habitat with the same rate, assuming that temperature may limit their performance. A critical read of the scientific literature results in surprisingly few documented examples of climate-change induced range expansion of native and non-native species. It should be remembered that long-term trends in the distribution and abundance of pest insects are notoriously difficult to document. Thus, it is possible that many more pest insects could have responded to climate change, or are likely to do so in the near future, than can be detected in the data bases presently available. It is also possible, however, that biological systems, including forest pests, are less sensitive to direct climate effect than previously thought, due to buffering effects of trophic interactions.



RNA interference of Varroa destructor salivary genes: a tool to disclose honeybee-mite immune interactions

A Becchimanzi¹, EM Campbell², G Di Prisco¹, AS Bowman², F Pennacchio¹

¹University of Napoli "Federico II" – Department of Agricultural Sciences; ²University of Aberdeen – School of Biological Sciences (Zoology)

During the last decade the dramatic dimension of honeybee colony losses attracted the attention of the public opinion and the increasing interest of the scientific community. It is now largely accepted that this syndrome has a multifactorial origin that consist in biotic and abiotic stressors. Several monitoring programs indicate that the large majority of losses are associated with the co-presence of the parasitic mite Varroa destructor and the Deformed Wing Virus (DWV). Varroa destructor is an obligate ectoparasite feeding on bee's haemolymph and has a severe impact on honeybee physiology. Varroa acts as vector of DWV, viral pathogen that aggravates the syndrome induced by mite infestation through an escalating immunosuppression. The virus depressive effect on host immune system could be further complemented by poorly explored effects of Varroa feeding and saliva injection on bees. This work aims to use RNA interference of V. destructor salivary genes in order to understand the mechanisms underlying the impact of the mite on honeybee immunocompetence. Merging transcriptomic and proteomic data, different targets from the sialome of the mite were selected. dsRNA for these targets were synthesized and administered to the mites by soaking, monitoring the gene silencing at different time points. Functional studies were performed assessing the effect of the silencing on mite's survival, while effects on honeybees immune response need to be evaluated.



The non-target effects of modern insecticides on natural enemies of pests

A. Biondi¹, G. Siscaro¹, A. Russo¹, N. Desneux², L. Zappalà¹

Combining selective insecticides and natural enemies of pestsis crucial for implementing sustainable integrated pest management packages. Pesticide risk assessment for natural enemies of insect pests inhabiting agroecosystemscan be challenging when (i) evaluating newer classes of pesticides that can cause sublethal effects rather than direct mortality, and when (ii) including other stressors such as high temperature regimes. Here, we present the results of several investigations aimed atdeepening the knowledge on behavioral and physiologicalsublethal effects of various modern insecticides toward parasitoids and predators of invasive insect pests.



¹University of Catania – Department of Agriculture, Food and Environment – Italy; ²INRA – InstitutAgrobiotech Sophia Antipolis – France

Molecular and functional characterization of *Hermetia illucens* larval midgut

M. Bonelli¹, D. Bruno², G. Tettamanti², M. Casartelli¹

The increase in global demand for meat and the management of organic waste are huge global issues. In addition to policies to contain the excessive meat consumption and the production of food waste, a possible perspective is to consider insects as agents for organic waste reduction and as source of protein for monogastric feed production. The larvae of *Hermetia illucens* (Diptera: Stratiomyidae) are good candidates for bioconversion of vegetal waste and feed production because they grow on different organic substrates and the dry-matter of the prepupa contains a very high percentage of protein with high nutritional value. The study of *H. illucens* midgut physiology is essential to understand the extraordinary feeding plasticity of this insect and to best exploit this ability. We characterized the digestive enzymes involved in the initial phase of digestion in the three regions of the midgut (anterior, middle and posterior). The proteolytic activity was assayed using chromogenic substrates and specific inhibitors. The total proteolytic activity is highest in the posterior midgut and the major activity is due to serine proteases. We measured the activity and the transcript levels of the two main serine proteases involved in insect digestion: trypsin-like and chymotrypsin-like proteases. Moreover, we determined the total amylolytic activity and the highest value was recorded in the anterior midgut. Thanks to our data, we propose the first model of the functional activity of *H. illucens* midgut. This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).



¹University of Milan - Department of Biosciences;

²University of Insubria - Department of Biotechnology and Life Sciences

The larval midgut of *Hermetia illucens* is characterized by a highly complex structural organization

D. Bruno¹, M. Bonelli², M. Casartelli², G. Tettamanti¹

The demand for food of animal origin is expected to increase by 70-80% within 2050, with a consequent rise in feed requirement. Another serious concern is represented by food waste disposal: in fact, it is estimated that 1.3 billion tons/year of food are globally wasted. The dipteran *Hermetia illucens*, also known as black soldier fly (BSF), is a promising insect species to tackle these critical challenges because of the ability of the larvae to grow on different organic substrates and their efficiency in the bioconversion process. Moreover, the high nutritional value of the larvae and pupae makes them an alternative protein source for the production of fish feed.

A deep understanding of the biology of the larval midgut, which is implicated in food digestion and nutrient absorption, is essential to better comprehend the extraordinary dietary plasticity of the larva.

In the present study, we performed a morphological characterization of the midgut of last instar larvae. Our results demonstrate that the larval midgut is composed of three distinct anatomical regions with different luminal pH. These regions are characterized by different cell types that accomplish digestion and absorption activities (columnar cells), acidification of the midgut lumen (cuprophilic cells), regulation processes (endocrine cells), and growth of the epithelium (stem cells). Moreover, we are investigating the expression of different enzymes along BSF midgut epithelium, which are involved in transport mechanisms.

This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).



¹University of Insubria - Department of Biotechnology and Life Sciences, Varese, Italy ²University of Milan - Department of Biosciences, Milan, Italy

Development of an e-trap for the monitoring of the olive fruit fly Bactrocera oleae (Gmelin) (Diptera: Tephritidae)

P. Calabrese, A. Sciarretta

Università degli Studi del Molise – Dipartimento Agricoltura, Ambiente, Alimenti; Via de Sanctis snc, 86100 Campobasso, Italy

The olive fruit fly *Bactrocera oleae* (Gmelin, 1790) (Diptera: Tephritidae) is considered the most serious pest of olive groves in many areas throughout the word, affecting the quality and quantity of oil and table olives during the infestations in late summer and autumn. The research project focuses on the comparison of some commercial or modified traps, baited with different attractants, to be selected for the development of a semiautomatic e-trap for the monitoring of B. oleae. Various traps and attractants have been evaluated for catch efficiency and selectivity toward predators and parasitoids. The experimental sites were located in an inner hilly area of the Campania region. Tests, using latin square experimental design, were carried out to compare yellow sticky panel, bottle trap and Jackson trap, activated with two different food attractants (ammonium carbonate or protein food bait). Jackson trap was modified using different colors of support/sticky surface and sizes. A linear relationship between catches and sticky surface area was observed. The all-yellow Jackson trap, activated with ammonium carbonate, showed the best performance in terms of catch number and resulted to have the lowest captures of natural enemies. Therefore, it was selected for the manufacturing of an automatic monitoring device, equipped with a camera, able to record images of the captured flies and send them, through the cloud, to a remote computer. Field tests were conducted to validate the ability of the e-trap vs conventional trap in capture olive fruit flies and to verify the image quality and transmission.



An innovative approach for the management of spotted wing drosophila (*Drosophila suzukii*): improve crop protection using an environmentally friendly formulation

I. Castellan¹, S. Schmidt², S. Angeli¹

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The management of pest insects in the field is an ongoing challenge for agriculture. It becomes even more complex when we have to deal with an invasive pest insect causing increasing damages to local crops. This is the case of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), an invasive fruit fly species threatening fruit farming and viticulture, arrived in Italy in 2008. The peculiarity of this species lies in its attack modality: females have a serrated ovipositor that can pierce the fruit skin surface to lay eggs. This makes it difficult to respect pre-harvest intervals with chemical agents. That is why we must rely on a different approach to tackle the problems caused by D. suzukii. Chemical ecology applications allow to modify the insect's behaviour in the field, improving the control and preventive measures. Within the EU ERDF-project DROMYTAL we aim to develop a new strategy that will reduce or eliminate the residues of chemical insecticides on the fruits, using target yeasts to attract the oviposing females, as yeast are already known to interact with drosophila species. The yeast strains will be analysed to obtain volatile profiles emitted from the headspace of veast cultures. The main methods utilized are based on headspace analyses and gas chromatography (GC-MS), to get deep into the volatile profile identification. The advanced technique of electroantennography (EAG and GC-EAD) will be utilized to test the selected volatiles on D. suzukii. The ultimate aim will be to find a right blend of volatiles to create a formulation potentially applicable in the field to keep the pest presence under control.



The role of cuticular proteins located at the surface of the acrostyle, an organ at the tip of aphid stylets, in plant-aphid interactions

M. Deshoux¹, B. Monsion¹, C. Webster¹, V. Masson²⁻³, K. Arafah³, S. Voisin³, P. Bulet²⁻³, S. Blanc¹ & M. Uzest¹

¹INRA Montpellier - UMR 385 BGPI - France ; ²Institute for Advanced Biosciences - CR Inserm U1209 - CNRS UMR5309 - Université Grenoble Alpes – France ; ³ Plateforme BioPark d'Archamps - Archamps Technopole - France

Aphids are among the most devastating sap-feeding insects on crops. They feed from sieve tubes using specialized piercing-sucking mouthparts, called stylets. Stylets are made of cuticle, composed mainly of cuticular proteins and chitin. To collect nutrients essential for growth, development and reproduction, aphids have evolved sophisticated mechanisms to overcome plant defenses. Such defenses are triggered by the mechanical injury of penetrating stylets and by the release of elicitor molecules secreted in the aphid saliva. In compatible interactions, aphid effectors are able to counteract these plant defenses, leading to sustained insect feeding.

The acrostyle is an organ located over the inner side of the tip of maxillary stylets. This organ is in contact with both plant cell content, plant sap and aphid saliva, and may have a key role in aphid-plant interactions during the feeding process. The proteins of the acrostyle are emerging from the cuticule at the stylet/fluid interface, thus are able to interact with proteins (at least transiently) originating either from the ingested plant sap or from the egested saliva.

In an attempt to define the physiological functions of the acrostyle, the main goal will be to search for interacting partners of the cuticular proteins at the surface of the organ within plant (defense molecules) and aphid saliva (effectors). Cuticular proteins with surface-exposed peptides have been recently identified. Several approaches are now developed to find their putative partners. Preliminary data showing an interaction between a cuticular protein and an effector will be presented.



Molecular and functional characterization of an immune gene of Spodoptera littoralis

<u>I. Di Lelio</u>¹, F. Astarita¹, P. Varricchio¹, S. Herrero², F. Pennacchio¹, S. Caccia¹

¹Università degli Studi di Napoli "Federico II" - Dipartimento di Agraria; ²Universitat de València-Departamento de Genética

A polydnavirus-derived gene (*gasmin*) has been found in the genome of the moth *Spodoptera exigua*, to which seems to confer protection against baculovirus infection, as suggested by *in vitro* studies on cell lines.

Here we report the finding of a *gasmin* homologue in the genome of *Spodoptera littoralis*, showing high sequence identity but different functional attributes that have driven its positive selection. By studying (1) the expression profile *in vivo* and its changes in response to different immune challenges, (2) the phenotypes induced by RNAi mediated gene silencing, (3) and a functional proteomic approach to assess the localization and fate of the gene product, we were able to demonstrate that this gene is a potent immune effector, being the encoded protein an opsonizing factor promoting phagocytosis.

These results indicate how important is the role of polydnaviruses in promoting horizontal gene transfer in insects and in the evolution of their genomes.



NF-kB signaling is impaired by neonicotinoid insecticides

<u>G. Di Prisco</u>¹, M. Iannaccone¹, R. Ferrara¹, E. Caprio¹, P. Varricchio¹, D. Annoscia², F. Nazzi², R. Capparelli¹, F. Pennacchio¹

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Honeybee colony losses are induced by a multifactorial syndrome, associated with reduced immunocompetence and increased load of parasites and pathogens, that can be induced by different stress agents, in particular by the parasitic mite Varroa destructor and the Deformed Wing Virus (DWV). Recent studies have demonstrated that neonicotinoids downregulate the immune barriers of honeybees, by negatively modulating a transcription factor in the NF-kB family, which is involved in the activation of a number of defense responses. The reduced efficiency of the antiviral barriers under the Toll pathway promotes replication of DWV, which is stably associated to honeybee populations, often with asymptomatic infections. Given the central role of NF-kB in immunity, we wanted to assess the impact of neonicotinoids on both cellular and humoral components, such as encapsulation, melanization and coagulation, which may be relevant not only for pathogen control, but also for their impact on feeding activity of Varroa and its fitness. Our results show a negative impact of neonicotinoids on these immune parameters, setting the stage for studies aiming to investigate if and how their sub-lethal doses can have any influence on Varroa and other pathogens. Because NF-kB modulates a number of conserved pathways in animals, this prompted us to analyze the impact of neonicotinoids on human cell lines, in order to assess any subtle effect on non-target organisms. The transcriptional profiles obtained and the associated immunosuppressed phenotypes indicate the occurrence of a potential risk, which, however, will have to be carefully assessed at the organism level.



Archaeoentomological studies provide information about the biodiversity of the Sardinian entomofauna in the last centuries

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The studies of insects from archaeological contexts can provide important information to reconstruct past events, climate and environments. Moreover, these studies can furnish useful information about changes in a region biodiversity. In this work, the archaeoentomological results from two Sardinian sites are reported. Insects have been collected from human mummified corpses recovered from a crypt located under the Sant'Antonio Abate Cathedral in Castelsardo (Sassari province), and from an urban mediaeval cesspit in Sassari city. In the first site, the 95% of the specimens were Diptera puparia whereas only few Lepidoptera cocoons and some Coleoptera fragments were isolated. The findings of Calliphoridae identified as Phormia regina and Calliphora vicina together with Muscidae puparia of Hydrotaea capensis indicated an initial colonization of the bodies in an exposed context prior to their burial into the crypt where the decomposition process got over. On the second site, Muscidae, Fanniidae and Sphaeroceridae puparia were found in different state of preservation (mineralization) correlated with changes in the water level into the cesspit. The species feeding on decomposing matter confirm the use of the well as a collecting point of domestic wastes. Human activities was proved by the collection of synantyhropic flies as Musca domestica, Muscina prolapsa and Stomoxys calcitrans and of the seaweed flies Thoracochaeta zosterae which testify the hygienic conditions of the cesspit. Phormia regina and T. zosterae are reported for the first time from Sardinia. For the first species an extinction phenomenon can be suggested, whereas for the second further field research is needed.



Inter-specific relationship among stored-product Coleoptera: role of VOC emissions

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Volatile Organic Compounds (VOCs) play a pivotal role routing food and host selection in insects. Among Coloptera, stored-product pests may exploit volatile emissions to discriminate and select trophic sources presenting different palatability values. Here, we focused the attractiveness of food sources toward an external feeder of kernels, Tribolium confusum J. du Val, which is generally a secondary pest of grains. Thus, the presence of previous infestation of primary pests, such as Sitophilus zeamais Motschulsky, enhances T. confusum population as well as other secondary feeders. The present study aimed to evaluate under laboratory conditions the preferences of T. confusum females toward differentially infested rice, highlighting the impact of intra- and inter-specific competition. Tested beetles positive oriented toward S. zeamais-infested rice in choice bioassays and preferred this source over intact rice. Furthermore, to assess the effect of intra- and interspecific competition, kernels concurrently infested by a primary (S. zeamais) and a secondary pest [T. confusum or Cryptolestes ferrugineus (Stephens)] were tested in nochoice bioassays, highlighting that both food sources were innately attractive for T. confusum females. Nevertheless, the presence of a secondary infestation greatly altered T. confusum responses in choice bioassays. Indeed, when a conspecific infestation occurs, females showed positive chemotaxis toward the double-infested rice, while they avoided the rice infested by C. ferrugineus, averting a far more competitive habitat. T. confusum behavioral responses were in agreement with VOC analyses, which identified 70 volatiles and emphasized significant differences among the tested food sources, highlighting the presence of putative attractive and repellent compounds.



Influence of cover crop management techniques on soil ecosystem services

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Cover crops may increase vegetational complexity within agro-ecosystems, leading to a positive impact on natural enemies by providing food and shelters. Two-year experiment was carried out in an organic vegetable system with the purpose to assess predation pressure under different soil management techniques. Field experiments were performed at C.R.E.A Horticulture Research Unit of Monsampolo del Tronto in Central Italy within two fields characterised by different cover crop (vetch & barley) and cash crop (tomato & zucchini respectively). Green manure and roller crimper were the selected cover crop termination techniques and they were compared with a synthetic biodegradable plastic mulched control. Predation pressure was evaluated by using artificial caterpillars built with plasticine. Marks on their surface were assigned to higher taxonomy ranks (chewing insects, birds and mammals). Frequencies of artificial caterpillars predated by chewing insects were correlated with the activity density of carabids with body length > of 15 mm. We found different responses between the two investigated fields. In vetch-tomato system, the frequency of predation was higher in roller crimper; a positive correlation was also found between frequencies of attacked artificial caterpillars and the activity density of carabids (>15 mm). In contrast, in barley-zucchini system neither differences among treatments nor significant correlation were found. These discrepancies were strongly affected by the crop system (cover crops-terminations-cash crops). In conclusion, artificial caterpillar method seems to be a practical and suitable method to measure ground-level predation.



Multiple symbiont acquisition and host shifts in a termite-protist mutualistic association

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Many animals and plants live in interaction with microorganisms that benefit their survival and reproduction. In these mutualistic symbioses, however, natural selection is expected to favour 'cheater symbionts' that invest less or not at all in the cooperation with the hosts. Several mechanisms have been proposed to explain the persistence of mutualisms despite the threat of cheaters. One of them, called 'partner fidelity', maintains an alignment of interests between the host and its microorganisms. In many insects, it has been hypothesized that partner fidelity is insured by a strict vertical transmission of the symbionts (from parents to offspring). However, more empirical evidences are required to test this hypothesis. One macro-evolutionary consequence of vertical transmission is a strict co-cladogenesis, which can be tested by using phylogenetic inferences.

The present study aims to test the hypothesis of co-cladogenesis between the subterranean termites of the genus *Reticulitermes* (Rhinotermitidae) and their mutualistic gut protists of the genus *Trichonympha* (Trichonymphidae). We inferred both the phylogeny of *Reticulitermes* species, based on mitochondrial regions (i.e., COI, COII, 16S), and the phylogeny of their *Trichonympha* based on the SSU rRNA 18S. Analyses revealed that *Reticulitermes* termites have acquired independently 3 to 4 distinct variants of *Trichonympha*, most probably from another termite lineage through horizontal transfers. Co-phylogenetic analyses revealed that the diversification of these symbioses not only includes co-speciation, but also host-switches. The hypothesis of a strict co-cladogenesis can thus be discarded. The implication of these findings for a more general understanding of mutualistic symbioses will be discussed.



ABC-transporter gene silencing inhibits insecticide detoxification in anopheline malaria vector

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Chemical insecticides still represent a main tool for pest control; the insurgence of different types of resistance against insecticides remains a major threat to human health and environmental quality. ABC transporters are major components of the "machinery" that determine insect resistance against xenobiotics; these proteins act as efflux pumps for cell detoxification. Our aim was to obtain a postranscriptional silencing of an ABC transporter gene, achieved trough species- and gene-specific siRNA (RNA interference method), combined with the insecticide permethrin, to induce an increase in Anopheles stephensi insecticide susceptibility and mortality. The ABC transporter more involved in the detoxification process, the ABC-G4, was picked out of all "defensome-genes" as a target for inhibition. We performed bioassays on larvae of An. stephensi, using oral-delivery pretreatment with siRNA against ABC-G4 plus permethrin, and with permethrin alone. Mortality and relative expression of ABC-G4 gene in wool body were analyzed at different time point (6h, 24h, 48h). The same experiment was carried out using the fluorescent siRNA-Alexa Fluor 488, separating intestines from the carcasses, in order to obtain information around siRNA absorption and distribution. The increased mortality in siRNA treated larvae confirms the role of ABC-G4 in permethrin detoxification and the potential of RNA interference as a tool for the development of novel strategies for vector control.



Human based genetic tools to refine genetic populations structure of honey bees (*Apis mellifera ssp* L.) (Hymenoptera: Apoidea: Apidae) colonies at regional scale

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The native distribution of Apis mellifera covers Europe, Africa and western Asia. Thereafter, importations, globalization of commercial exchanges and human management has extended honey bees populations worldwide, excluding Antarctica. It has also led to the admixture of native subspecies, particularly in our temperate regions, and to the common use of managed hybrid populations. Understanding the population structure of hybrid colonies and the intensity of admixture at local and regional scale might help to guide conservation and breeding strategies. Single Nucleotides Polymorphisms (SNPs) data were generated with genotyping-by-sequencing method (GBS) on 285 samples of pooled honey bees workers from different apiaries in Wallonia region (Belgium) and Gabon (Africa). For fine-scale stratification of honeybees populations we employed a newly developed fine-scale SNP-based structure detection tool in humans, called IPCAPS, which was built on principles of iterative pruning Principal Component Analysis (ipPCA). We found that Gabonese honey bees populations are more genetically diverse than Belgian honey bees populations. However, by computing population genetic distance values (Fst) between samples, it seemed that our Gabonese honey bees belong to one clearly defined subspecies, whereas Belgian honey bees should follow an admixture gradient from two hypothetical ancestral groups (lineages). In conclusion, we believe that the cost-effective approach we adopted could help beekeepers and conservation policy makers to design evidence-based standards to preserve native subspecies as well as hybrid populations and to select specific honey bee populations of interest.



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New strategies in organic pest insect control by the characterization of bioactive plant volatile organic compounds of agricultural crops

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This PhD project will aim to collect, identify, quantify and verify the activity of volatile organic compounds (VOCs) emitted by the main fruit crops (such as grapevine, pome and stone fruits) in order to use these compounds in insect pests monitoring and control. Volatile compounds originated by undamaged and insect-damaged plants will be collected by headspace sampling as closed-loop-stripping-analysis (CLSA), solid phase micro direct headspace (DHS) and characterized by (SPME) and chromatography-mass spectrometry (GC-MS) or monitored by proton transfer reaction time of flight - mass spectrometry (PTRToF-MS). In particular the herbivore-induced plant volatiles (HIPVs), which can play a key role in mediating multitrophic plant-insect will be evaluated by techniques such as gas chromatographyelectroantennographic detection (GC-EAD), wind tunnel test and open field lures comparison in order to contribute to the characterization of volatiles blends useful for future strategies in integrated pest management (IPM). Combined volatiles lures will be tested in commercial orchards with standard traps and with smart trapping system, which allows the computer identification of the insects and the remote check of the catches. bringing such informatics technologies in the field experiments. Finally, the use of kairomones in addition or substitution of the sex pheromone can increase the target pest catches and could be useful also for beneficials (parasitoids in particular) monitoring.



High synchronization of *Drosophila suzukii* activity along elevational gradients

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Drosophila suzukii (Matsumura, 1931), also known as Spotted Wing Drosophila (SWD), is a polyphagous invasive crop pest native of South-East Asia. The species is widely distributed on the Italian territory due to its large array of host plants and high tolerance to different climatic conditions. The aims of this work were to test 1) the abundance, fertility and emergence rate of SWD along steep elevational gradients when potential host plants are available 2) the synchronization of time series across locations and within elevational gradients. We selected 12 transect covering an overall elevation gradient of 2100 m: 8 transects in the Prealps, ranging between 100 and 1300 m a.s.l. and 4 in the Dolomites, ranging between 1000 and 2200 m a.s.l. Every 100 m ca. elevation gain we identified a sampling site (tot. 115), where we placed a trap lured with selective SWD attractant, monitored every 2 weeks from June to November 2015. Female fertility was determined by checking the development stage of the ovaries. Fruits of elderberry (Sambucus nigra L.) were collected along the Prealps transects to assess the emergence rate of the fly. In autumn, we noticed a decrease in SWD abundance together with an increase in female fertility at high elevations. Due to its high dispersal potential and mobility, the insect revealed a high synchronization activity across both different locations and elevations. These findings are highly relevant to define its population dynamics, then giving the possibility to forecast and manage the outbreaks of the pest.



Impact of Halyomorpha halys (Stål) on grapevine in Veneto region

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The Brown Marmorated Stink Bug (BMSB), Halyomorpha halys (Stål) (Heteroptera: Pentatomidae), a polyphagous stink bug native of eastern Asia, is becoming an invasive pest in Europe and North-America. In North-America, the European grapevine Vitis vinifera L. is considered as a potential host plant for this stink bug. However, no information are available on the impact of BMSB on this crop in Europe. Here we investigated the occurrence of this pest in vineyards of Northeastern Italy also considering various cultivars. We also assessed direct damage due to BMSB infestation by performing a cage experiment. The infestation level of BMSB was monitored from July to harvest in multicultivars vineyards by using visual and beating sampling methods. Cage experiment was performed on Cabernet Franc, Merlot, Pinot gris, and Glera cultivars by manipulating bunches infestation density and timing. The effect of infestation density and timing was assessed on bunches weight, the incidence of damaged berries and the must quality. The results are discussed in the context of grape IPM.



An insect pheromone primes plant defenses with community wide effects

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Plants frequently employ induced, rather than constitutive, defences against herbivores and pathogens, presumably as an adaptive response to the unpredictability of attack by particular antagonists. Plants may further accelerate defence deployment by "priming" appropriate defences in response to environmental cues that reliably predict impending attack. However, the population- and community-level ecological consequences of such priming remain relatively unexplored. We discovered that the volatile emission of the male gall fly Eurosta solidaginis, primes the anti-herbivore defences of its host plant, tall goldenrod (Solidago altissima). More recently, we determined that E,S-conophthorin, the dominant compound in the emission, elicits a priming response equivalent to that elicited by the overall blend. To understand community level effects of priming in this system, we used field experiments to explore effects of the priming cue on neighbouring ramets at varying distances from the emission source. Following exposure, we quantified levels of foliar herbivory, and plant growth and flower production. We observed a linear increase in leaf damage with distance from the emission source, consistent with the hypothesis that the intensity of priming corresponds to the intensity of exposure to the fly emission. Unexpectedly, ramets nearest the emission source also exhibited a short-term increase in growth-rate. Responsiveness of S. altissima ramets to herbivore-associated cues across a distance of 1m or more is highly relevant in this system, where individual ramets frequently grow within a few cm of one another. The resulting mosaic of ramets primed to varying degrees is likely to influence herbivore (and consequently predator) distributions.



Drosophila suzukii spillover from natural habitats to vineyards and cherry orchards

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The invasive pest *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) was reported for the first time in Italy in 2009, where it is causing heavy damage on cherry, soft fruits and some susceptible grape cultivars. The building up of *D. suzukii* populations is promoted by natural habitats rich in host plants, and by the landscape fragmentation. Characterizing the spillover of adults from natural habitats to cultivated areas is essential to develop sustainable IPM strategies. We sampled, using traps lured with food baits, D. suzukii adults at the margin and in the centre of 20 vineyards close to forests. Moreover, in 7 cherry orchards we observed the D. suzukii captures at different distances from the forest and at different heights from the ground level. Results showed that D. suzukii adults are more abundant at the margin of vineyards/orchards than in the centre of cultivated area. We also noticed that the captures in the cherry orchards strongly declined with increasing distance from the forest margin. Pest abundance decrease also with increasing height from the ground. The patterns observed varied across the crop phenological development stages indicating that *D. suzukii* preferred natural habitats when crops were not suitable for reproduction. Differently, when fruits or berries were ripe, D. suzukii colonized the cultivated habitat. Our findings contribute to implement IPM strategies diversified according to presence and distances with natural areas, based on the modification of orchard configuration, use of insect-proof net or different insecticide application.



First records of *Synthesiomyia nudiseta* (Diptera: Muscidae) from forensic cases in Italy

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The knowledge, at the regional scale, of the fauna associated with carrions and cadavers plays a fundamental role in forensic cases for the correct estimation of the time since death. In the last years global warming and globalization have affected the insect distribution. This phenomenon is affecting also the species of forensic interest associated with cadaver decomposition. The species distribution shift, in the forensic context, has been mainly observed in Diptera of different family: Calliphoridae, Stratiomyidae and Phoridae. In the last decade the presence of the carrion feeding species, Synthesiomyia nudiseta (Diptera: Muscidae), was reported from forensic cases in Spain and in the last year from Italy where the species was collected from 5 bodies in different decomposition stages in the Genoa district. All the records concern indoor cases with the presence of other species belonging to the first colonization waves (e.g. Calliphoridae, Sarcophagidae). Different hypothesis about the presence of the species in Italy can be suggested, but the molecular analysis and the trade importation records support the hypothesis of an introduction trough commercial exchanges with Asian countries instead of a variation in the species distribution area from the Iberian Peninsula.



New acquisition about the role of *Colomerus vitis* in the transmission of Grapevine Pinot gris virus

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Grapevine Pinot Gris Virus (GPGV) is a new Trichovirus associated with symptoms of chlorotic mottling and leaf deformations in grapevine (*Vitis vinifera*).

Preliminary studies indicate that GPGV can be transmitted by the eriophyid mite *Colomerus vitis* (Pagenstecher). Acquisition and transmission by an arthropod vector is central to the infection cycle of the majority of plant pathogenic viruses. In this work we carried out transmission trials and analyzed the virus to detect which strains of the GPGV is transmitted by *C. vitis*.

Specimens of *C. vitis* were collected in GPGV symptomatic vineyards and mites were extracted from infested buds and leaf erinea. A pool of 10 mites for sample was subjected to RT-PCR to ascertain the presence of GPGV. Total RNAs of C. vitis were extracted and GPGV detection was carried out by RT-PCR and real-time PCR (RT-qPCR). To detect GPGV strains, PCR products were both sequenced and compared with GenBank, and digested by BAM HI.

Transmission trials were carried out under controlled conditions (22°C, 70 % relative humidity, 16:8 L:D) using GPGV infected buds and leaf erinea infested by *C. vitis*. Buds and leaf erinea were placed onto GPGV free vines. After transmission trials the appearance of erinea was observed and each plant was analyzed with the above described methods to assess the presence of GPGV and qualify the strain. Both strains of GPGV were found in *C. vitis* as well as in the GPGV positive vines obtained by transmission trials.



Immunological properties of engineered *Asaia* symbionts: implications for the control of mosquito-borne diseases

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The genus Wolbachia collects intracellular bacteria, which commonly infect arthropods. It is well-known that Wolbachia is capable of inducing the production of reactive oxygen species and innate immune effectors in mosquitoes; this immune stimulation is likely involved in the reduction of arthropod vector capacity, toward a variety of pathogens. In fact, the presence of Wolbachia has been associated with the inhibition of virus and parasites development in arthropods. Among Wolbachia molecules, the Wolbachia surface protein (WSP) has been shown to induce immune responses in mosquito cell lines, and Th1 polarization in mammalian hosts, after stimulation of innate-immunity receptors. Due to the intrinsic features of Wolbachia bacterium e.g. not culturable in cell-free media, its use in vector-borne diseases control is limited; for this reason we realized a chimeric bacterium of the genus Asaia, symbiont of Diptera vectors, to express WSP protein. The immunological effects of this engineered bacterium have been tested evaluating the stimulation of the innate immune response in mosquito cell lines and in vivo in presence of Asaia producing WSP. Investigations on the immunological properties of WSP in mosquitoes could generate novel insights into the insect-bacterium relationships, thus providing novel ideas toward the development of tools for the control of mosquito-borne



diseases.

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Modelling the shifts in the timing and spatial distribution of *Vitis* vinifera, Lobesia Botrana and Trichogramma Sp. phenology in a warming climate

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Plants and insects depend on climatic factors (temperature, solar radiation, precipitations, relative humidity and CO₂) for their development. In the context of a warming climate, strong impacts on population dynamics, adaptation, limits of development and phenological stages can be expected. This literature review aims at obtaining an overview of validated facts, models, historical and observed data with the objective of understanding whether the current range of hosts and parasites might evolve under global warming conditions. It is expected that a warming climate might alter tritrophic relations leading to stable conditions, expansion or to extinction of some species, but the knowledge on their timing and distribution in the future is still unclear. This study aims at emphasizing that under climatic change, and at different latitudes, tritrophic relations might evolve in synchrony or asynchrony according to bioclimatic regions. In order to investigate these issues, we shall use modelling techniques, which are a major tool to predict the shifts in plant and insect phenologies and to implement Integrated Pest Management. By applying a sensitivity analysis and different temperature conditions, models can help predict the exact window of overlap between the trophic levels, thereby possibly opening the way for optimal use of biological control agents against insect pests.



Influence of landscape complexity and vineyard management on leafhoppers abundance in North-Italian vineyards

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Different species of leafhoppers (Homoptera Cicadellidae) can threat grapevine production. Among these, Empoasca vitis (Göthe), Zygina rhamni Ferrari and Scaphoideus titanus Ball are the most important in vineyards of North-eastern Italy. The role of landscape complexity and pest management practices are often matter of discussion for their impact on the abundance of both pests and natural enemies. The aim of this study was to investigate the effects of landscape complexity and management strategies (organic vs. conventional) on the abundance of the three leafhoppers. Their presence was assessed in 27 vineyards located in the Conegliano-Valdobbiadene DOCG area from June to September 2016. The presence of E. vitis was more abundant in conventional vineyards in complex scenarios in early season, while in late season it was higher in the organic ones within extremely simplified landscapes. A higher number of Z. rhamni was recorded in organic vineyards in complex scenarios in late summer. The presence of S. titanus was more abundant in organic vineyards within complex landscapes. The different amounts of semi-natural habitats that characterised the landscape complexity and pest management strategies can influence leafhoppers populations densities. Insecticide applications did not reduce effectively leafhoppers populations in organic vineyards. Moreover, the presence of non-cultivated vegetation around small size vineyards could offer shelters from insecticide treatments and favour a subsequent colonisation of organic ones where low persistent insecticides are used. This aspect deserves further investigation since semi-natural areas can also harbour natural enemies like predators and parasitoids.



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ABSTRACTS



Toxic activity for contact and inhalation of some compounds of essential oil of *Humulus Iupulus* (L.) against *Sitophilus granarius* (L.)

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Essential oils (EO) of aromatic plants that exhibit repellent and/or toxic activity against insects often share some active substances but with different concentrations. In preliminary assays, EO of *Humulus lupulus* (L.) was shown to possess a strong contact toxicity against the adults of the granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae), with LD $_{50}$ and LD $_{90}$ values of 13.3 and 40.2 µg/adult. The main components of *H. lupulus* EO were found to be the sesquiterpenes α -umulene (21.1%), caryophyllene (12.6%), selinene (11.6%), and farnesene (9.5%) and the monoterpene ß-myrcene (6.6%).

In this study, the contact and inhalation toxicities of ß-myrcene, farnesene and ß-caryophyllene towards the granary weevil adults was evaluated. Contact activity was assessed by topical application of increasing concentrations of each test compound on the pronotum of adult weevils whereas inhalation toxicity was determined by exposure of insects to increasing concentrations of the same compounds in tightly-closed cylindrical glass containers (600 mL).

All compounds tested showed both contact and fumigant toxicity against *S. granarius* adults. Contact mortalities induced by farnesene and \mathbb{B} -myrcene were significantly higher than that of control starting from 26.7 and 50.1 $\mbox{\mu g/adult}$ and reached 100% at 107.0 and 200.2 $\mbox{\mu g/adult}$, respectively. The strongest contact toxicity was observed for farnesene with LD50 and LD90 values of 53.4 and 80.6 $\mbox{\mu g/adult}$, respectively.

Fumigant mortality by &B-myrcene, &B-caryophyllene, and farnesene showed significant differences compared to control starting from 40.0, 79.8 and 40.0 mg/L and reached 100% at 106.6, 140.1 and 160.0 mg/L, respectively. The highest fumigant toxicity was observed for &B-myrcene with LC50 and LC90 values of 54.2 and 87.0 mg/L.



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Possible side effects of sugar supplementary nutrition on honey bee health

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In the last decade, during the autumn-winter period, honey bee colony losses have been reported mostly related with pathogen and parasites, notwithstanding other factors such as landscape deterioration impacting the foraging activity.

To support honey bee colonies, beekeepers normally supply homemade syrups which could however cause negative side effects, including the formation of hydroxymethylfurfural (HMF) by the dehydration of certain sugars. However, literature data on this subject are unclear, in particular both the toxicity of HMF for bees and its concentration in the syrups.

For these reasons, we tested the survival of uninfested and mite infested bees fed with sublethal doses of HMF as estimated according to the literature. Then we quantified the concentration of HMF in homemade syrups produced at different temperature and pH and supplied these syrups to newly emerged bees to assess their survival.

We show that doses of HMF similar to those reported as sublethal in the literature are non-toxic also for infested bees; however, the amount of HMF that can be found in homemade syrups, which increases with temperature and acidity, can be much higher and causes significant bee mortality.



Preliminary bioassays on the susceptibility of stone fruits rootstocks to Capnodis tenebrionis (L.)

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Capnodis tenebrionis (L.) (Coleoptera: Buprestidae), the so called Mediterranean flatheaded root-borer, is an economically important phytophagous pest species mainly on stone fruit trees (apricot, plum, cherry, peach and nectarine). Chemicals and Entomopathogenic nematodes are used for the control of adults and neonate larvae, respectively. Further control means are under investigations in order to have more options within Integrated Pest Control strategies. This study is aimed at investigating the susceptibility of rootstocks to the larvae of C. tenebrionis. Two bioassays were carried out during 2016 and 2017. A first bioassay was based on the evaluation of a potential antixenosis action expressed by neonate larvae infesting twigs of rootstocks (Marianna 26, Barrier, Adesoto, Mylaboran 29C, GF677, Garnem, Cab 6P, Max Ma60 and Colt). This bioassay allowed to process a high number of different rootstocks in a short time. It has a preliminary value. The second bioassay assessed the antibiosis influence of the rootstocks through the breeding of larvae (since the neonate ones) on artificial diets containing bark flour of Adesoto, Cab 6P, Colt, Garnem, GF677, Max Ma60, Montclar and 29C rootstock. The first bioassay showed that Colt, Mylaboran 29C and GF677 were the most susceptible rootstocks to larval infestation of C. tenebrionis and Max Ma60 was less favorable to the pest. Concerning the effects of the diet, larvae reared on a diet containing Montclar, Cab 6P and GF 677 bark flour had a mean daily increase of their weight higher that those reared on cortex tissues of other genotypes whereas Garnem and Colt had a lower increase.



SELAPIS Project

<u>Grégoire Noël^{1,2}</u>; Gil Leclercq^{1,2}; Julien Bebermans¹; Frédéric Colinet², Nicolas Gengler² et Frédéric Francis¹

Honey bees populations (*Apis mellifera* L.) were domesticated by human populations to better benefit from its many products (honey, pollen, wax, propolis, royal jelly), as well as to use it as a pollination auxiliary in agriculture. However, more than a decade, high losses of honey bee colonies have been noticed in several countries, including Belgium. One factor of this decline is attributed to the mite *Varroa destructor* (Anderson & Trueman, 2000), considered as the main threat for beekeeping. By the inefficiency of the current chemical treatments, the beekeepers are increasingly in demand for honey bee lines tolerant to this parasitic mite. The first goal of this research project is to provide new insights about honey bees diversity in Wallonia (Belgium) and particularly about tolerance against parasitic mite. Secondly, in this diversity, the project plan to select tolerant honey bees strains for achieving transferable progress in the regional beekeeping sector.



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Effects of raw propolis on host/parasite fitness: the case of *Apis*mellifera - Varroa destructor

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Animal self-medication plays a main role among the variety of behavioral defense mechanisms evolved by animals against pathogens and parasites (Lozano, 1998). The conditions defining this adaptive behavior are: 1) the substance in question must be deliberately contacted; 2) the substance must be detrimental to one or more parasites; 3) the detrimental effect on parasites must lead to increased host fitness. A recent study show that *Apis mellifera* is able to increase resin foraging rates in the presence of *Varroa destructor*. Therefore, the second and third criteria need to be clarified yet.

In order to understand if mite-infested colonies could benefit from increasing the collection of resin, laboratory bioassay were carried out to test the acaricidal or narcoleptic effect of propolis on *V. destructor*. Different treatments were compared: crude propolis, and extracts of propolis in both distilled water and ethanol. In addition, we investigated the effect of propolis on *A. mellifera* adult fitness. For this purpose, the longevity of adult bees artificially infested with the mite and kept in experimental cages was monitored over time. The following experimental groups were compared: 1) infested adult bees provided with crude propolis; 2) infested adult bees without crude propolis; 3) uninfested adult bees provided with crude propolis; 4) uninfested adult bees without crude propolis.

Our preliminary results show that honeybees experimentally-infested with *Varroa* mites, kept in cages containing propolis, live significantly longer than those held in cages without propolis. Moreover a narcoleptic and acaricidal action of crude propolis was observed.



Insights on insect vector-plant-phytoplasma interactions: the Flavescence dorée of grapevine

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<u>Background</u>. Flavescence dorée of grapevine (FD) is a phytoplasma-associated disease transmitted by the leafhopper *Scaphoideus titanus*. The disease spreads through primary infections (from gone-wild vines surrounding the vineyard to cultivated grapevines) and secondary infections (from vine to vine within the vineyard). To start looking for disease resistance in grapevine, FDp transmission experiments to the main Piedmontese cultivars were carried out with the vector *S. titanus*. Susceptibility was estimated by measuring the proportion of infected plants and the phytoplasma load, as estimated by qPCR.

PhD research project. The research will address the possibility that susceptibility/tolerance would possibly be related to the vector fitness/feeding behaviour on the different grapevine genotypes and/or to the plant genetic characteristics. For this, Electric Penetration Graph (EPG) studies will be conducted on the most susceptible and tolerant cultivars, and vector fitness will be described by measuring *S. titanus* adult longevity and female prolificacy on the same cultivars. Moreover, the possibility of manipulating vector-FDp interactions using RNA interference will be investigated. For this purpose, dsRNA complementary to insect genes presumably involved in phytoplasma transmission will be produced and delivered to the leafhoppers by injection and feeding. Gene silencing and its effect on vector transmission ability will be evaluated. The expected results, together with the ones obtained by plant physiologists on the identification of grapevine genetic traits possibly involved in resistance, will greatly enhance the comprehension of the epidemiology of FD and open new control perspectives.



Colomerus vitis (Acari: Trombidiformes: Eriophyoidea): some morphometric, biomolecular and biological aspects comparing deutogyne versus protogyne forms

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Eriophyoid mites overwinter by a winter form (deutogyne) morphologically distinct from the spring-summer form (protogyne). Deutogyne is well distinct and known for a part of eriophyoids. Grapevine erineum mite, *Colomerus vitis* (Pagenstecher), is widespread in the main world viticultural areas, was recently demonstrated vector Grapevine pinot gris virus. Its females overwinter mainly under outer scales of buds and a deutogyne was described but not perfectly distinct from the protogyne.

A morphometric, biomolecular and biological approach was applied in order to better characterize deutogynes versus protogynes. Buds or leaves of Luisa infested by *C. vitis* were sampled 6 times from the same vineyard between December 2015 and January 2017. Females were studied for about 70 traits commonly used for taxonomic identification. Kruskal-Wallis test was applied for the measured populations. ITS1 was investigated in individuals collected from buds (winter) and erinea (spring). Groups of protogynes and deutogynes were separately submerged in water or vaseline, at 5/25±1°C, and mite survival was assessed every week/day, respectively. Data were analyzed by regression analysis.

Length of foreleg seta I' and hindleg seta ft', and numbers of smooth dorsal semiannuli were significantly different between protogynes and deutogynes. ITS1 analysis confirmed the homogeneity of these populations. The study demonstrated the presence of population composed by protogynes and deutogynes in July and September 2016. In April and May 2016 were collected only protogynes, whereas in December 2015 and January 2017 were collected only deutogynes, as expected. Deutogynes showed a higher survival than the protogynes in all experimental conditions.



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ABSTRACTS



PMI estimation: a challenge for forensic pathologists

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No problem in forensic pathology has been investigated in detail as that of the Post-Mortem Interval (PMI) determination. The experts have spent so many efforts to get a reliable and objective method of assessment but the results are still inadequate especially in the forensic practice. In the recent past only Forensic Entomology (FE) has provided very useful and innovative information documented by 1.000 scientific peer-reviewed articles approximately from 50 countries published in less than 50 years. The assessment of death chronology based on the entomological evidence can be predicted with accuracy during the early and late PM period respectively based on the oldest specimen associated to the body or scene (early PMI) and the composition of the arthropod community as it relates to expected successional patterns (late PMI). The precision of the entomological method has revealed superior to the qualitative estimates commonly made by forensic pathologists depicting the morphological changes related to the stage of decay. In FE different models of calculation correlating the age of immature specimens to environmental temperatures have been developed. However, several variables may still influence the human decomposition and the development of an insect deeply affecting the accuracy of the forensic estimates. Furthermore, a large variation in the definitions of terms and intervals (such as Period of Insect Activity or Pre and Post Colonization Intervals) has added confusion in the forensic practice and research. The presentation will focus on the need of a common language to be used in FE with a special regard to the standardization and harmonization in order to avoid controversy in forensic practice and/or in courtroom proceedings. Respecting the different disciplines and competences, the complex ecology of the decay process and the role which insects play in the breakdown of corpses suggest that the collection and interpretation of entomological evidence associated with dead bodies should be done preferably by both the forensic entomologist and pathologist correlating circumstantial information and findings noted at the crime scene and at the autopsy. When a close interaction of these two front-line experts is missing, the reliability of PMI calculations can easily fail especially for those death cases where insects are associated with the remains.



Chemical ecology of egg parasitoids in crop protection

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In the last decade, there has been growing attention to the role of semiochemicals in the field of induced defenses involving the release of parasitoid-attracting volatiles by the plants to conserve and/or enhance natural enemies' efficacy in sustainable agriculture. However, the existing literature on plant resistance traits against insects is highly biased, almost investigating plant traits against feeding stages, while plant traits against nonfeeding stages, as the eggs, have received less attention. Exploitation of egg-induced plant defenses, so called 'early herbivore alert', could be a promising strategy given that insect herbivores are killed before the crop feeding stage, thus keeping the crop damage to a minimum. Here, I provide an overview on egg-induced defense traits. I will first briefly discuss direct defense traits targeting directly the herbivore. These include cell growth or cell death that lead to eggs desiccating, being crushed or falling off the plant. I will then discuss indirect egg-induced defenses, which consist of plant chemical cues recruiting parasitoids that kill the egg or the hatching larvae. Such chemical cues can either be volatile compounds, also called oviposition induced plant volatiles (OIPVs), or contact chemical cues (CCs). To date, the use of semiochemicals integrated with natural enemies in IPM is still limited, despite the fact that important basic research has been done in recent years to elucidate the interactions between semiochemicals and natural enemies in a multitrophic context. Finally, I will conclude discussing the aspects the have limited the implementation of herbivore-induced plant defenses in IPM so far, and the real prospect for developing novel and ecologically-sustainable pest management strategies to protect crops from damaging insect pests.



Smart plants or naive insects?

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Plant-pollinator relationship represents a fundamental ecosystem service. Rather than a coevolution of communication signals between animals and plants, these latter have adopted pre-existing signals in animal behaviour/communication. Nowadays, plant-pollinator relationship has not been considered as a symbiotic interaction but a sort of arm-race, with each partner evolving optimizing strategies for increasing its fitness by imposing a cost to the other partner. Orchids are renewed for their peculiar pollination strategies based on pollinator deception and absence of reward. Orchid floral deception is based on mimicking of forms and colours of rewarding flowers or, in the most special case, on the sexual attraction of pollinators with the orchid flower mimicking their female partner both in shape and scent. In this most extreme case, orchid flowers have evolved visual and olfactory clues to parasitize pre-existing sexual communication channels within animal species.



Insects and microorganisms interactions and the induction of plant defence

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Insect-plant interactions are modulated by a number of factors regulating plant metabolism and response. In fact, the attacking herbivore is the first source of molecules active on plants, with the secretions necessary for its feeding activity. Among these, salivary secretions and gut regurgitate play a key role. Thus, the insect feeding produces a mechanical damage that activates plant signalling, and this is reinforced by elicitors delivered at the feeding site. The network of molecular pathways underlying the plant metabolic response is further conditioned by the microbiota associated with plants.

In this presentation the focus is on these issues, by presenting case studies where this complex network of interactions is analyzed from a multitrophic perspective.



Hidden information within mummies and other burials: Paleo-entomology of insect-vectors for investigating past epidemics

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In recent years there was a new, strong interest for the cemeteries of ancient epidemics and new archaeological and molecular methods of looking at this question were adopted, and many mass and multiple burials were explored. Several cases are worth to be mentioned: in England the accurate excavations of the Black Death cemetery (1349), at East Smithfield in London, with 560 individuals and in France the Observance convent (1720-1722), at Marseille, with 179 individuals, the trenches of the Capucins of Ferrières in Martigues, at Bouches-du-Rhône, with 33 individuals of same period, the cemetery of hospital of Fédons (1590), at Lambesc, with 133 individuals, and other important sites. In Italy, two important sites were discovered in the last years: of the large plague cemetery of Saint Michael at Alghero (1582-1583) in Sardinia, with 198 individuals, and the plague or epidemic fever of Lucca in Tuscany (first half of 17th century), with 80 individuals.

Molecular analyses clearly demonstrated that the plague epidemics were caused by different strains of *Yersinia pestis*.

Regarding the insect-vectors, only the genus *Pediculus* was intensively studied so far, but a great research work remains to be done in order to identify and describe the insects involved not only in the plague pandemics but also in epidemic typhus and in some diffuse endemic infections, as for example malaria and Chagas' disease.

In conclusion, the integration of bioarcheology with paleo-microbiology and paleo-entomology will offer a potent tool for understanding the epidemiology of epidemics, eventual effects of other diseases on the emergence of plague and human-pathogen and insect-vector coevolution, addressing questions of great interest for different researchers, as historians, physical anthropologists and geneticists.



Plant Response to Stress: Can this be exploited for Crop Protection?

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A major challenge to both Science and Society is to increase agricultural productivity by an estimated 70% to feed an additional 2.3 billion people by 2050. Strategies to increase crop production must be both resilient and environmentally sustainable, requiring a paradigm shift in current practices. The commercialization of Bt-expressing Biotech (transgenic) crops in the mid-1990s has gone some way to meet these needs. However, it is clear that other technologies and molecules are required to meet future demands. One such approach is to exploit endogenous resistance mechanisms, which form part of the plants' defence armoury. These defences can be either constitutive or induced, the latter often involving extensive reprogramming of defence-signalling pathways. Although endogenous resistance in many crops is currently not adequate to combat heavy pest infestations in an agricultural environment, increased knowledge of these mechanisms provides increased scope for enhancing endogenous defence against phytophagous insects in crops by conventional breeding strategies. The level of partial resistance against pest species achieved using inherent genetic resources may well be sufficient to give adequate protection to a crop under a suitable IPM programme, especially with an increased contribution from predators and parasitoids of the pest. Functional genomics provides a powerful platform to study these responses. Examples from different cropping systems with a view to their subsequent exploitation in breeding programmes to enhance crop resistance to insect pests will be presented.



Are leaf-miners and gall-inducers part of a continuum of plantmanipulators

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Gall-inducing insects are iconic examples in the manipulation and reprogramming of plant development, inducing spectacular morphological and physiological changes of host-plant tissues within which the insect feeds and grows. Despite decades of research, basic mechanisms of gall formation remain unknown. Recent research suggests that some aspects of the plant manipulation shown by gall-inducers may be shared with other insect herbivorous life histories. The ability of leaf-mining insects to manipulate their host plant physiology ask whether leaf-miners can also be considered to be plant reprogrammers. Understanding the evolution and adaptive significance of plant manipulation needs to contrast feeding strategies of different species in an evolutionary framework. It also needs to understand how insects manipulate the physiology and the anatomy of their host-plant. We focused our study on Gracillariidae (Lepidoptera), the majority of which are leaf miners. This family contains around 2000 species but includes only three galligenous species. Among them, Caloptilia cecidophora induce a gall on leaves of Glochidion obovatum (Phyllanthaceae). We conducted a complete histological study of the development of C.cecidophora galls. It appears that first and second instars of C.cecidophora are leaf-miners while later instars turn into gall-inducers. Late larval instars induce the development of new tissues absent from the normal leaf but found in complex galls like those made by Cynipidae. By showing a mix-strategy between leaf-mining and gall-inducing and a convergence in complex gall structures, our results shed light on the origin of gall induction by insects and help to understand the mechanisms of gall morphogenesis.



Host tree-insect interactions in northern forest ecosystems in a changing climate

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In recent years, the magnitude and severity of forest insect outbreaks have been increasing. In northern boreal forests, mild winters are responsible for host shifts, changes in voltinism and range expansion of forest insects. My research program investigates the outbreak dynamics of native and exotic forest insects, particularly, factors that mediate transition between endemic and epidemic states. Currently, I am studying the northward expansion of spruce budworm on to a secondary host, black spruce, including changes in phenology and insect performance. We found that in naturally occurring warm microclimates in the forest, there is tighter synchrony between larval emergence from diapause and budburst of host species. Using a T-face system, we are artificially heating infested saplings to test whether phenological traits in spruce budworm and its host trees are heritable. We predict that as the climate warms, black spruce will become more suitable as a host for spruce budworm.



Signaling networks underlying plant defense responses at individual and community level

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Reactions of plants to insect pests include the activation of a local and systemic defence response essentially based on transcriptional changes that are mainly controlled and coordinated by phytohormones. The attacked plant perceives the invading organism by recognizing herbivore-associated elicitors that selectively induce different defence responses. These latter are enhanced by endogenous plant molecules associated with plant damage (DAMP - Damage Associated Molecular Patterns), that include small peptides present in several plant species. Peptide DAMP are released from larger precursors and bind to membrane receptors to activate signaling cascades that induce local and systemic immunity. The level of protection is conferred by the deep transcriptomic modifications induced by insect damage, which underpin a wealth of metabolic changes. Beside producing new metabolites that are toxic or repellent for insect herbivores or that are able to recall their natural enemies, the metabolic profile of attacked plants can alert neighboring con-specific plant and prime them for a more effective response against a stress agent present in the environment (defense priming). This presentation will focus on the role played by small peptides and their precursors in plant immunity activation and in plant-to-plant communication.



Forensic entomology and funerary archaeoentomology: a source of answers

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In forensic entomology, necrophagous insects are useful in studying postmortem interval (PMI), postmortem transfer (the movement of a body from one location to another after death), presence of drugs or poisons and, in the last years, as well in identifying the victim and/or the suspect. Many species may be used to estimate the minimum PMI (mPMI), according to the stage of cadaver decomposition, body exposure, geographical region, and season.

Funerary archaeoentomology is the application of the principles and techniques used in forensic entomology to human and animal remains, tombs, mummies and other burials of archaeological interest. The two disciplines, forensic entomology and funerary archaeoentomology, are separate and well distinct despite sharing the same bulk of knowledge related to insect colonization of bodies and carrions, and using some common techniques for the collection and analysis of the samples. In fact, it does not make any sense referring to forensic entomology (from the latin *forum*, related to the court) in an archaeological context, being the word forensic referable only to a legal mandate. In an archaeological context, the study of insects associated with a burial may provide reliable information about the taphonomy, funerary practices and the sanitary conditions of the body. If several extrinsic and intrinsic factors can affect the body colonization by insects in the archaeological framework, the cultural context and the funerary traditions and practices have also to be considered to interpret the entomological findings.

This presentation aims to explain how the entomological findings are useful to provide the solution to unanswered questions both in the forensic and in the funerary-archaeological contexts.

