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BOOK OF ABSTRACTS

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PLENARY LECTURE ABSTRACTS

From fragrance to function: Unraveling specificity in green leaf volatile signaling

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Plants can release substantial amounts of volatile compounds from their leaves into the surrounding air. When subjected to insect attacks, the composition of these volatiles undergoes significant changes. These alterations not only impact the behavior of insects interacting with the plants but also influence the metabolism of the plants themselves, as well as neighboring plants.

My research activities have been dedicated to a specific group of plant volatiles known as “green leaf volatiles” (GLVs), which are emitted immediately following herbivory. We identified a class of enzymes present in both plants and insects that play a crucial role in ecological interactions. These enzymes are responsible for converting the highly abundant green leaf volatile *Z*-3-hexenal into *E*-2-hexenal. The distinct effects of these two compounds, along with their derivatives, have significant implications for the behavior of herbivorous and predatory insects, as well as the metabolic processes of plants.

In this talk, I will provide an overview of how plants achieve specificity in their GLV signaling, explore the influence of insects on this signaling specificity, and discuss the consequences of these interactions for both plants and their biotic counterparts.

Keywords; *Green leaf volatiles; VOCs*

Mass spectrometry an indispensable technique for chemical ecological explorations

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Mass spectrometry is a powerful analytical technique to characterize chemical signals used by organisms to communicate with one another. In addition, mass spectrometry enables us to decipher the biosynthetic pathways that lead to the formation of these chemicals used to attract or repel other organisms, signal danger, or mark territories. Deciphering biosynthetic pathways not only enables us to better understand the ecological interactions but also lead to developing novel pest control strategies and improve crop yields. As technology advances and new techniques are developed, mass spectrometry will continue to play a crucial role in our understanding of the natural world and the chemical interactions that shape it. In this talk, I will present many examples from my 40-year exploration on semiochemical characterization using mass spectrometry as my primary analytical tool.

Keywords: *Mass spectrometry; instrumentation; semiochemicals*

Nature-based crop protection solutions for below-and above-ground plant pests

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Agriculture is the backbone of most African economies, and it employs 60% of the labour force, mainly small holder farmers. The sector is dominated by staple food crops including cereals and grain legumes, horticultural crops such as vegetables and fruits, and several commercial crops, namely cocoa, coffee, cotton and cashew, to name a few. However, crop production is constrained by a myriad of pest infestations and exacerbated by climate change and anthropogenic activities. To address these challenges, our research at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, focuses on developing IPM packages based on nature-based solutions as alternatives to the use of synthetic pesticides. The focus of this lecture is on our discovery and application of semiochemicals based on an understanding of the chemical ecology of key pests of some of these crops, with examples drawn for below- and above-ground pests including plant parasitic nematodes, legume pests and tephritid fruit flies.

Keywords: *Plant parasitic nematodes; legume pests; tephritid fruit flies*

Olfaction in blood-feeding arthropods: Evolutionary and functional insights

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Blood-feeding evolved independently several times in Arthropoda, the largest and most diverse animal phylum. From ticks to flies, arthropods display robust olfactory-driven behaviors as they track down a mate or a host and explore for a suitable habitat. However, appreciation for multimodal signaling is growing, and studying senses across arthropods offer unique insights into the origins and function of this signaling. I will present findings from our laboratory's ongoing work, drawing upon two examples wherein olfaction acts in concert with other modalities. The blacklegged tick, *Ixodes scapularis*, is equipped with only ca. 20 olfactory sensilla in and around a structurally simple sensory apparatus, the Haller's organ, that additionally contains other sensory structures contributing to a unique host-seeking strategy defined as 'questing'. I will discuss how questing is manifested in ticks, thereby leading them to a successful exploitation of a host for blood-feeding. In the second example, I will introduce our ongoing work on exquisite mate communication strategies in sand flies. Male phlebotomine sand flies produce pheromones that serve two purposes: they act as an aggregation pheromone by attracting conspecific males and also serve as a sex pheromone. In addition, males produce songs — an acoustic signal generated by vibrating their wings — during copulation. Fly populations across Brazil are shown to utilize unique combinations of these songs and pheromones to communicate. Drawing insights from the recently completed genomes, I will present detailed analyses of the chemoreceptors [odorant receptors (ORs), gustatory receptors (GRs) and ionotropic receptors (IRs)] that show unique patterns. Comparison with other sequenced Diptera revealed a large and unique expansion in sand flies where over 80% of the ~140 ORs belong to a single, taxonomically restricted clade. Analysis of single nucleotide polymorphisms (SNPs) and gene copy number in the chemoreceptors from 63 *Lutzomyia longipalpis* individuals provided insights into the populations that employ unique combinations of sex-aggregation pheromones and songs.

Keywords: *Haller's organ; questing; circadian biology; aggregation pheromones; sand flies*

AWARD LECTURE ABSTRACTS

How very simple molecules can occupy a chemical ecologist’s life

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The cuticular hydrocarbons present on the cuticle of all arthropods are very simple chemical substances. Nevertheless, they are always found in very complex mixtures and can reflect many biological, ecological and historical histories. Initially, they were thought to serve as chemotaxonomic traits, but it was soon shown that they were responsible for different types of individual recognition, sometimes through minute changes in their proportions. These modifications may reflect physiological changes, different ecological niches, hybridism or parasitism, but may also be the result of perfect mimicry. They may also be the cause of one of the most impressive insect invasions on several continents. However, after more than 35 years working on those molecules, we still do not understand how cuticular changes can be so rapid, or what happens biochemically and genomically, even though more or less recent experiments have helped us to understand this. So simple molecules do not mean simple science!

Keywords: *Cuticular hydrocarbons; cuticle; chemistry*

Metal matters! Co^{2+} and Mg^{2+} ions control chain length of prenyl diphosphates in insects

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Terpenes are a huge group of metabolites in all major divisions of life that can be divided into classes (e.g. C_{10}, C_{15} and C_{20}) depending on the number of C_5 units included in their structures. Short chain isoprenyl diphosphate synthases (scIDS's) condense two C_5 units to generate the precursors of each size class, such as C_{10}-geranyl diphosphate (GDP), C_{15}-farnesyl diphosphate (FDP) or C_{20}-geranylgeranyl diphosphate (GGDP). The reaction requires for activation a trinuclear metal cluster, usually containing Mg^{2+} or Mn^{2+} (1). To understand the influence of the metal cluster on the chain-length of the products, we studied a member of the short chain IDS family (PcIDS1) in juvenile horseradish leaf beetles, *Phaedon cochleariae*. In the presence of Co^{2+} or Mn^{2+} the PcIDS1 produced 96% GDP (C_{10}) and 4% FDP (C_{15}) from isoprenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP). In contrast, with Mg^{2+} only 18% GDP but 82% FDP were detectable. Using RNA interference, the PcIDS1 was shown to be the only active enzyme associated with the *de novo* synthesis of defensive monoterpenoids (chrysomelidial) in the leaf beetle larvae. As an FDP synthase the enzyme is responsible for the biosynthesis of juvenile hormone required to control the development of the insect. The detection of Co^{2+}, Mn^{2+} and Mg^{2+} in the fat body of *P. cochleariae* larvae suggests together with *in vitro* activity studies that the flow into C_{10} vs. C_{15} isoprenoids in these insects is regulated by the metal co-factors. Consequently, metal ions may control the terpenoid metabolic flux at a branch point with both potential evolutionary and ecological implications (2). Comparative studies with active enzymes of other insects demonstrate, that this type of regulation is widespread in the insect kingdom (storage pests, aphids, leaf beetles, parasitic wasps).

Keywords: *Terpenoid biosynthesis; metal cofactors; chain length regulation*

1. Aaron JA & Christianson DW (2010) Trinuclear metal clusters in catalysis by terpenoid synthases. Pure Appl. Chem. 82(8):1585-1597.
2. Frick S, et al. (2013) Metal ions control product specificity of isoprenyl diphosphate synthases in the insect terpenoid pathway. Proceedings of the National Academy of Sciences USA 110:4194-4199.

What I learned from Jim Tumlinson when I was his PhD student… and beyond

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This lecture will honor the exceptional contributions of James (Jim) Homer Tumlinson III to the field of Chemical Ecology in general, but also to the scientific development of those who had the good fortune of working with him. Jim worked his way up from a modest upbringing on his family's cattle and cotton farm in rural Mississippi to become one of the most innovative, respected, and productive chemical ecologists to date. His professional life started with a short but impressive military career, after which he decided that he was more suited to become a scientist. I will highlight several of his main achievements in chemical ecology, starting with his astonishing PhD research that led to the eradication of the cotton boll weevil. I will show how he often was one step ahead of his peers and that his work has been of great importance to crop protection. Many of the pheromones that he and his team identified are still being used worldwide for monitoring. I will finish with his group's switch to highly impactful work on plant–insect interactions. With several memorable anecdotes, I will illustrate how Jim positively affected my approach to research, as well as that of others, including the next generation.

Keywords: *plant–insect interactions*; *chemical ecology; biography*

Floral fragrance for pollinators and beyond

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To attract pollinators, flowers use a variety of signals. Visual signals have long been assumed to be of primary importance for pollinator attraction, and only in the last decades we have discovered the beautiful diversity of chemical signals involved in plant–pollinator interactions. In my talk I will elaborate on some of the insights we have gained recently into the chemical ecology of floral fragrance. Whereas flowers often present a reward for their pollinators, deceptive flowers don't, but attract pollinators with out-of-context signals, for example chemical compounds that insects use for mating or search for oviposition substrate. Such mimetic flowers are often pollinated in a highly specific way and chemical variation is typically involved in attraction of different specific pollinators. In the European orchid genus *Ophrys*, an example for sexual floral mimicry, alkenes and long chain esters are often important for mediating such specificity of pollinator attraction. In rewarding flowers, certain compounds can act as honest signals, i.e. those that are correlated to the sugar content of the flower reward. Such honest signals are learned by generalist pollinators and used by them to decide which flowers to visit. Phenylacetaldehyde, a very common floral scent compound, is an example of an honest signal in flowers of *Brassica rapa*, and bumblebees rapidly learn to use this signal. We have shown that this and other floral scent compounds have medium to high heritability and evolve rapidly under pollinator-mediated selection. Floral scent also impacts interactions other than pollination, for example plant–herbivore and other trophic level interactions. In a plant–herbivore–crab spider system we have shown that bee pollinators positively select for increased scent emission, but crab spiders, that also use floral scent for finding flowers where they hunt for insects, remove this selection on scent. Crab spiders, however, feed not only on pollinators but also on herbivores, thus, when herbivore pressure is high, crab spiders are beneficial and plants are selected to attract them to herbivore-invested flowers, which is achieved by herbivore-induced floral scent emission.

Keywords: *Floral scent; crab spiders; herbivory; pollination*

Reflections on the Pherobase

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Almost 150 years ago, the first published observation of the sexual attraction of male moths to their females was made. A century later, bombykol the sex pheromone of silkworm was the first pheromone identified and the word "pheromone" was coined for the chemicals conveying information in interspecific interactions between individuals of the same species. Since then, pheromones have been identified from species across the entire animal kingdom, that extend from bacteria to elephants. The research on chemical communication has been expanded to cover chemicals that are involved in interspecific interactions between organisms. This has resulted in the identification of tens of thousands of compounds that convey information in intra- and interspecific interactions between organisms which were termed "infochemicals" or "semiochemicals", with the latter being most commonly used by researchers.

With the accumulation of semiochemical discoveries in the past 60 years, there was a need to convert these discoveries into electronically searchable database entries. The Pherobase addresses this need and has been publicly available to the scientific community on the world wide web for around two decades. Currently, the Pherobase lists semiochemicals of almost 7000 species and the behavioural functions of about 15000 compounds. In addition, mass spectral data, NMR, and synthesis literature for more than 1500 semiochemicals are available. A separate section provides a list of applications of semiochemicals in pest management that can be categorized by region. Furthermore, the Pherobase lists about 2500 floral volatile compounds and 1700 plant species. Recently, a Kovats calculator and search was introduced, which enables users to calculate the Kovats retention index for a given compound and search Kovats values of over 35,000 organic compounds.

The Pherobase has proven to be a useful tool in semiochemical identification, understanding the evolution of chemical signals, pest management, and monitoring and detection of harmful pests. Over the years, the Pherobase has not only been used by chemical ecology researchers but also been accessed by researchers working in other fields including food chemistry, the pharmaceutical industry, and flavour and fragrance science.

Keywords: *Semiochemicals; pheromones; database*

Developing semiochemical-mediated, behaviorally-based management programs for pests in tree fruit and stored products

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Semiochemical-mediated, behaviorally-based management tactics include those that use volatiles at a distance to manipulate the behavior of the pest population to protect the commodity of interest. This includes a suite of tactics, namely attract-and-kill, mating disruption, push–pull, among others. Over the last 10 years in my career, I have focused on developing attract-and-kill in apple orchards against *Halyomorpha halys* (Hemiptera: Pentatomidae), as well as against a variety of stored product insects. In deploying high doses of *H. halys* aggregation pheromone + a pheromone synergist along the perimeter of apple blocks, attract-and-kill successfully reduced damage by 2–7-fold, while at select baited trees, over 10,000 individuals were killed. The crop area treated with insecticide against *H. halys* was reduced by 97%. Against stored product insects, we used an interception trap design, and found an SPB lure (Insects Limited) was most attractive in the wind tunnel and release–recapture assays, although increasing the number of lures did not increase attraction by insects. We found that long-lasting insecticide netting was a successful kill mechanism, preventing progeny production. Over 2 years, interception traps deployed for 48-h periods on the perimeter of commercial food facilities in two US States captured over 3,000 insects, representing 14 stored product insect taxa. Overall, we demonstrate a successful proof-of-concept for attract-and-kill in tree fruit against *H. halys*, and show significant progress in developing a behaviorally-based strategy against stored product insects. Ultimately, adoption of behaviorally-based tactics may reduce insecticide usage where they are adopted for the pests driving pest management programs.

Keywords: *Pest management; attract-and-kill strategy; behaviour*

SYMPOSIUM 01: SEMIOCHEMICALS AND THEIR APPLICATION IN PEST MANAGEMENT

Abstract Number: 23

Fire ant bait specificity: current status and opportunity for development using semiochemicals

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Fire ants are a significant pest, causing about $7 billion dollars of damage in the United States annually. Toxic bait remains a widely used tool for fire ant control. Many ants are beneficial by providing various ecological services that are essential to the health of our ecosystems. In the case of fire ants, native competing ants are also an important factor in preventing/slowing down their infestation/reinfestation. Conserving beneficial ants is therefore crucial and requires highly specific fire ant bait products. However, despite the tremendous efforts, low specificity remains a major problem in fire ant bait technology, because all the bait active ingredients are broad-spectrum insecticides and most fire ant bait products in the market use soybean oil as a food source that attracts many non-target ant species. Many approaches have been proposed to alleviate this problem, such as using biological control agents and RNA inference instead of conventional synthetic insecticides, developing new bait carriers and formulations, and optimizing the timing of bait application and application rate. Incorporation of ant pheromones into the bait can make it more specific by facilitating the finding of the bait and encouraging fire ants to carry them back to the nest. Recently we discovered for the first time that fire ants feed their nestmates with their own venom. Even more interesting is that adding a venom alkaloid into the soybean oil at high concentrations, such as 6.6 mg/g (6600 ppm), did not significantly affect the feeding preference of fire ants to the soybean oil. This exciting discovery immediately motivated us to investigate how piperidine alkaloids affect the feeding preference of native ants that compete with fire ants for food sources. Remarkably, at 6.6 mg/g, piperidine alkaloid almost completely stopped little black ants and thief ants from foraging on soybean oil. By adding piperidine alkaloids into fire ant bait products, making them unattractive (unpalatable) to other ant species, we demonstrate an innovative method to preserve native ant populations and maintain the health of our ecosystem without sacrificing the benefits of fire ant bait products.

Keywords: *Bait specificity; fire ant; venom alkaloids*

An essential oil component as a building block for pheromone synthesis

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The spherical mealybug *Nipaecoccus viridis* (Newstead) is an insect pest of several major crops including soybeans, grapes and citrus varieties. Virgin females of *N. viridis* release two volatiles, 2,2,3,4–tetramethyl–3–cyclopentene–1–methanol (γ–necrodol) and γ–necrodyl isobutyrate – a monocyclic terpenoid alcohol and a related carboxylic ester [1].

In the course of the pheromone identification, trans–3,4,5,5–Tetramethyl–2–cyclopentene–1–methanol (trans–α–necrodol) and trans–α–necrodyl acetate, found in the commercially available essential oil of Spanish lavender, *Lavandula luisieri*, were rearranged to γ–necrodol that was then used to synthesize γ–necrodyl isobutyrate. GC–MS and NMR data confirmed the identifications. However, the chirality of trans-α-necrodol obtained from essential oil of Spanish lavender, *Lavandula luisieri*, which is crucial for pheromone application in pest control, was unknown. Our objective was to determine the chirality of *trans*-α-necrodol in the essential oil of Spanish lavender that can serve as a precursor in the synthesis of the spherical mealybug pheromone to control the pest.

To determine the chirality of the pheromone, we synthesized γ-necrodyl acetate enriched in (+)-(S)-enantiomer and separated the enantiomers using a lipase enzyme. Then we compared the retention times and mass-spectra of the synthetic and natural components with chiral GCMS analysis.

We confirmed that the natural components, both in the mealybug and in the lavender essential oil, consist of the (−)-(R)-enantiomer [2]. Bioassays conducted in the lab and field show that males are attracted to (−)-(R)-γ-necrodyl isobutyrate pheromone alone.

Pheromones of mealybug species usually require multi-step syntheses that are complicated, which has limited their being used widely in pest management. However, since mealybug pheromones are usually built of terpenic structures, we believe that syntheses of these pheromones can be facilitated by using appropriate essential oil components as preformed synthetic precursors.

Keywords: *Essential oils; mealybugs; pheromones*

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Behavioral response of the invasive spotted-wing Drosophila to anthracnose-infected fruit volatiles

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Spotted-wing drosophila, *Drosophila suzukii* (Matsumura), is an invasive pest of soft, thin-skinned fruits such as raspberries, blueberries, and strawberries. Behavioral manipulation of *D. suzukii* using attractants and repellents is being researched as an alternative to chemical control due to the pest's high likelihood of developing resistance to insecticides. A novel source of potential repellents for *D. suzukii* are volatiles emitted from blueberry fruits infected with *Colletotrichum fioriniae*, a main causal agent of anthracnose fruit rot, an important disease of blueberries. Our earlier studies demonstrated the detrimental effects of anthracnose-infected blueberry fruits on *D. suzukii* attraction, oviposition, and development. Following these studies, 14 differentially emitted volatiles from infected fruits were identified and then tested in two different laboratory choice assays for repellency effects. These assays resulted in the discovery of two anthracnose volatiles that repelled *D. suzukii* from choosing blueberry fruits and that were comparable in activity to known *D. suzukii* repellents such as 2-pentylfuran, geosmin, and octenol. Follow-up studies showed that the response of *D. suzukii* to these volatiles is dose- dependent. The findings from our study can aid in the discovery of novel repellents for *D. suzukii* that could be incorporated into push–pull systems to manage this pest.

Keywords: *Behavioral control; invasive pest; repellents*

Microbiome of fruit flies as a novel source of female attractants for pest management

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The oriental fruit fly (OFF), *Bactrocera dorsalis*, is one of the most destructive pests of fruit and vegetable crops worldwide with ~400 hosts and $20 billion in economic losses from crop damage and trade disruption. Male fly-focused approaches such as the male annihilation technique using methyl eugenol, a potent male attractant, and the sterile insect technique, have been successful in preventing OFF establishment in the US. However, with increased frequencies of OFF invasion, there have been outbreaks from undetected breeding populations of OFF. In this study, we report on our efforts to develop improved means to monitor female OFF populations. Protein liquid baits have been used to monitor female populations of OFF. However, they are considered weak attractants, are difficult to maintain, and attract many non-target insects. Complementary to the current research on developing host fruit and protein food odor-based OFF attractants, microbial volatiles may afford an additional opportunity and compounds for female attraction. Microbes associated with insects have been shown to have attractant and repellent attributes with regard to other dipteran species, depending on the isolate and species involved in the interaction. Here, we report results from prospecting the gut and ovipositor microbiome of *B. dorsalis* as a novel source of female attractants.

Keywords: *Attractants; microbiome; semiochemicals*

Development of attract-and-kill strategy for management of cocoa pod borer

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The yield of cocoa bean in Southeast Asia has been significantly affected by infestation of an insect pest of the cocoa pod, the cocoa pod borer (CPB) (*Conopomorpha cramerella*), and pod losses can be up to 80% of the crop. Until now, application of synthetic pesticides has been the only conventional intervention for growers to control CPB populations. To develop a semiochemical-based alternative management method, an attract-and-kill strategy was evaluated in a Malaysian cocoa plantation. Our modified synthetic method resulted in more economical production of the sex pheromone of CPB, which made this approach possible. The effectiveness of the attract-and-kill strategy was tested in 2-year experiments. Our results demonstrated that the killing stations [8 cypermethrin treated killing stations per ha of cocoa with high dose (100 µg) and low dose (33.3 µg) CPB-pheromone loadings respectively] with lower dose loadings significantly reduced the number of CPB-infested cocoa pods ($p = 0.021$), while those with high dose were ineffective in reducing pod loss due to the mating disruption effect of high concentration of pheromone present in the cocoa field. This useful information will help growers and scientists to develop a management strategy based on natural product into an efficient, safe, and environmentally friendly CPB-control alternative, producing high quality chocolate products for worldwide consumers.

Keywords: Theobroma cacao*; integrated pest management; sex pheromone*

Abstract Number: 192

Pheromone-based trapping method for pea leaf weevil

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The pea leaf weevil, *Sitona lineatus*, is emerging as a serious pest of pulse crops in Montana. Lentils, peas, and beans have increased in acreage in the last 7 years in this area. In the past two years, pea leaf weevil numbers have increased significantly. Currently, monitoring programs are based on damage to growing plants' leaves, with larvae of weevils showing most injury and leaf damage being independent of yield loss. The study was undertaken to develop known pheromone attractants into monitoring systems for pea leaf weevil. This pheromone trapping has not been developed for extensive monitoring and trapping programs in peas despite its potential and the relative ease of optimization required. Testing different trap types, trapping distance, timing, and lure concentration are the next steps toward developing this system into a quantifiable easy-to-use tool for farmers. Current management strategies rely on the accurate monitoring of pest populations. Pheromone-baited traps will help in monitoring and mass-trapping the weevil population. Pheromone-baited pitfall and ramp traps in pulse crops caught significantly more pea leaf weevils than ground or delta traps. Pitfall traps baited with gray rubber septa captured significantly more adults than traps baited with membrane formulations. All these findings can be used to improve pest monitoring and should be considered when developing an integrated pest management program.

Keywords: *Montana; pea leaf weevil; pheromones; pulse crops*

Abstract Number: 93

Odour world of the fruit piercing moth, *Eudocima materna*: A hard puzzle to put together

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The adult fruit-piercing moth (FPM), *Eudocima materna* (L.), is a major pest of commercial fruit crops throughout the tropical and subtropical regions of the world. Although several integrated management strategies are being advocated for this nefarious pest, they are not economically feasible due to which the control of FPM moths remains challenging. An alternate sure-shot way of managing these dreaded moths is through the utilization of semiochemical-dependent management methods. However, the information on isolation and identification of semiochemicals involved in the FPM moth's attraction to its host plant (both oviposition as well as feeding) and conspecifics are meagre. Therefore, In the present study, we aim to understand the semiochemical-dependent behaviors of *E. materna* to decipher the attractant cues involved in olfaction. In addition, *in silico* molecular docking was performed using putative odorant binding proteins (OBPs) of *E. materna* to predict the most efficiently binding compounds for elucidating behaviorally potent active cues. Detailed olfactometer assays and electrophysiological studies (GC-EAD/MS) revealed a bouquet of chemical cues involving majorly esters that *E. materna* preferred. Further *in silico* molecular docking revealed that certain OBPs (namely 6, 7, 10,13,15,18 and 19) showed the highest binding affinity (K_d) to identified EAD-active compounds confirming FPM's attraction to these compounds. The role of these identified semiochemicals has been discussed in light of trapping FPMs under field conditions.

Keywords: *Lepidoptera; sap feeder; semiochemicals*

Identification of behavior-modifying odors for Mediterranean fruit fly isolated from tea tree oil

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The Mediterranean fruit fly, or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most economically important pests of fruit crops worldwide. Trimedlure (TML), a parapheromone attractive to males, is the standard lure used to monitor medfly populations. Although an effective attractant, TML has a relatively short field-life, is expensive, and is supplied by only one manufacturer. Additional research is thus needed to identify alternative attractants to TML. In a previous study we developed a thin-layer chromatography (TLC) method for separating and assaying fractions of tea tree essential oil (TTO) that are attractive to male medflies. Five distinct TTO fractions were separated initially and two were found to be attractive to males. In the current study, four antennally-sensitive monoterpenes from fraction 1, and two alcohols from fraction 3 were isolated and identified via gas chromatography coupled with electroantennographic detection (GC-EAD) and gas chromatography coupled with mass spectrometry (GC-MS). Olfactory responses to volatile emissions from synthetic EAD-active compounds were also quantified by electroantennography (EAG). In small-cage assays the two alcohols were more attractive than TTO, and the monoterpene was less attractive than TTO. However, male flies spent more time contacting and clustering around the monoterpene than TTO or the two alcohols. Developing economical alternatives to TML for monitoring medfly populations could reduce programs costs involved in managing this serious agricultural pest.

Keywords: Ceratitis capitata*; electrophysiology; essential oil*

Efficacy of natural products as oviposition deterrents for invasive fruit flies

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Oviposition deterrent activity of a natural product-based putative oviposition deterrent mixture (OviX; patent pending) was evaluated against two major invasive fruit flies, *Bactrocera dorsalis* (oriental fruit fly, OFF) and *Drosophila suzukii* (spotted wing drosophila, SWD) and bioactive key-deterrent compounds were identified. Two-choice assays using guava-juice infused agar (for OFF) and fresh raspberries (for SWD) treated with OviX deterred OFF and SWD oviposition in a dose-dependent manner with 87% and 99% reduction at 20mg dose for OFF and SWD, respectively, compared to a solvent control (hexane). When individual components of OviX were tested, four compounds significantly reduced OFF oviposition (negative compounds), two compounds had no effect (neutral compounds) and other compounds stimulated (positive compounds). Two-choice tests with the blend of four OFF "negative" compounds failed to elicit the same level of oviposition reduction as OviX, whereas the addition of two "neutral compounds" recovered oviposition deterrence in parallel with OviX. Subsequent subtraction tests showed that only one of the two neutral compounds was necessary and the resulting 5-component blend with a neutral compound plus the four negative compounds showed the same level of OFF oviposition deterrence as OviX. For SWD, only two individual compounds were negative, while other compounds did not affect oviposition. Laboratory two-choice tests showed that the blend of two SWD "negative" compounds was as effective as OviX, indicating the two negative compounds as the key oviposition deterrents for SWD. These results indicate that developing synthetic chemical oviposition deterrents from natural products could be effective against invasive fruit flies, providing their biodegradability and safety to the environment.

Keywords: Bactrocera dorsalis*;* Drosophila suzukii*; oviposition deterrent*

Abstract Number: 124

Development of pheromone-based tools for wireworm management in the northwest region of the U.S.

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Wireworms (Coleoptera: Elateridae) are serious pests of many crops in the United States, including those that are important for food security such as cereals and vegetables. In the Pacific Northwest, growers are susceptible to wireworm damage from several different species and are heavily reliant on insecticides to manage existing populations. However, recent deregistration of effective pesticides has resulted in a resurgence of populations, leaving growers with minimal options for wireworm management. The below- ground life of wireworms makes them difficult to detect, and there has been minimal research into detection of the adults (click beetles). Thus, it was important to research alternative methods to detect and manage wireworms.

Until recently, there were no pheromone tools available for use in managing wireworms in the U.S., excluding the three invasive *Agriotes* species (*A. sputator*, *A. lineatus*, and *A. obscurus*). The need for such tools resulted in collaborative research that has led to sex pheromone and sex attractant identifications of many species across nine genera, including pest species in the genera *Limonius, Melanotus,* and *Selatosomus.*

Research on elaterid pheromones began by field-screening dozens of published and unpublished pheromones of click beetles from Eurasia and North America. These field tests were conducted in several locations throughout the states of Oregon and Washington. Additional applied research on *Limonius* species was also done to optimize trapping, which included lure type, trap type, and trap placement. The results of this research are promising and can be incorporated into additional studies for wireworm management.

Keywords: *Click beetle; pheromone; wireworm*

Development of female attractant for *Bactrocera dorsalis*

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There is a clear need for a new and more potent attractant for surveillance of female oriental fruit fly (OFF). Worldwide, OFF is one of the most destructive invasive pests of fruit that can become a serious trade-barrier once established. Current OFF surveillance programs in the US rely on a large number of traps baited with methyl eugenol and liquid protein bait (torula yeast, TY). Although methyl eugenol is a highly potent attractant, it only attracts males and cannot monitor OFF females. TY-baited traps can attract both sexes and thus have been used for detection of female OFF. However, TY is not strong enough to meet program needs as a standard female attractant; furthermore, TY traps are difficult to maintain, attract many non-target insects, and change attractiveness with time. The lack of suitable sensitivity of TY traps for OFF females appears to be related to their inability to attract females ready for oviposition. In bioassays using cohorts of 14~16-day old mated OFF females, some preferred traps baited with TY while some preferred traps baited with host fruit. Interestingly, the mated females that preferred host fruit odor had 2X more mature eggs in their ovaries and laid 2X more eggs than mated females that preferred TY odor, suggesting great potential for using host fruit odor-based lures to monitor "oviposition-ready" mated females and improve the efficacy of OFF detection. In this talk, we report our current research on developing a chemical lure that is as attractive to female OFF as a preferred host fruit and significantly more effective than TY in catching oviposition-ready females in the field.

Keywords: *Female attraction; fruit fly; physiological status*

Semiochemicals and their application in insect pest management

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Insect pests cause significant losses to the tune of more than $ 540 Billion. This is going to increase considerably due to climate change. Models assessing the agricultural impacts of global warming rarely consider crop losses to insects. Global warming has led to increasing in metabolism followed by higher breeding rates. This has led to Insecticides providing varying degrees of success due to higher economic threshold levels (ETLs) prevailing in the crops leading to a quick build-up of insecticide resistance. Further, insect epidemics such as locusts across crops and the entry of alien invasives like Tuta in tomato, fall armyworm in corn, and pink bollworm in cotton are posing challenges for the current IPM techniques. Governments worldwide emphasize the need for safe and sustainable technologies that can transform pest management practices. As the technology developed by ATGC scales down the dependence on pesticides, it will address the problems associated with the use of insecticides like the buildup of pesticide residues on marketable commodities, occupational exposure to applicators and bystanders, and ecological imbalance due to loss of natural enemies. A chemical ecology-based approach is an emerging platform which can deliver the ideal solution without harming the ecosystem. Insects rely on chemical signals emitted from the same or different species in their local geographical areas for their life history. Semiochemicals are chemicals that have a specific message toward their recipients and can be effectively used for modifying their behavior.

Exploiting the potential of semiochemicals is an eco-friendly approach to managing insect pests, considering its compatibility with biocontrol agents; however, tools using semiochemicals have been largely limited due to a lack of proper diffuser which mimics natural rates of insect release. Meso- and microporous materials are novel carriers/dispensers for the volatile signaling molecules with controlled spatiotemporal release rates. A delivery matrix made of micro/mesoporous sieves with pore channels was developed for loading semiochemicals ranging from sex pheromones, aggregation pheromones, anti-aggregation pheromones, and attractants and has been effectively deployed for mating disruption, attract and kill, and push–pull strategies. Our advanced diffusers have been tested in crops like pink bollworm, fall armyworm, diamondback moth, tomato pinworm, fruit flies, weevils, *Heliocoverpa*, carob moth, and citrus leaf miner to name a few. The technology has been deployed for more than 50 lepidopteran pests globally. The results have been outstanding and the technologies have limited crop damage to less than 5% without any insecticides compared to 15% to 80% damage with conventional insecticide-treated farmer's practices.

Keywords: *Mating disruption; pheromone; semiochemicals*

Semiochemical-based toxic baits for controlling snails and slugs

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Snails and slugs are serious pests of a variety of nursery, horticulture, and ornamental crops. The economic loss attributed to snails and slugs in 2021 was more than USD 711 million around the globe. Snail and slug problems have increased recently due to the increase in acreage under the zero-tillage cropping system. Currently, molluscicide bait products are the primary method of snail and slug management. The lack of a strong attractant that can draw the snail and slug away from host plants is a major limitation behind the development of a reliable and effective gastropod bait. The identification of snail and slug attractants has previously been restricted to whole foods such as beer, yeast, bread dough, vegetables, etc. The use of these attractants shows promise in attracting snails and slugs in controlled environments, but their field use is often impractical in many settings due to food disintegration in outdoor environments. In order to overcome this limitation, we are developing a semiochemical lure- incorporated bait that is significantly more attractive than alternative food sources available in the environment like daylily. In addition, our results demonstrate that the bait incorporated with the attractant is four times more attractive than commercial bait available on the market today.

Keywords: *Attractant; bait; snail*

SYMPOSIUM 02: CHEMICAL ECOLOGY OF TEPHRITID FRUIT FLIES

Abstract Number: 33

Application time of kairomone traps is crucial in reducing fruit fly infestation in mango orchards

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Tephritid fruit flies are economically important pests of horticulture. Among fruit flies, *Bactrocera dorsalis* is the most notorious and a challenge to the global horticulture industry. Although techniques like MAT (male annihilation technique) and BAT (bait application technique) are developed for controlling *B. dorsalis*, a lack of understanding on the field behaviour of *B. dorsalis* and the time of application of these control measures make them appear ineffective. Here, we studied the impact of time of application of BAT and MAT on infestation levels of *B. dorsalis* in mango (*Mangifera indica* L.) orchards. We used BAT in the form of a season-long female fruit fly bait station (FruitClean®FTS) and MAT in the form of a season-long male fruit fly lure station (FruitClean®MLS) to assess the effect of time of application of the selected control measures in reducing infestation of *B. dorsalis* in mango orchards. Seven different combinations were evaluated: plots without bait or lure stations (Control), FruitClean®MLS in orchard with immature fruits (MLS-IM), FruitClean®MLS in orchard with mature fruits (MLS-M), FruitClean®FTS in orchard with immature fruits (FTS-IM), FruitClean®FTS in orchard with mature fruits (FTS-M), FruitClean®FTS and FruitClean®MLS in orchard with immature fruits (FTS+MLS-IM) and FruitClean®FTS and FruitClean®MLS in orchard with mature fruits (FTS+MLS-M). These experiments were conducted during the Indian mango season for 4 consecutive years (2017-2020). Results show that FruitClean®FTS and FruitClean®MLS in mango orchards with immature fruits had significantly reduced *B. dorsalis* population and infestation by 95%. This indicated that time of application of bait and lure is crucial in controlling fruit flies.

Keywords: *Fruit flies; kairomone; mango*

Endosymbionts: A hidden dimension in tephritid–plant interactions

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Niche is a compartment that specifies boundaries in the ecological system to a certain biological entity. Herbivore insects behave within their ecological niches that may be defined in different ways. Of major interest is a reproductive niche created by existing populations so that they thrive and co-exist. Therefore, niche partitioning exists among different organisms as a measure of resource distribution to provide sustainability. Reproductive isolation in general is brought about by reproductive incompatibility or geographical separation. Ovipositional niches exist as an additional level of compartmentalization that prevents population disruption among closely related insect communities. Studies in a few biological systems have revealed that microbes play an important role in the development of such biological niches. Our studies with tephritid fruit flies, namely the Oriental fruit fly *Bactrocera dorsalis* and the guava fruit fly *Bactrocera correcta*, have helped in understanding this interaction from a semiochemical perspective. Olfactometer behavioural assays suggest that the volatile profile of a fruit infested with larvae of one population is an ovipositional deterrent for the other. This was then confirmed to be influenced by the microbes that are associated with eggs. Microbes such as *Klebsiella oxytoca, Enterobacter cloaece, Citrobacter species* and *Serratia marcescens* were isolated from *B. dorsalis*-infested fruits while *Elizabethkingia anopheles, Klebsiella oxytoca* and *Enterobacter bugandensis/ Klebsiella grimontii* were isolated from the fruits infested by *B. correcta*. We speculate that microbes alter the volatile profile of the fruits and thus prevent the competing population from ovipositing in the same fruit. Further GC-EAD/GC-MS studies revealed several important cues that can be employed as ovipositional deterrents. Such niches thus help the populations co-exist without displacing each other.

Keywords: *Microbe; niche partitioning; tephritids*

Attraction of non-methyl eugenol-attracted males of the Oriental fruit fly to beta-caryophyllene

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In controlling the highly destructive Oriental fruit fly, *Bactrocera dorsalis*, the use of male attractants such as methyl eugenol (ME) remains the gold standard in programmes such as the male annihilation technique (MAT). This is because ME remains the most potent attractant for this highly invasive fruit pest species. Consumption of ME by male *B.* dorsalis has been shown to result in result in significantly higher mating success for females compared with non-ME-fed males. Recently, work in our laboratory has been focused on developing non-ME-attracted male Oriental fruit fly lines but supplemented with semiochemical exposure. Non-ME-attracted males offer advantage that they will not be attracted to the MAT trap devices containing toxicant-laced ME. We hypothesized that non-ME-attracted males can be raised from the field, and that those males can regain mating advantage when exposed to certain semiochemicals. This follows our recent work which demonstrated that although pure isolines of non-ME-responding males were not attained, reduced responder males were obtained instead, until the 10th generation of rearing. When those males were assayed for their attraction to the sesquiterpene compound beta-caryophyllene (CP), no loss of attraction to CP was observed when compared to laboratory males that were used as controls. Males were attracted to feed on CP. Further, those CP-fed males showed that they were able to mate significantly earlier and more successfully compared with control males. This suggests that in releases of sterile male flies to compete with wild males for mating, and not getting captured in MAT devices, non-ME-attracted sterile males but supplemented with compounds such as CP can be considered for further field trials. This warrants further investigations.

Keywords: Bactrocera dorsalis*; beta-caryophyllene; methyl eugenol*

References:

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Interactions between Jarvis's fruit fly (*Bactrocera jarvisi*) and the orchid *Bulbophyllum baileyi*

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Orchids use a variety of tactics to attract pollinators. One of the most interesting tactic is the presentation of floral chemicals which act initially as an attractant and then as a pheromone reward for the pollinator. Daciniphilous orchids attract male tephritid fruit flies which feed upon the flower to obtain the chemicals which are then incorporated into the male pheromone blend. Male flies visiting a flower can have the orchid pollinarium placed on them and so, when visiting a subsequent flower, can transfer the pollinia to complete the pollination cycle. We studied the interaction between Australia's only endemic daciniphilous orchid *Bulbophyllum baileyi* and Jarvis's fruit fly (*Bactrocera jarvisi*). This orchid is known to produce the phenylbutanoid zingerone and its reduced form zingerol; we found that *B. jarvisi* responds electrophysiologically to both compounds. To better understand the role of plant chemistry in fly behaviour we used gas chromatography-mass spectrometry to sample the chemical profile of whole flowers within and across days, as well as to quantify the abundance of chemicals in different floral parts. Zingerone and zingerol levels varied between flower parts, with the highest levels for both compounds in the sepals, especially the lateral sepals. This matches with observed fly behaviour tending to occur on these flower parts. We were not able to detect methyl eugenol, raspberry ketone or cue-lure in any of the flower parts. Twelve replicates of ten male *B. jarvisi* caged with a single flower were observed over an entire day; the number of flies on the petals was highest at 8:00 am and dropped steadily throughout the day. There were, however, no differences in overall levels of zingerone and zingerol across time or over the three days of flowering. The presence of these floral compounds in these orchids supports the proposed role of pollinator for this fruit fly species.

Keywords: *Daciniphilous; pollination; zingerone*

Bactrocera dorsalis management by semiochemicals for simultaneous application of SIT and MAT

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The Oriental fruit fly (OFF), *Bactrocera dorsalis*, is an invasive fruit pest of economic and quarantine importance. OFF males are strongly attracted to methyl eugenol (ME), which is instrumental in male annihilation technique (MAT) and sterile insect technique (SIT) programmes that are often implemented sequentially for fruit fly population suppression and eradication. The proposed simultaneous implementation of MAT and SIT aims to reduce operational costs and achieve fruit fly eradication in a more timely manner. However, sterile males released to the field may also respond to MAT's ME-baited traps and, thus, reduce the efficacy of SIT. Therefore, for MAT to be carried out in tandem with the SIT programmes, developing a protocol to reduce the response of *B. dorsalis* to ME is desired. We seek to determine the effects of ME-based diet feeding by immature males in suppressing lure response, enhanced mating performance and longevity. ME-based diet (0– 5%) was offered to immature males for 48 hrs, and their response to ME and mating performance was tested at 3, 7, 11 and 15 days after treatment (DAT) while the fly mortality rate was observed for 60 DAT. Results showed all treatments showed a significant reduction in lure response for up to 11 DAT, with the highest reduction recorded for the 5% ME diet. The mating performance of all treated males increased significantly compared to controls at 3- and 7-DAT. By 15 DAT, only treated males of the 5% ME diet showed a significantly higher mating success than controls. There was no significant difference in mortality between treated and control males up to 30 DAT. However, males with a diet of 5% ME showed significantly higher mortality at 40 DAT and beyond. This finding suggests that ME-based diet feeding by immature *B. dorsalis* male flies potentially contributed to the simultaneous implementation of MAT and SIT programmes.

Keywords: Bactrocera dorsalis*; methyl eugenol; reduced lure response*

Cuticular chemistry of Queensland fruit fly: footprints and their role in chemical communication

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The study of infochemicals produced by fruit flies has mainly focused on volatile pheromones released by males to attract females. Little is known about cuticular compounds (CCs) and their role in close contact communication. CCs have been used as a taxonomic tool, but their biological functions are little understood. We have studied the biological function of CCs in Queensland fruit fly (Qfly). Further, we have found that Qflies leave chemical 'footprints' where they walk. The deposited chemical cues might have a role as markers of mating arenas in Qfly, might be used by females in oviposition decisions, and might inform both conspecifics and enemies about local presence and abundance of flies. We studied sex, age and mating status variation in the CC profile of Qfly, and also assessed variation in CC profile of different body parts in males and females by using Gas Chromatograph-Mass Spectrometer analysis of hexane extractions and solid phase microextraction. Attraction of flies to headspace emitted by conspecifics of different sex and mating status were run using a Y-maze olfactometer. Footprints were collected by allowing flies to walk inside a bottle and extracting with hexane. Behavioural responses of conspecifics to footprint extracts were studied using four arm olfactometers. We also evaluated the persistence of footprints under laboratory conditions as well as the effect of age, sex, mating status and recent yeast consumption on the footprint chemical profile.

The cuticular chemistry of Qflies varies with age, sex, and mating status and also varies across body parts of individual flies. The cuticular profile of mature flies is sexually dimorphic and immature, virgin and mated females each have a different cuticular profile. It is hence likely that CC chemistry in part functions in signalling of sex and maturity. Males are highly attracted to the headspace of virgin and mated females but not to the headspace of immature females. The footprints left by individual flies closely matches their cuticular profile and recent yeast intake affected the chemical composition of the footprint. Footprints persist days after deposition. Low volatility and non-polar structure of fruit fly CCs suggests that footprints might be quite persistent.

Keywords: *Cuticular chemistry; footprints and role in chemical communication*

Electrophysiological responses of two Australian fruit fly species to protein bait lure compounds

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Protein bait lures have been successfully used to control pest tephritid fruit flies in integrated pest management (IPM) and area-wide management (AWM) programs around the world. However, some baits cause blemish to certain commodities such as mangoes. To solve the issue, farmers aim to apply baits to leaves and avoid fruit as much as possible. This is possible in small orchards, where handheld equipment is used, but not in large orchards where speed is required to complete the job. A recent study tested bait application to mango tree trunks at 1 to 1.5 m above ground level, but the process was slow and laborious. An alternative to protein baits is the development of bait stations derived from compounds found in these baits. In this study we first identified volatiles from over half a dozen liquid protein lures using headspace volatile sampling and GC-MS analysis. Major volatile groups identified include small organic acids, alkylpyrazines, arenes, and alcohols. Additionally, ammonia evolving from each lure was analysed using a solid-state sensor. Secondly, we used electroantennographic detection (EAD) to assess the electrophysiological responses from two Australian pest fruit fly species, the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) and Jarvis' fruit fly, *Bactrocera jarvisi* (Tryon). The EAD responses from mature, protein-fed and immature protein-deprived male and female flies from the two species were assessed. The results showed responses to most compounds tested compared to the blank control, with especially strong responses to the alkylpyrazines, and no response to the commercially available lures. Results for both species, sexes and stage of maturity were similar. Future research includes continuation of rotating olfactometer bioassays against the two species as well as the Oriental fruit fly, *Bactrocera dorsalis* (Hendel).

Keywords: *Electroantennogram; electrophysiology*

Neuromodulation and host shifts in the tephritid fruit fly *Rhagoletis pomonella*

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Host shifts are considered a key generator of insect biodiversity. For insects, adaptation to new host plants often requires changes in larval/pupal development and adult behavioural preference toward new hosts (Rajus *et al*., 2021). We are studying the neural mechanism for ecological speciation in *Rhagoletis* spp. (Diptera: *Tephritidae*). This speciation is being observed over the past 300 years in North America. The introduction of domesticated apples (*Malus pumila*) by European colonists around 1800–1850 caused a shift in host preference by these flies from their native host downy hawthorn (*Crataegus mollis*). Various behavioral and electrophysiological analyses have found that preference for the odor of the host is a key factor mediating host choice. We have identified a neuronal switch at the first synapse in the chemosensory system in the brain associated with differential host choice behaviour (Tait *et al*., 2021). This suggests that any changes in host preference must occur via network-level changes in the brain. In addition to behavioural preference, these races exhibit differences in pupal diapause timing with respect to their hosts.

In insects, metamorphosis brings profound reorganization and transformation of the nervous system. Neurotransmitters and neurohormones are known to play a role in both development and behavior. As such, any changes in neuromodulation could link developmental synchronization with adult behavioral response to host plants, leading to host shifts and potentially the emergence of new species. To test this hypothesis, we have used a variety of chemoanalytical, morphological, and immunohistochemical techniques to examine adult brain development, life history timing, and corresponding neurotransmitter levels in *Rhagoletis pomonella* (Kharva *et al*., 2022). We found that diapausing apple race pupae exhibited adult brain morphogenesis three weeks faster after an identical simulated winter than the hawthorn race, which correlated with significantly lower titres of several neurochemicals. Similarly, the non-diapausing apple race and lab-reared colony apple flies also showed a lower level of neurochemicals in the brain. In summary, faster brain development and changes in neurochemicals can be coupled in this speciating system, providing new hypotheses for the origins of species through host shifts.

Keywords: *Host choice;* Rhagoletis pomonella*; neurotransmitter*

SYMPOSIUM 03: CHEMICAL DEFENCES IN PLANT–INSECT INTERACTIONS

Abstract Number: 31

Defense in the Neotropical ant-plant *Tococa*: Working for bed and breakfast

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Ant-plants and their associated ants are classic examples of a defensive mutualism. The so-called myrmecophytes provide ants with pre-formed nesting sites and/or food whereas the ants protect their hosts against insect and vertebrate herbivores. The importance of ants as biotic defenders and the rewards the ants receive in exchange have been subject of many ecological studies. However, knowledge about possible metabolic adaptions of myrmecophytes to their partners as well as the myrmecophytes' general response to herbivory is still limited. In this field study, we elucidated both constitutive and inducible defense mechanisms of the Amazonian ant-plant *Tococa quadrialata* living in association with *Azteca cf. tonduzi* ants via targeted and untargeted metabolomics. Furthermore, by comparing ant-deprived with ant-colonized plants we were able to identify additional effects that the presence of ants may have on the metabolism of *Tococa* plants and hence, their performance. Taken together, our results provide novel insights into this defensive mutualism and reveal how myrmecophytes benefit from their symbionts.

Keywords: *Myrmecophytes; plant defense; ellagitannins*

Metabolomic basis of differential defense responses in maize genotypes on fall armyworm herbivory

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Insect herbivore damage is devastating for agriculture productivity. Fall armyworm (FAW; *Spodoptera frugiperda*) is a serious threat to maize and causes crop loss. Though leaf damage-based phenotyping techniques have identified resistant maize genotypes, the metabolic basis of its resistance/susceptibility is mainly unknown. To fill this knowledge gap, we characterized the maize metabolic responses to FAW infestation in resistant and susceptible genotypes. In this study, we have applied metabolomic profiling by liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) in conjunction with chemometrics and statistical analytical to delineate primary and secondary metabolites critical to impart FAW resistance. We identified key metabolic plasticity related to host plant resistance in maize genotypes, BML 6 (susceptible), CML 71 (resistant) and DMRE 63 (resistant) to FAW feeding, by chemometrics and statistical-frame-work-aided metabolomics analysis. Using an information theory framework, we found that global metabolite alteration in CML 71 and DMRE 63 is directed towards specialization, which leads to the biosynthesis of herbivory-specialized metabolites, including both primary and secondary metabolites. Significant induction of jasmonates (JA, JA-Ile) and benzoxazinoids (BXs), specifically HDMBOA-Glc and HDM2BOA-Glc accumulation contributes to higher defense in resistant genotypes. Our study reveals a combined constitutive and induced metabolic basis of resistance/susceptibility of maize to FAW herbivory.

Keywords: *Herbivory; maize; secondary metabolites*

Abstract Number: 119

Direct and indirect defenses in sorghum against sap-feeding aphids

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Aphids are important pests of plants that cause substantial loss in plant productivity. The sugarcane aphid (SCA; *Melanaphis sacchari*) is a relatively new and devastating pest of sorghum (*Sorghum bicolor*) in the United States. Previously, we identified varied levels of resistance to SCA in founder lines of the sorghum nested association mapping (NAM) population. Compared to wild-type plants, SCA proliferation was lower on SC265 and higher on SC1345 plants. Monitoring of aphid feeding behavior using electrical penetration graph (EPG), a technique to unveil the feeding process of sap-feeding insects, revealed that SC265 plants restricted SCA feeding from the phloem sap. Our preliminary results also indicate that the constitutive and SCA feeding-induced volatiles were significantly higher in SCA-resistant SC265 plants compared to SCA-susceptible SC1345 plants. Moreover, recent transcriptomic profiling of sorghum plants reveals that the SCA-resistant SC265 line is better adapted to activate early defense signaling mechanisms in response to SCA feeding. Taken together, our results indicate that SCA feeding, in addition to altering direct defenses can also modulate indirect defenses in sorghum.

Keywords: *Sorghum; aphids; plant defenses*

Abstract Number: 123

Egg–leaf interactions of two fall armyworm strains

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The fall armyworm *Spodoptera frugiperda* is a generalist noctuid moth and pest to a variety of plant species. It occurs as two strains that differ predominantly in host distribution and timing of mating at night. Despite the generalist nature of the species, the larvae of the so-called corn-strain are found primarily on larger grasses like corn or sorghum, while the so-called rice-strain is mainly found on smaller grasses like rice or pasture. The mechanism of this differential host association remains unclear, but some studies show strain-specific differences in oviposition preference. However, differences in larval preference or performance are inconsistent between studies. Hence, this study focuses on the life stage between oviposition and larva, the eggs, and their interaction with different plants. Differences in overcoming plant defenses or eliciting plant defenses may be especially crucial in this early life stage and could help explain the observed host association. We address differences in plant volatile emissions following oviposition as well as differences in egg structure, color and adhesion. The results are evaluated for their potential to establish isolation barriers between the two strains and for their potential for pest control mechanisms.

Keywords: *Volatile organic compounds; eggs; host differentiation*

Abstract Number: 226

Herbivory and defence responses across ontogenetic trajectories in *Mucuna pruriens* (L.) DC.

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The tropical legume *Mucuna pruriens* L. (DC.) is an ecologically and economically important species native to North-Eastern India that accumulates high amounts of L-3,4-dihydroxy phenylalanine (Levodopa or L-DOPA). The plant thus displays remarkable immunity to attack by insects and small mammals suggesting the key role of L-DOPA in defence. However, a few herbivores feed on it, and appear to have evolved resistance to L-DOPA toxicity. Although much research has focused on the medicinal properties of L-DOPA, little is known about (a) L-DOPA-mediated plant-insect interactions and (b) its interface with other plant defence phenotypes. To address these, we first determined the richness and abundance of herbivores in two *M. pruriens* varieties with contrasting phenotypes, and assessed their damage vis-à-vis expression of five defence traits: trichome density, trichome length, specific leaf area, water content, and L-DOPA content. We used two tissues (leaf and pod) across three ontogenetic stages. We recorded 47 insect species on this species of which 21 showed chewing activity. Of the two varieties, var. *pruriens* with higher L-DOPA and trichome contents experienced less herbivory damage across all the ontogenetic stages. The intermediate-age leaves with higher water content, specific leaf area, and lower L-DOPA content showed greater damage as compared to other tissues in both the varieties. Further, we quantified L-DOPA variability across 124 accessions from 16 Indian states with different geographic locations and climatic conditions. The wild variety, var. *pruriens*, showed uniformly higher L-DOPA content as compared to the cultivated variety, var. *utilis*, irrespective of its geographical origin. Lastly, we investigated the effect of L-DOPA on the generalist herbivore *Spodoptera litura*. The insect showed reduced larval and pupal mass upon feeding on an artificial diet spiked with L-DOPA. The L-DOPA did not affect the survivorship and eclosion rate of *S. litura*, indicating that *S. litura* might have mechanisms to partially tolerate or detoxify L-DOPA. Our study thus provides insights into the ecological role of L-DOPA in plant–insect interactions and the circumstances under which natural selection may favour the manifestation of specific combinations of defensive traits impacting herbivory in this plant.

Keywords: *Herbivory;* Mucuna pruriens*; L-DOPA*

SYMPOSIUM 04: NEMATODE CHEMICAL ECOLOGY IN AGROECOSYSTEMS

Abstract Number: 177

Playing with plant and pest parasites: Getting to the root of nematode chemical interactions

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Plants have a significant impact on soil-dwelling organisms and their community composition through the release of organic molecules. These molecules serve as cues for successful foraging, prompting soil-dwelling organisms to detect and respond to them. However, it remains unclear how specific these responses are and how they evolve over time. This talk will focus on chemotaxis of soil nematodes and explore the ecological and evolutionary aspects of this phenomenon. Apart from CO_2, numerous other chemical compounds from different classes have been demonstrated to induce chemotaxis in nematodes. This suggests that plants, which coexist within a complex nematode community comprising beneficial entomopathogenic nematodes, plant-parasitic nematodes, and microbial feeders, face selection pressure to produce specific molecules in the rhizosphere that maximize their fitness. However, how selection operates and how underground signaling evolves in this context remain largely unknown. With examples from our own lab and other recent studies we will look at potential barriers, paths forward, and other interesting interactions in between.

Keywords: *EPN; plant defense; behavior*

Using a gel-based formulation of entomopathogenic nematodes to control the fall armyworm in Africa

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The fall armyworm (FAW), *Spodoptera frugiperda* Smith, is a devastating pest of maize from the Americas that recently invaded and spread across Africa and Asia. This pest causes severe plant damage in maize cultivation and can significantly reduce yield, thereby threatening food security and the livelihood of millions of farmers. The invasion of FAW has resulted in unprecedented increases in chemical insecticides use in Africa and Asia. The negative impacts of the pesticides have prompted the call for safer and more sustainable control practices.

With this in mind, we have developed a gel formulation containing entomopathogenic nematodes (EPN) that can be applied into the whorl of maize plants. Using a locally isolated EPN strain, we have shown in laboratory assays and in field trials in Rwanda that a low dose of EPN formulated in the gel can drastically reduce FAW infestation and plant damage, which translated into an increased yield.

We are now exploring ways to increase the efficacy of the EPN-gel formulation. One approach is to incorporate UV protectants into the formulation to enhance the longevity of the biological control agents. In another approach, we explore the possibility to incorporate attractants and feeding stimulants to entice FAW caterpillars to feed on the gel formulation, as an attract-and-kill strategy. Finally, we are testing different application approaches to meet the specific needs of both small- and large-scale maize cropping systems. Our first results clearly demonstrate that timely application of our EPN gel formulation offers an effective, safe and sustainable control method against the FAW.

This research was funded by the University of Neuchâtel, the European Research Council Advanced Grant (788949) as well as by CABI, through the UK Department for International Development, the Directorate-General for International Cooperation of The Netherlands and the Swiss Agency for Development and Cooperation under CABI's Action on Invasives Programme and the Plantwise Plus Programme.

Keywords: *Integrated pest management; biological control*

Abstract Number: 107

1-Nonene as a putative apneumone in EPN *Steinernema feltiae* and larval cadaver interaction

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Entomopathogenic nematodes (EPNs) are obligate parasites of insects and are widely applied as environmentally-friendly agents to control economically important insect pests. Despite the fact that EPNs are widely applied for insect pest control, still many aspects of insect–nematode interactions are not completely understood. Hence, a better knowledge of how EPNs locate and discriminate among potential insect hosts may be useful for enhancing the efficacy of EPNs as biocontrol agents.

Volatiles are emitted not only by healthy insect larvae but by larvae infected with EPNs as well. It is known that EPN-infected larvae release different blends of volatiles depending on the EPN and insect species. However, 1-nonene was the only volatile common to all infections. In this study, we have tested the effect of 1-nonene on *Steinernema feltiae* infective juveniles (IJs) (free-living non-feeding stage of EPNs surviving in the soil and searching for suitable insect hosts), widely applied for insect pest control. 1-nonene is not soluble in water, hence the response of the nematodes to the solvent (ethanol) in a two-choice test was recorded. To our knowledge, we revealed for the first time, that ethanol was highly attractive to *S. feltiae*; the attractivity was similar to *Galleria mellonella* larvae, the model insect host of EPNs. Different concentrations of 1-nonene in ethanol affect EPN in a dose-dependent manner and can either repel or attract *S. feltiae* depending on the concentration of the compound. Uninfected insect cadavers do not release 1-nonene, and its concentration increases following infestation; thus only fresh potential prey becomes attractive to IJs. This indicates that nematodes can distinguish between different qualities of the cadaver and that 1-nonene plays an important role in the behavior of the EPN. As the compound is released by a cadaver (non-living organis) and is beneficial for IJs, such a compound is classified as an apneumone. Our results contribute to a better knowledge of EPNs which are widely used as biopesticides.

Keywords: *Entomopathogenic nematodes; 1-Nonene; apneumone*

Cover crop legacy influences belowground natural enemy preference toward herbivore-infested roots

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Western corn rootworm (WCR), *Diabrotica virgifera virgifera*, can cause significant yield losses in maize. One promising approach for controlling root pests like WCR is the use of entomopathogenic nematodes (EPNs), which are soil-dwelling organisms that parasitize insects. Cover cropping is another strategy used to control root pests, as it can alter soil feedbacks. In this study, we investigated if cover crops also influenced natural enemy behavior by studying the preference of *Steinernema feltiae*, an EPN known to parasitize WCR. Two-choice olfactometers were used to evaluate the attraction of *S. feltiae* towards either WCR-infested or control plants grown in soils with four different cover crop backgrounds: pea, triticale, radish, or fallow. Results showed that preference of *S. feltiae* towards WCR-infested maize roots was influenced by the preceding cover crop, where attraction toward infested plants was stronger in plants grown after triticale compared to other treatments. These findings suggest that EPN behavior can be influenced by soil legacies and that cover crop species selection should be considered as a factor in efficacy of biocontrol, reducing the need for chemical pesticides.

Keywords: *Cover crops; entomopathogenic nematodes; multitrophic interactions*

Abstract Number: 153

Vitamin C-mediated interactions between nematodes and aphids

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The interactions between above- and belowground herbivores are deemed important to overall terrestrial ecosystem functioning. Yet, as these organisms, bound by space and time, are not often 'seen' interacting, their influence on each other is mostly overlooked. Amongst the herbivores of *Brassica nigra*, the survival of a specialist aphid, *Brevicoryne brassicae* was negatively affected by prior infection of the root-lesion nematode (RLN), *Pratylenchus penetrans*[1,2]. Metabolic profiling revealed reduced levels of ascorbic acid (vitamin C) in the leaves of RLN-infected plants. Ascorbic acid is a compound with multiple roles in essential metabolic processes of both plants and animals. Aphids, on their own, are incapable of vitamin C synthesis and are therefore reliant on plants to obtain this essential dietary requirement[3, 4]. To confirm the importance of ascorbic acid on aphid performance, we first evaluated aphid survival on *Arabidopsis thaliana vtc2-1* mutant. This mutant line produces 75% less vitamin C compared to its wildtype background Col-0[5]. We compared aphid survival on *vtc2-1* with that on *P. penetrans*-infected Col-0 plants (ColPp). Survival on ColPp plants was lower compared to uninfected Col-0 plants at 3 days-post inoculation (dpi). At 14dpi, the number of surviving aphids in the *vtc2-1* plants also reduced, similar to the number of surviving aphids on ColPp plants.

We hypothesize that in this RLN–aphid interaction ascorbic acid is induced as a reactive oxygen species scavenger during nematode infestation at the roots, thereby limiting its availability for shoot-feeding aphids. To further understand how plants deploy ascorbic acid to mediate this nematode–aphid interaction, we utilized gene expression and targeted metabolomic analyses to map the changes in enzymes and upstream compounds in the vitamin C biosynthesis and recycling pathways.

Keywords: *Metabolomics; simultaneous herbivory; antioxidant*

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Abstract Number: 169

Riddles in the dark: root-knot nematodes facilitate performance of the root-herbivore *Delia radicum*

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Plants form the basis of most natural communities and simultaneously interact with various organisms. These organisms are often spatially separated because of feeding preferences and the presence of physical and chemical traits in different plant tissues. Nevertheless, they may interact indirectly via systemically induced responses. Such plant-mediated interactions occur when one herbivore changes the plant's nutritional quality or defence status, which in turn may affect the performance of a second herbivore sharing the same host plant. Although numerous studies have addressed plant-mediated interactions among aboveground herbivores and between above- and belowground organisms, examples of belowground plant-mediated interactions are scarce. However, since soil-borne organisms also interact with distinct root classes and may trigger responses in distal root parts, we expected that plant-mediated interactions occur belowground as well. To confirm this hypothesis, we experimentally assessed the performance of the cabbage root fly (*Delia radicum*) on *Brassica rapa* plants infected by root-knot nematodes (*Meloidogyne incognita*). In addition, we studied the plant's defence response to either organism independently, but also how *M. incognita* feeding on the fine roots influences the defence response in the taproot, the feeding site of *D. radicum*. We observed a higher emergence of *D. radicum* adults from eggs hatching on roots of nematode-infected plants, which suggests that *M. incognita* infection facilitates *D. radicum* performance. This increased performance coincided with the differential production of several classes of secondary metabolites, including glucosinolates, indole alkaloids and coumarins. Furthermore, we measured an increased accumulation of proteins and an attenuated decrease in carbohydrate levels in the taproot of nematode-infected plants in response to *D. radicum* herbivory. Our results provide evidence that root-feeding organisms may influence each other's performance via plant-mediated interactions, here caused by nematode-induced changes in the accumulation of primary and secondary metabolites in distal tissues. In addition, we argue that root systems should not be considered homogeneous organs but rather as structures with different sub-classes with distinct defence traits and responses to herbivory.

Keywords: *Plant-mediated interactions; induced defences; ecometabolomics*

SYMPOSIUM 05: BIO-ORGANIC CHEMISTRY: SYNTHESIS, METHODS, AND APPLICATIONS IN CHEMICAL ECOLOGY

Abstract Number: 201

The action of plant glucosinolate toxins and the reaction of insect herbivores: chemical approaches

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The interactions between glucosinolate toxins of Brassicales plants and insect herbivores have been frequently studied, but important questions remain about the mode of action of glucosinolates in insects and whether glucosinolates defend against piercing-sucking herbivores, as well as against chewing herbivores. Research on these topics can benefit from chemical approaches.

The textbooks tell us that glucosinolate toxicity arises because the electrophilic isothiocyanates, the major products formed from glucosinolates after hydrolysis, covalently bind to proteins via sulfhydryl groups and the amino groups of lysine residues, and so inactivate them. However, after administering ^{14}C-radiolabelled glucosinolates to feeding insects, only a small portion of the label was found in the protein fraction. The majority of isothiocyanates appeared instead to bind to the free thiol glutathione and to other low molecular weight compounds. An untargeted metabolomics search identified tetrahydrobiopterin as a new substrate for isothiocyanate conjugation in insects. This purine derivative is a cofactor for aromatic amino acid hydroxylases. As a consequence of the decline in tetrahydrobiopterin, tyrosine and dopamine levels decreased, causing reduced cuticle thickness, and the level of the purine nucleotide GTP decreased, causing DNA damage.

Most studies of glucosinolate-insect herbivore interactions have not focused on phloem-feeding insects because such herbivores are not usually believed to be negatively impacted by glucosinolates. Phloem feeders, such as aphids and whiteflies, have slender piercing-sucking mouthparts, which result in only limited plant damage and thus avoid triggering glucosinolate hydrolysis. However, we determined that glucosinolates are indeed hydrolyzed by feeding of the whitefly *Bemisia tabaci*. This worldwide, phloem-feeding crop pest was also found to detoxify glucosinolates by stereoselective addition of glucose moieties, which prevents their hydrolytic activation to isothiocyanates. Administration of ^{13}C-labeled sugars demonstrated that glucosylation was accomplished not via the expected UDP-glucosyltransferases, but via glycoside hydrolase family 13 enzymes catalyzing transglucosidation reactions. These enzymes appear to have been recruited from progenitors that form higher order sugar oligomers in order to reduce the osmotic pressure caused by feeding on phloem sap with its high concentration of sugar. The use of sugar residues in detoxification reactions may be especially favored in phloem feeders since they have a large excess of sugar available for metabolism.

Keywords: *Isothiocyanates; phloem-feeding insects; transglucosidation*

Terpene synthases: Metal keys for precursors and furthering biosynthetic diversity

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Terpenes constitute the largest and most diverse class of natural products, with currently over 80,000 known members. These terpene carbon skeletons can be attributed at least in part to highly promiscuous enzymes known as terpene synthases (TPS). TPS activity interestingly can be influenced by substrate geometry, which is built from standard isoprenoid units of different lengths such as geranyl (GPP) and farnesyl pyrophosphate (FPP). The characterization of a metal-dependent, bifunctional isoprenyl diphosphate synthase from the leaf beetle showed inter- and intramolecular cooperative effects in the homodimer structure through a synthetic substrate analog. These cooperative effects strongly depend on the provided metal ions and regulate the biosynthetic flux of terpene precursors to either biological defense or physiological development. Strikingly, a unique chain length determination domain adapts to form GPP or FPP by altering enzyme symmetry and ligand affinity between both subunits. Balancing the concentrations of terpene precursors in accordance with environmental and developmental demands is an essential cellular process.

Keywords: *Terpene synthases; biosynthesis*

Abstract Number: 198

Biological production of pheromone precursors in recombinant organisms

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The majority of sex pheromones are fatty acid derivatives such as desaturated fatty alcohols and desaturate fatty acyl acetates of C12–C18 carbon chain length with one to three double bonds. The use of sex pheromones is an alternative technology for pest control in agriculture, horticulture, and forestry, and in contrast to insecticides, is biologically safe and environmentally friendly. Currently, although sex pheromones are being chemically synthesized at a commercial level, their chemical synthesis requires expensive substrates and catalysts and generates hazardous wastes. In contrast, the biocatalysis method using recombinant yeast and plant systems are more efficient, have reusability, are cost-effective and eco-friendly. In the present study, we demonstrated the possibility of producing pheromones or a pheromone precursor component in immobilized yeast *Saccharomyces cerevisiae*, *Pichia pastoris* and transgenic plants by the concerted expression of a suite of biosynthetic enzymes such as fatty acid desaturases (FADs) and fatty acid reductases (FARs). Overall, our study demonstrates for the first time that the combined approach of immobilization and permeabilization provides recombinant cells that produce a better yield of pheromones or precursors in a more cost-effective way.

Keywords: *Pheromones; bioproduction; recombinant yeast*

Abstract Number: 114

Exploring the sequence and applications of macrocyclic cysteine-rich plant peptides

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Natural products including small molecules, peptides and macromolecules are valuable sources for applications in agriculture and medicine. Of particular interest among these versatile compounds are peptides from plants, cone-snails, snakes, spiders, scorpions, fungi, and bacteria. Cyclotides are one such unique class of gene-encoded, ribosomally synthesized macrocyclic peptides (26–37 residues) produced by several plant species as a form of their defense strategy. In addition to the end-to-end circular backbone, they form a cyclic cystine knot arrangement comprising a conserved six-cysteine framework. This knotted arrangement along with the circular backbone renders these cyclotides exceptionally stable against enzymatic, chemical and thermal degradation. I will be presenting our recent work that discusses the chemistry and therapeutic potential of cyclotides from *Clitoria ternatea* that is used in traditional Indian medicine as an antioxidant and enhancer of cognitive functions. Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder characterized primarily by extracellular plaques of beta-amyloid peptide (Aβ) deposits and neurofibrillary tangles in the brain. The origin of neurotoxicity is often associated with the aggregation of Aβ monomers into oligomers leading to subsequent formation of fibrils in the pathogenesis. Thus, inhibiting Aβ aggregation as both a preventive and therapeutic method for the treatment of AD becomes the starting point of the majority of research in the field. Emerging evidence shows that transgenic *Caenorhabditis elegans* which expresses the human Aβ (1–42) can be a suitable in vivo model for investigating the toxicity of Aβ. Cyclotide-rich fractions (CRF) from *C. ternatea* tissues were observed to delay Aβ-induced paralysis in transgenic *C. elegans* CL4176 strain expressing human muscle-specific $A\beta_{1–42}$ gene. Additionally, CRF significantly improved Aβ-induced defects in chemotaxis behavior in transgenic strain CL2355, expressing the human $A\beta_{1-42}$ in neuronal cells. Furthermore, Aβ deposits in the transgenic strain CL2006 treated with CRF were significantly reduced. The reactive oxygen species (ROS) assay showed that this protection is likely mediated by reduction of intracellular oxidative stress. These results show that cyclotides from *C. ternatea* could be novel leads for inhibition of Aβ aggregation and potent antioxidants, and a source of a new pharmacophore against neurodegenerative diseases.

Keywords: *Plant peptides; mass spectrometry; neurodegenerative diseases*

SYMPOSIUM 06: CHEMICAL ECOLOGY OF ENDOSYMBIONTS

Abstract Number: 102

Endosymbionts contribution to the cuticle synthesis of pest beetles

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Beetles are distinguished by their hard cuticle that protects them against physical injuries, predators, parasites, and environmental stresses. Tyrosine is a key metabolite in cuticle synthesis and its modifications (sclerotization and melanization), but insects have to obtain it from their diets as they lack the capability to synthesise it on their own. However, several beetle families harbour bacterial endosymbionts that can biosynthesize the precursor of tyrosine, thus contributing to cuticle synthesis, pigmentation and hardening in nutritionally unbalanced environments. Bostrichidae beetles are generally pests of wood or stored food, and they consistently harbour the symbiont *Candidatus* Shikimatogenerans bostrichidophilus. This symbiont mainly retains genes involved in the shikimate pathway, which produces the precursor to tyrosine. In addition, some subfamilies of these beetles host a second symbiont, *Candidatus* Bostrichicola ureolyticus, that retains genes involved in urea recycling and the biosynthesis of peptidoglycan, lysine, methionine, and vitamins. This additional symbiont likely complements the function of *C*. Shikimatogenerans bostrichidophilus, providing the insects with advantages for developing in nutrient-deficient environments.

The goal of our study is to investigate the interplay between symbionts and their host, using *Dinoderus porcellus*, which possess a dual symbiont, as our model system. We specifically test whether *Bostrichicola* recycled nitrogen from urea is incorporated into tyrosine synthesized by *Shikimatogenerans*, and later in the host cuticle, resulting in a targeted exchange of metabolites between the host and the two endosymbionts. Furthermore, we investigate the contributions of both endosymbionts to host physiology, and their regulation, by supplementing the beetle's diet with single amino acids, selective antibiotics, or pharmacological inhibitors. We evaluate the impact on symbionts and host, by monitoring both symbionts' titers and measuring the host cuticle's thickness and melanisation. Correlating diet nutrition, symbiont titer and cuticle measurements will allow us to draw conclusions about the contribution of endosymbionts to host physiology, but also to get insights into possible regulatory mechanisms involved in this tripartite interaction.

Keywords: *Grain pest beetles; endosymbionts; cuticle synthesis*

Endophyte-assisted insect resistance in *Brassica juncea*

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Plants and endophytes have a complex and intricate relationship where endophytes contribute to the host plant's fitness, growth, and defence in exchange for plant carbon and nitrogen resources. However, the role of endophytes during insect-induced responses is not well understood, except for grasses. To address this knowledge gap, we conducted a comparative analysis of the transcriptome, physiological responses, and molecular responses in *Brassica juncea* plants with (E+) and without (E–) seed-borne endophytes following herbivory by *Spodoptera litura*.

We found that E– plants had a trade-off of increased rapid growth with biomass, but this change did not affect the root:shoot ratio, indicating equivalent nutrient uptake. A significant increase in antioxidant enzymes and serine protease inhibitors was observed in undamaged E– plants. These plants showed a downregulation in jasmonic acid (JA) responsive factor (MYC2), but an upregulation in Pathogenesis-Related protein 1 (*PR1*) and indole glucosinolates (*CYP79B2*), thus indicating salicylic acid (SA) signaling response. However, all these responses declined upon herbivory. E– plants also showed an increased transcriptome expression of thionin, mitogen-activated protein kinases, annexin 1, and annexin 3 involved in systemic acquired resistance against pathogens. A decline in post-transcriptional processes was also observed in these plants.

In contrast, E+ plants showed elevated serine protease inhibitor upon insect damage. These plants also showed upregulation of *MYC2,* gibberellin responsive genes and gibberellin responsive gene (*GA20ox1*) expression, indicating a predominant JA signaling response.

Thus, contrary to the JA-based response upon herbivory in E+ plants, E– plants, which mainly had SA-based signaling responses, showed a compromised JA response. This resulted in reduced early responses in E– plants, making them vulnerable to herbivory.

This study provides direct evidence of the role of endophytes in growth as well as resistance in *B. juncea.* Endophytes play a crucial role in managing biotic and abiotic stress in field-based applications, making them an important focus of investigation. By understanding the complex interactions between plants and endophytes, we can develop effective tactics to enhance plant resistance and adaptation to stressors.

Keywords: *Biotic stress; plant–insect–endophyte interaction; plant signaling*

Abstract Number: 161

Emissions from microbial volatile organic compounds (MVOCs) attract female melon flies

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The melon fly, *Zuegodacus cucurbitae* (Coquillett), is a ravaging pest of cucurbit crops worldwide. The behavioural manipulation by a parapheromone, cue lure, to attract male flies is being advocated as a potential component of IPM to manage *Z. cucurbitae* in the field. Many studies are being carried out to attract both sexes of tephritid flies using various methods. Previously, few studies have highlighted the role of endosymbionts in modulating the behaviour and ecology of several tephritids, implying that microbial emissions (MVOCs) can be a source of potential semiochemicals. However, not much attention was given to exploring the volatile metabolites of endosymbionts as a technique to attract *Z. cucurbitae* females in the field. In this context, an attempt has been made to understand the behavioural interactions of the melon fly and its endosymbionts, specifically focusing on the identification of the odorous metabolites of bacterial isolates that are responsible for female attraction. Bacterial isolates were obtained from the gut, rectal gland, and reproductive parts of both sexes of melon flies from field-collected as well as laboratory-reared populations. Higher bacterial diversity was found in field-collected melon flies than in laboratory-reared melon flies. A total of 54 bacterial isolates were obtained and the most prevalent bacteria were *Enterobacter* spp., *Klebsiella* spp. and *Citrobacter* spp. Detailed olfactometer and gas-chromatography linked to electroantennographic detection (GC-EAD) assays involving bacterial volatiles were conducted to determine the attraction potential of these isolates to female melon flies. Of all bacterial volatiles tested, those from *Citrobacter portucalensis*, *Klebsiella oxytoca*, *Klebsiella* sp. and *Enterobacter* sp. significantly attracted female melon flies in laboratory studies but also captured significant numbers in the field, with most of them being females. Further, GC-EAD analyses showed 17 active compounds from the volatile profiles of these four bacteria and this research emphasises the necessity of understanding the melon fly's microbiome in deciphering its behaviour and ecology. Also, this study highlights the need for further research on the role of odorous microbial metabolites in the behavioural management of *Z. cucurbitae*.

Keywords: *Bacterial volatiles; melon flies; insect olfaction*

Abstract Number: 163

Transmission-enhancing effects of a plant virus depend on endosymbionts of aphid vectors

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Aphid-transmitted viruses frequently alter traits of host plants and aphid vectors in ways that enhance virus transmission. Meanwhile, aphid interactions with host plants can also be modulated by the presence of aphid endosymbionts, whose fitness interests may diverge from those of viruses, but few studies to date have examined such interactions. Moreover, the interface between disease transmission and microbial ecology is only beginning to be explored. We explored how different aphid endosymbionts influenced the effects of pea enation mosaic virus on interactions between pea aphids (*Acyrthosiphon pisum*) and fava bean plants. In addition to examining virus effects on plant chemistry, we assessed how effects of virus infection differed for clonal aphid lines harboring each of four facultative endosymbionts (or only the obligate endosymbiont, *Buchnera aphidicola*).

Metabolomic analyses revealed that virus infection downregulates hormonal defenses of plants against aphids. Subsequent assays revealed striking interactions between virus and aphid endosymbionts, with virus effects frequently apparent only for aphids harboring specific endosymbiont strains. Two strains in particular, *Regiella insecticola* Ri and *Hamiltonella defensa*, exhibited strong interactions with the virus across a range of assays, and their presence was often associated with effects that would be expected to favor virus transmission. For example, aphids harboring these strains exhibited significantly higher population growth or biomass on virus-infected plants, despite being the worst performing lines on uninfected plants. Similar patterns emerged in behavioral assays, where aphids harboring these strains frequently exhibited virus effects on dispersal and host-plant preferences that appear conducive to virus transmission, while such effects were absent for aphids harboring other endosymbionts. Additional analyses of metabolites revealed that aphids harboring these endobacteria had higher levels of critical amino acids, sugars, and neuromodulator molecules when feeding on virus-infected plants, which indicate some of the mechanisms involved on the combined effects of virus and endosymbionts on aphid traits.

Altogether, our results demonstrate that the presence of different facultative aphid endosymbionts can modulate virus effects on aphid–plant interactions, with potential implications for disease transmission. More generally, these findings contribute to a growing body of work documenting the important role of microbial symbionts in shaping inter-specific ecological interactions.

Keywords: *Symbiosis; pathogens; plant–insect interactions*

SYMPOSIUM 07: MULTIDISCIPLINARY APPROACH TO PEST MANAGEMENT

Abstract Number: 2

In search of an attractive kairomone for biological control by mass trapping of *Eurytoma amygdali*

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Eurytoma amygdali End., the almond wasp, is an important pest in organic almond orchards potentially causing high crop failure in the southeast of France and in several countries in the Middle East. Currently there is no biological control method against *E. amygdali* and only one effective pesticide is authorized in France.

In order to develop a biocontrol strategy against wasp infestation, we have identified and quantified by GC-MS the volatile organic compounds (VOCs) of the almond tree (*Prunus dulcis*) in spring, when females are attracted by almond trees to lay eggs in almonds in development, under environmentally-controlled conditions. Emissions of VOCs from almond trees were low and mainly composed of sesquiterpenes. Other VOC classes have been identified as monoterpenes, aldehydes, alcohols, esters and anisole.

To find the volatiles most likely to attract *E. amygdali* during the day we have monitored VOC emissions according to temperature and light gradients by PTR-ToF-MS and GC-MS, with five varieties of *P. dulcis* during flowering and fruit and leaf ripening. Emissions increased significantly with temperature but not with light. Depending on the almond variety and the phenological stage, the composition and the quantities of volatiles emitted were different.

Thirty-eight compounds were then tested by electroantennography (EAG) to observe their detection by the female antennae and identify the most active VOCs. The compounds best detected by females are not those most emitted by the almond tree. Only five compounds did not elicit significant EAG responses when compared to control stimuli and no compounds from the panel were much more active than the rest of EAG-active compounds.

We are currently testing the attractiveness on wasps of mixtures of the VOCs most emitted by the almond tree and the most active in EAG in order to develop a kairomone trap, a means of biocontrol by massive trapping against the almond wasp.

Keywords: *Almond; volatiles;* Eurytoma amygdali*; biocontrol*

Abstract Number: 170

Pearl body-associated multitrophic interactions and their application for pest control

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Plants have developed a multitude of traits to defend themselves against herbivorous pests. Production of food rewards to attract beneficial insects is one such trait. Plants produce a diversity of food rewards such as extrafloral nectar (EFN), floral nectar and pearl bodies. Although there is a vast array of studies investigating the role of EFN in plant defence, the role of pearl bodies is relatively understudied. Particularly studies are lacking on how multitrophic interactions influence pearl body production and in turn increase or decrease the consequent plant defence. Exploring interactions associated with food rewards in agricultural crops can provide missing insights for development of pest control measures.

Pearl bodies are produced by several plant species as reward for ants and in exchange, ants defend these plants against insect pests. Sap-sucking pests such as aphids also excrete honeydew as food reward for ants, leading to potential conflict where ants could preferentially defend either the plant or the aphid. We conducted a set of field and greenhouse experiments to explore plant–aphid–ant interactions, their influence on pearl body production and resulting performance of plants, across different plant varieties. Our study system was the okra plant and our field site were located in Cameroon. Okra is a widely consumed vegetable worldwide; in Cameroon okra is usually cultivated by small scale farmers. Okra is attacked by the ant-tended cotton aphid. The plants produce pearl bodies, which are predominantly found on the underside of the leaves and form from epidermal cells.

We will discuss our results and show that pearl body production and its variation across okra varieties can alter ant–plant interactions in the field. Our results further show that plants can adapt their production of food rewards in order to maximize defence, and minimize negative effects on other physiological traits. Finally, we will discuss how knowledge of such food reward-associated interactions can be integrated in management of small-holder farms in order to enhance biological pest control.

Keywords: *Plant defence; ants; aphids*

Abstract Number: 225

Molecule to management: feasibility of RNAi against *Ips typographus* (Coleoptera: Scolytinae)

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Norway spruce (*Picea abies* L. Karst) is the primary forest production species in Europe and the Czech Republic. However, there has been a radical reduction in *P. abies* population due to climate change and outbreaks of the European spruce bark beetle, *Ips typographus*. Existing conventional methods for managing *I. typographus* failed to stop the recurrent outbreaks, so efficient alternatives are needed for efficient bark beetle management. Research on the RNA interference (RNAi) tool has gained significant interest in controlling coleopteran forest insect pests due to its sequence-specific gene-silencing mechanism and efficiency. However, there is no progress in using RNAi against European tree-killing bark beetles (Coleoptera: Curculionidae, Scolytinae). With the genome sequence availability, we postulate that RNAi can be an efficient and aggressive method to control *I. typographus*. To determine the functionality and bio-pesticide potency of RNAi against *I. typographus*, target gene silencing was performed by force-feeding dsRNA. Further RNA-seq studies revealed RNAi-core machinery gene expression dynamics in *I. typographus* and its downstream consequences. We used nano-formulations to protect dsRNA for environmental application and evaluate their efficiency against *I. typographus*. Our findings showed the potential application of RNAi technology against *I. typographus* and other coleopteran wood-boring forest pest management.

Keywords: *RNA interference;* Ips typographus*; Norway spruce*

SYMPOSIUM 08: EVOLUTION AND MECHANISMS OF SOCIAL SIGNALLING IN SOCIAL INSECTS

Abstract Number: 75

Seven decades of ant chemical ecology – retrospect and prospects

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Ants are renowned for being a great factory of natural products. They are endowed with multiple exocrine glands that produce a dazzling array of chemicals, and almost every facet of their social biology is governed by pheromones. Most chemically identified ant pheromones can be categorized as releaser pheromones, e.g., alarm and trail pheromones, but only a few as primer pheromones. An overview of the chemical identities and glandular sources of the various pheromones reveals a clear lack of functional pattern. Pheromones having the same biological function can have diverse chemistry and originate from different glands. It appears that chemical communication in ants has evolved many times independently, haphazardly using both existing biochemical pathways and exocrine glands.

Ant pheromone studies in the past decade or two focused mostly on cuticular hydrocarbons. These are spread throughout the body surface and function as nestmate recognition pheromones. Recent reports indicate also that they may function as primer queen pheromones. However, the ease of hydrocarbon extraction and identification may create the syndrome of "*the streetlight effect" - looking under a lamppost for a key that was lost on the other side of the street.* Considering the fact that ant pheromones are highly chemically diverse, it is not inconceivable to hypothesize that queen pheromones, when present, have likewise evolved independently multiple times, and show similar chemical diversity.

Keywords: *Ants; pheromones; hydrocarbons*

Abstract Number: 80

Brood signals in bumble bees and how they shape sociality

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A hallmark of sociality is reproductive division of labor between reproductive and sterile females. These interactions are often mediated by pheromones. While the role of the queen in regulating worker reproduction in social insects attracted much of the attention in selected model organisms, the role of the brood and its semiochemicals remained poorly understood.

Here, we used bumble bees to examine the various roles of brood in maintaining and manipulating the structure of a social insect colony. We show that worker reproduction is inhibited by young larvae and is stimulated by pupae in a dose-dependent manner, and that workers are responsive to both unique behaviors and chemical signals produced by young larvae and pupae. We further show that the impact of the queen on worker reproduction is partial without the brood, and that the presence of brood regulates large-scale processes at the colony level such as the beginning of the competition phase and the production of gynes and males. Examining the chemical mechanisms underlying these effects reveals signals associated with hunger and age in larvae and with the pupal case, and differential attraction of workers to selected compounds. These studies point overall to a significant impact of the brood on the social behavior of bees and demonstrate the need to examine the effects of brood and the chemical mechanisms underlying these effects more thoroughly across other species.

Keywords: *Brood; pheromones; social insects*

Abstract Number: 117

Task-specific cuticular hydrocarbon profile in workers is an ancestral trait of honey bee species

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Honey bee societies exhibit age-based division of labour (DOL) with young bees performing within-hive tasks like tending larvae (nursing), while older bees transition to outside-hive tasks, culminating in foraging. This transition is a hormonally regulated process of behavioural maturation.

The diversity of extant honey bee species evolved in the Asian tropics. Except the Western honey bee species, *Apis mellifera*, all other extant honey bee species are endemic to Asia. Honey bees show two distinct nesting behaviours. Phylogenetically ancestral open-nesting species build single exposed combs which are surrounded and shielded by a curtain of bees, while cavity-nesting species build multiple combs within cavities, leading to differential investment in nest defence, thermoregulation, and worker–brood ratios. This differential investment provides the opportunity to perform comparative studies on how variation in nesting behaviour influences patterns of behaviour and physiology across species in this monophyletic group.

In this project, we performed cross-species comparative studies on cuticular hydrocarbons (CHCs) with the open-nesting honey bee species *Apis florea* and *Apis dorsata* and the cavity-nesting honey bee species *Apis cerana*. In social insects, task-based differences in CHC profiles could help in optimizing DOL dynamically in response to changing environments. Task-specific CHC profiles could also provide insights into underlying patterns of DOL across different species. We specifically tested: (i) if there are task-specific CHC profiles in these three Asian honey bee species, and (ii) if so, does nesting behaviour correlate with patterns of variation in the CHC profiles across task?

Our results show that task-specific CHC profiles are present in the three species that we studied, which suggests that this is a conserved, ancestral trait in honey bees. Additionally, results from our work show that nurses from open-nesting species form two sub-clusters, with one cluster forming near the day-olds and another forming near the foragers. We propose that these two sub-clusters are due to differences in the dynamics of behavioural maturation across nesting phenotypes, with the dynamics being slower and more variable at the worker population level in open-nesting species. We are currently collecting age- and task-based CHC samples from open- and cavity-nesting species to explicitly test this hypothesis.

Keywords: *Asian honey bees; comparative biology; cuticular hydrocarbons*

Abstract Number: 172

Biosynthesis of Dufour's gland signals in *Bombus impatiens* – insights from RNAseq and proteomics

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Pheromones regulating social behavior are one of the most explored phenomena in social insects; however, the genetic basis of pheromone biosynthesis is still poorly understood. Here we examined the gene and protein expression associated with pheromone biosynthesis in the Dufour's gland of the social bee *Bombus impatiens*, where the compounds of this gland, mostly esters, terpenes, and hydrocarbons, are involved in reproductive signaling. We conducted chemical, RNAseq, and proteomic analyses of the gland in queens, gynes, reproductive queenright and non-reproductive queenless workers. The gland contains unsaturated wax esters specific to queens, saturated esters specific to workers, and terpenes and terpene-esters found exclusively in the queen caste, predominately in gynes. The worker esters are produced in higher quantities by queenright compared to queenless workers. Ester and terpene compounds are assumed to be synthesized *de novo* in the gland; however, their genetic basis is unknown.

A whole-transcriptome gene expression analysis of the gland showed distinct transcriptomic profiles, with thousands of differentially-expressed genes between queens, gynes and workers but not between the worker groups. According to their chemical phenotype, workers and queens express genes associated with key enzymes in the biosynthesis of wax esters. Genes related to terpene biosynthesis were upregulated mostly in gynes, but also by other female groups although terpenes are produced mainly by gynes. Proteomics using the same female groups recovered over hundreds of proteins, of which over 100 were differentially expressed. Many of these were found between the worker groups, including key genes in fatty acid biosynthesis. Protein expression associated with terpenes was mostly upregulated in gynes. Overall, the proteomic data matched more closely with predictions based on chemical phenotype than the transcriptomic data. We discuss the biosynthesis of esters and terpenes and whether the results of the RNAseq and proteomic data differ due to differential regulation at the mRNA or protein level, or methodology. Overall, our data provide strong candidate genes for functional validation by crossing the data sets and demonstrate gland-specific regulation of chemical signals associated with social behavior.

Keywords: *Social insects; chemical signals; biosynthesis*

Origin of linoleic acid biosynthesis in Blattodea

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Fatty acyl desaturases (FAD) are involved in primary and secondary metabolism in the vast majority of organisms. These enzymes catalyze the introduction of unsaturated bonds into the acyl chains of fatty acids. Important insights into FAD functions have been obtained from studies on different insect species, in which they often participate in the production of fatty acyl-derived pheromones.

In our work, we focused on characterizing FADs in termites and cockroaches (Blattodea) with respect to their involvement in the biosynthesis of linoleic acid. From public databases and our own NGS sequencing data, we reconstructed a comprehensive phylogenetic tree of blattodean FADs in the context of FADs from other insects. Using AlphaFold, we generated 3D structures of selected FADs to explore the geometry of their substrate tunnels and predicted their desaturation activities and substrate specificities. Subsequently, candidate FADs were functionally characterized. Our results revealed that termites possess a Δ12 FAD that is responsible for the biosynthesis of linoleic acid from oleic acid. Termite ancestors inherited this FAD from their cockroach ancestors, in which it evolved roughly 160 million years ago via gene duplication and neofunctionalization of a ubiquitous Δ9 FAD. The discovery of the Δ12 FAD provides a better understanding of how termites survive on a diet deficient in fatty acids. Additionally, the newly described Δ12 FAD may be potentially involved in the biosynthesis of unsaturated fatty acyl pheromones known to occur in termites.

Keywords: *Fatty acyl desaturases; linoleic acid biosynthesis; Blattodea*

SYMPOSIUM 09 : HERBIVORY-INDUCED PLANT VOLATILES IN PLANT PROTECTION

Abstract Number: 103

Attraction of a parasitoid to volatiles of Zambian maize accessions attacked by the fall armyworm

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The fall armyworm (FAW, *Spodoptera frugiperda*) is an invasive pest present in Africa since 2016, causing substantial losses in maize yields. In this new ecosystem, indigenous parasitoids have been identified that can use FAW as a host, such as the larval endoparasitoid wasp *Cotesia icipe*. These natural enemies have the potential to be used in biological control strategies, as alternatives to the excessive and harmful use of synthetic pesticides. Like many other parasitoids, *C. icipe* likely uses herbivore-induced plant volatiles (HIPVs) to locate hosts. If so, its biological control potential may depend on selecting the right maize variety, one that releases highly attractive HIPVs under FAW attack. With this in mind, we used ten maize accessions from Zambia to test whether (*i*) the accessions differ in HIPVs emissions upon FAW larvae attack, and whether (*ii*) the accessions differ in their attractiveness to naïve parasitoid females of *C. icipe*. For this, we collected, analysed and compared the FAW-induced volatiles of the ten accessions. We found that the HIPVs blends differed considerably in quantity but also in composition. While green leaf volatiles were emitted in similar quantities by all accessions, there were significant differences in emission of terpenoids, with some accessions emitting large quantities, some accessions emitting low quantities, and some accessions emitting only low quantities of mono- and sesquiterpenes but higher amounts of homoterpenes. Accessions with overall high terpenoid emissions also emitted more linalool and indole. To test the attraction of *C. icipe* towards the HIPVs, a 6-arm olfactometer was used. Three of the maize accessions were consistently more attractive to *C. icipe* females than the other accessions. Among the three most attractive accessions, the volatile profiles were highly variable with one accession emitting overall high quantities of volatiles and the two other accessions emitting smaller quantities of volatiles, indicating that the parasitoids are not simply attracted to large quantities. Additional assays are underway to more precisely pinpoint the reason for the differential attractiveness. Choosing an attractive maize accession could be an effective strategy to increase the effectiveness of *C. icipe* in the biological control of FAW larvae.

Keywords: *Tritrophic interactions; larval endoparasitoid; herbivore-induced plant volatiles*

Abstract Number: 147

Chemotypic variation in wild cotton populations (*Gossypium hirsutum*) from the Yucatán Peninsula

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Wild cotton plants (*Gossypium hirsutum*), when grown under controlled laboratory conditions, exhibit structural diversity in their volatile chemical profiles. Much of this is due to the diversity of volatile terpenoid compounds that are stored in pigment glands. Two chemotypes were described; one chemotype contained higher levels of the monoterpenes γ-terpinene, limonene, α-thujene, α-terpinene, terpinolene, and p-cymene, while the other chemotype was distinguished by higher levels of α- and β-pinene. Chemotype distribution also followed a geographic gradient. In order to assess whether the same chemodiversity patterns were displayed in mature plants growing in their natural environment, we performed chemotyping on eight populations of wild cotton that were growing along the northern Yucatán Peninsula coastline. In addition to collecting samples for chemical analyses, we surveyed for insect presence in order to evaluate whether there was a relationship between chemotype and insect/herbivore damage, and if so, whether it followed geographic lines.

Keywords: *Chemotypic variation; wild cotton (*Gossypium hirsutum*): Yucatán Peninsula*

Abstract Number: 154

Host plant and conspecific body odour cues elicit behavioural responses in ash weevils

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Olfactometer bioassays and electroantennogram studies were conducted to understand the behavioural response of ash weevil, *Myllocerus subfasciatus*, a folivore pest of eggplant to its host plant and conspecific body odour cues. Host plant volatiles from eggplant [both healthy plant volatiles (HPVs) and herbivore-induced plant volatiles (HIPVs)]; hexane extracts of whole-body wash of males (MBWs) and females (FBWs); whole body volatiles of males (MBVs) and females (FBVs); and hexane extraction of thoracic glands of males (MTGs) and females (FTGs) were used as odour sources in both electrophysiological and behavioural assays. The results revealed that both sexes of ash weevils showed greater response to HPVs and HIPVs. EAD-active individual synthetic standards and synthetic blends from HPVs [n-undecane; n-pentadecane; β-caryophyllene, hexyl-1-decanol, 2-hexadecanol (100 ng/µL) and their synthetic blend] and HIPVs [n-undecane; ethyl benzoate; n-pentadecane, 2-hexyl-1-decanol, 2-hexadecanol (100 ng/µL) and their elicited significant response in both sexes. Similarly, conspecific body odour cues from MBVs, FBVs, MBWs, FBWss, MTG, and FTGs induced significant responses in the *M. subfasciatus* weevils. From the MTGs, we identified few aggregation pheromonal compounds viz., 2-methyl-4-heptanol and 2-nonen-1-ol which elicited behavioural responses in males. This demonstrates the efficacy of eggplant as well as conspecific odour cues that can lure adult male and female weevils of *M. subfasciatus* under laboratory conditions. The chemical compounds identified in the host plants and conspecifics body odours may provide information on developing future semiochemical-based management for the ash weevil *M. subfasciatus* in eggplants.

Keywords: *Ash weevil; host plant cues; conspecific cues*

Abstract Number: 211

Odor sensors to detect and identify agricultural pests for crop protection

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Plants respond to biotic stress by releasing specific blends of volatile compounds. Particularly intriguing are the so-called herbivore-induced plant volatiles (HIPVs), which various plants release in large quantities when they are attacked by herbivores. Natural enemies of herbivorous pests may use these plant-provided signals to locate plants with potential prey or hosts. Currently, there is an urgent need to develop agricultural technologies with less detrimental environmental impact, such as reducing the excessive use of harmful agrochemicals. An early and precise detection of attackers would be a way to drastically reduce the need for pesticides because by knowing exactly when an individual plant is being attacked, and by whom, could help farmers to take control measures promptly and accurately. In the current study, we tested whether odor-sensor devices could be applied for this purpose. We addressed the question whether plant odors can be used to monitor the presence of pests and diseases on crop plants. For this, we sampled volatiles from healthy plants, plants infected with the anamorphic fungus *Glomerella graminicola*, and plants infested with caterpillars of the lepidopteran pests *Spodoptera frugiperda* or *S. exigua*. This was done with two commercial hybrids of maize, Delprim and Aventicum. Using two techniques, one involving piezoresistive membrane surface stress sensors and the other proton-transfer reaction mass spectrometry, we found that, under laboratory conditions, both techniques distinguished with 80 to 90% accuracy, between maize plants that were either healthy, infested by caterpillars, or infected by a fungal pathogen. Under field conditions, the mass spectrometry sensor was readily able to recognize plants with simulated caterpillar damage from undamaged plants. In addition, a deep-learning model using the Extreme Gradient Boosting algorithm could predict the damage status of a plant with an average accuracy of up to 80%. With these two very distinct odor detection devices we demonstrate the feasibility of developing real-time pest-infestation diagnostics based on plant volatiles.

Keywords: *Volatiles; agriculture; sensors*

SYMPOSIUM 10: CHEMICAL AND MOLECULAR ECOLOGY OF PLANT–HERBIVORE–NATURAL ENEMY INTERACTIONS

Abstract Number: 74

Predator sensing by plants: Consequences for tri-trophic interactions

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Tri-trophic interactions between plants, herbivores, and herbivore natural enemies are key drivers of ecosystems and determine yield in agriculture. Evidence is accumulating that plants perceive the presence of herbivore natural enemies and respond to it. Yet, it remains unknown how relevant the response is for multitrophic interactions. In this study, we investigated how the maize response to the presence of entomopathogenic nematodes (EPNs), predators of root herbivores, affects the performance and behaviour of root and leaf herbivores under laboratory and field conditions. Upon exposure to EPNs, maize plants exhibited changes in the metabolomic profiles of root exudates, root, and leaf tissues. Additionally, prior exposure of maize plants to EPNs altered the distribution of the stem borer *Ostrinia nubilalis* in the field. Preliminary assays suggest that this shift in herbivore population dynamic relies on changes in female oviposition site selection, but not on larval performance. We discuss the potential adaptive value of the plant response and how the interplay between plants and natural enemies of herbivores may help to increase the efficiency of EPNs as a biocontrol agent.

Keywords: *Tri-trophic interactions; herbivory; entomopathogenic nematodes*

Abstract Number: 77

Perception of host plant VOCs by two races of cherry fruit flies *Rhagoletis cerasi*

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Larvae of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae), damage sweet and sour cherry fruits, and thus belong to pests of economic importance. This tephritid is distributed throughout Europe and western Asia and develop on host plants from two genera: *Prunus spp*. (cherries) and *Lonicera spp*. (honeysuckle). Two sympatric races can be distinguished, and this was supported by genetic analysis. Thus, this species can be at the stage of splitting into two species, which makes it an interesting model for research.

Data on the reactions of these insects to the VOCs of different host plants are limited. They could be useful for understanding speciation in the pest species. VOCs from the headspace of sour cherry, *P. cerasus*, fruits and those of honeysuckle, *Lonicera xylosteum*, were analysed. Gas chromatography–mass spectrometry (GC–MS) resulted in the identification of 51 compounds emitted by cherry. Terpenes and esters prevailed in two aspects: in the highest diversity of the compounds, and the amount of the total VOC emissions (62.3%). Among the single VOCs, ethyl octanoate prevails, followed by (*E*)-4,8-dimethyl-1,3,7-nonatriene. GC–electroantennographic detection (GC–EAD) revealed 14 EAG-active compounds and those were identified. In Y-tube olfactometer tests. The following EAG-active compounds attracted *R. cerasi* females in a similar way to the odour of sour cherry fruits: (*E*)-*β*-ocimene, linalool, and (*Z*)-3-hexenyl 3-methylbutanoate. Results of comparative analyses on honeysuckle fruits will be presented, highlighting the similarities and differences.

Keywords: *Pest insects; host plant; attractants*

Bioluminescence as a regulator of multitrophic interactions in belowground ecosystems

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Bioluminescence is the chemical production and emission of light by living organisms. This trait has evolved multiple times independently and occurs in more than eight hundred genera across the tree of life. While bioluminescence is well-studied in aquatic ecosystems, less is known about its ecological and evolutionary significance in terrestrial ecosystems, and almost nothing is known about the role of bioluminescence in belowground ecosystems. My group uses bioluminescent *Photorhabdus* bacterial symbionts as a model to understand the biological relevance of bioluminescence in the soil. *Photorhabdus* symbionts live in association with *Heterorhabditis* entomopathogenic nematodes. These nematodes penetrate soil-dwelling insects, move towards the insect hemocoel and release their *Photorhabdus* bacterial symbionts. Following the infection, *Photorhabdus* bacteria reproduce, produce toxins and immune suppressors that kill the insect host. Nematodes then feed on bacteria-digested insect tissues, and reproduce inside the insect cadaver before emerging as infective juveniles to search for a new host. During the colonization process, *Photorhabdus* bacteria produce bioluminescence, which results in a characteristic glow of the infected cadavers. How this type of bioluminescence impacts the behaviour, performance, and physiology of other soil-dwelling organisms including entomopathogenic nematodes, plants, and predatory and scavenging insects, remains unknown. During my talk, I will present our findings in this context and will show that this unique bacterial trait is a powerful regulator of multi-trophic interactions in belowground ecosystems.

Keywords: *Soil ecology;* Photorhabdus *bacteria; hidden regulators*

The smell of trouble: Exploiting the ecology of fear for plant protection

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Predator–prey interactions are significant drivers of animal communities, population dynamics, and ecosystem function. Traditionally, we consider how predators influence prey populations via their consumptive capacity, but predators also affect prey populations by triggering changes in prey behavior and physiology through non-consumptive effects or predation risk effects. Predation risk detection and response contribute significantly to the co-evolutionary arms race between predators and their prey, making non-consumptive effects a critical element of predator–prey interactions. With insects, olfactory cues are commonly used to convey information between organisms, and it has been shown that insects may also tap into odor cues as a means to determine predation risk. Our research explores the non-consumptive effects of natural enemies from two different feeding guilds, ladybeetles and parasitic wasps, on the behavior and performance of aphids. Additionally, we are identifying the importance of olfactory cues as a mechanism of detection of predation risk for aphids. We ask these questions using the herbivorous green peach aphid (*Myzus persicae* (Sulzer)), the predatory multi-colored Asian lady beetle (*Harmonia axyridis*), aphid parasitic wasps (*Aphidius colemani*), and collards (*Brassica oleracea*) as a system. Our research has demonstrated that *H. axyridis* odor cues stimulate significant changes in aphid hostplant decisions, feeding behavior, and population size. However, with *A. colemani*, it appears that odor cues may not stimulate similar changes in aphid behavior, despite observing significant non-consumptive effects of parasitic wasps on aphids when multiple stimuli are available such as physical contact. Throughout this work, we find disparities between the responses of aphids with or without wings, which highlight how phenotypic plasticity may be a critical defense for aphids. Further, our results raise interesting questions of whether the anti-predator responses that we observed are maladaptive or benefit aphid survival in the end. By investigating both the mechanisms of detection of predation risk and the triggered consequences in prey behavior, we are furthering our understanding of how non-consumptive effects influence animal populations and opening up research exploration in the manipulation of risk-induced behaviors for applied purposes.

Keywords: *Predation risk; non-consumptive effects; semiochemicals*

SYMPOSIUM 11: CHEMICAL ECOLOGY OF VENOMS AND POISONS

Abstract Number: 204

Venom and the molecular ecology of exochemicals

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Ecology is the science of context – an organism's relationships with other organisms make it what it is, both synchronically (in any given moment) and diachronically (over developmental and evolutionary time). Venom is a secretory mixture of toxic molecules which mediate interactions between venomous organisms and target organisms: venom is an intrinsically ecological trait and toxins are *ecomolecules*. Thus, the composition of venom is not only shaped by encounters between predators and prey in which it is deployed, but the structure–function relationships of individual toxins are shaped by encounters with their molecular targets. In fact, all biomolecules are ecomolecules in that their *functions* arise only as a consequence of interaction with other molecules in specific contexts, i.e., within *molecular ecologies*. Toxins and other *exochemicals* are special, however, in that they transcend the molecular ecologies internal to the organisms that produce them and enter the molecular ecologies of target organisms. In this talk I will discuss the central role of context in shaping function and articulate the usefulness of an *ecological stance* for the study of toxins and venom, using diverse examples from molecular to organismal evolution. I will utilise a dynamical systems perspective to describe toxins and other exochemical molecules as a method of chemically hacking another organism's physiology in order to disrupt or recalibrate its target states. This framework unites venom toxins, the exochemicals utilised in symbiotic relationships including parasitism, and the human use of toxins *as tools* (i.e., drugs), under the shared rubric of targeted manipulation of regulatory pathways contributing to the homeorhetic maintenance of biochemical states in order to shift them from one regime, or basin of attraction, to another.

Keywords: *Venom; toxin; context*

Abstract Number: 206

To eat or not to eat: the evolution of toad toxin resistance in Serpentes

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The coevolution of predators and their prey is often shaped by an ever-escalating arms race. One such example is the arms race between the poisonous bufonid toads and their predators. Bufonid toads secrete bufadienolides that block the Na+/K+-ATPase pump, resulting in lethal cardiotoxicity. Therefore, only a handful of animals have evolved resistance to toad bufadienolides. Certain snakes, for example, have acquired resistance to these toxins via specific substitutions (Q111L and G120R) in their α-subunit of Na+/K+-ATPase. While this phenomenon is well-documented in reptiles worldwide, our understanding of the prevalence of bufadienolide resistance in snakes of the Indian subcontinent remains unexplored. To this end, we sequenced the α-subunit of Na+/K+-ATPase from diverse Indian snakes. Our evolutionary reconstructions indicate that bufadienolide resistance has independently originated nine times in squamate reptiles. We examined mechanisms underlying the interaction between bufadienolide and its target receptor using molecular docking and simulations. In contrast to our current understanding, we show that the rarely documented genotype of resistant and susceptible substitutions (i.e., L111 and G120) may offer intermediary resistance. Thus, our findings provide novel insights into the emergence of toad toxin resistance in snakes and the complex molecular dynamics that underpin this adaptation.

Keywords: *Bufadienolides; toxin resistance; Na+/K+-ATPase*

Abstract Number: 208

Investigating ontogenic variability in medically important Indian snake venoms

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Venoms of snake species exhibit significant intraspecific variation, which is influenced by various factors such as their trophic ecology, gender, and body size. Snakes are generally considered gape-limited predators, and this constraint may force juvenile individuals to feed on smaller prey until they grow large. In this study, we investigated the ontogenic variation in two medically most important Indian snakes, the common cobra (*Naja naja*) and Russell's viper (*Daboia russelii*). We analysed the proteomic composition, biochemical activities, and lethality of venoms collected from snakes of these species across their developmental stages, from neonates to adults. Our findings reveal that the relative abundances of toxin families and their activities in *D. russelii* venoms significantly vary between young and adult individuals, while only minor variations are observed in *N. naja* venoms. We theorise that the different venom formulations secreted by juvenile and adult *D. russelii* may represent adaptive solutions for effectively disrupting the different physiologies of their prey. A light interference-based toxin-receptor binding experiment also revealed similar prey-specific neurotoxicity patterns for adult and juvenile *N. naja* venoms. Our study comprehensively assesses the ontogenic variability in the venoms of these two medically important Indian snakes, with important implications for snakebite management and antivenom development.

Keywords: *Venom proteomics; ontogenic venom variation; venom evolution*

Abstract Number: 210

Examining venom diversity and its correlates among scolopendrid centipedes

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Venoms have evolved many times in the animal kingdom and are considered a key trait contributing to the evolutionary success of many groups. Examining evolutionary and ecological drivers of venom diversity is crucial, though it remains a less explored aspect of venom research. Among terrestrial predatory arthropods, centipedes represent some of the oldest living lineages of venomous animals, with more than 3000 described extant species. The centipede family Scolopendridae comprises 400 species worldwide, with high diversity in tropical regions. In this talk, I would like to discuss the preliminary results of our ongoing work on documenting centipede venom diversity at the macroevolutionary scale (order: Scolopendromorpha; family: Scolopendridae) and exploring the ecological (diet and geography) and morphological (venom gland morphology) drivers.

We used an integrated proteo-transcriptomic approach to document the venom diversity (proteins and peptides). We generated individual venom-protein profiles for members of the six scolopendrid genera, representing 70–80% of the genus-level diversity (n=41). The Scolopendridae venoms were predominantly neurotoxic and varied in diversity and abundance across genera. Currently, we are assembling the data for diet through metabarcoding and characterizing the morphology of the venom gland through microscopic examination of scolopendrid centipedes. We hope to contribute to our understanding of ecological and evolutionary drivers of venom diversity.

Keywords: *Tropical forest; soil predatory arthropods; proteo-transcriptomic approach*

Abstract Number: 223

Ant venom evolution: insights from the genetically hyperdiverse greenhead ant

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Ant venoms have emerged as rich sources of extremely diverse peptide toxins, contrasting previously widespread misconceptions that they are primarily acid-based. While this diversity makes ant toxins attractive as pharmacological tools, the eusocial lifestyle of ants also provides opportunities for studying how venom evolution may be affected by selection at different levels of biological complexity. Here, I will present some of our recent work on the composition and function of ant venoms and their toxins, with a focus on the genetically hyperdiverse Australian greenhead ant, *Rhytidoponera metallica*, whose colonies contain some of the highest known intra-colony genetic variance for a eusocial animal. I will also present our most recent findings on the genomic architecture and evolutionary mechanisms that underlie the surprisingly complex toxin arsenal of this species. Although it represents an extreme case of genetically diverse colonies, *R. metallica* provides insights into how selection at both individual and colony levels contributes to driving the evolution of adaptive traits in eusocial animals.

Keywords: *Ant venom; greenhead ant*

SYMPOSIUM 12: CONSEQUENCES OF PLANT DOMESTICATION ON PLANT METABOLITES AND HERBIVORE–NATURAL ENEMY INTERACTIONS

Abstract Number: 43

Domestication effects on the nutritional quality of plant guttation and tri-trophic interactions

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Guttation is the process of secretion of droplets from xylem and phloem sap at the tips or edges of leaves of some vascular plants, including highbush blueberries. Highbush blueberry is a crop native to the northeast United States that has been domesticated for only 100 years and it was mainly selected for high yields and bigger fruit. In a previous study, we showed that guttation from domesticated blueberry plants contains high levels of carbohydrates but also contains proteins. As a result of domestication, we hypothesized that guttation from domesticated blueberries differs from their wild ancestors in chemical composition, which in turn affects the fitness of herbivores and their natural enemies. In this study, we compared the nutritional content of guttation from domesticated and wild blueberry plants and found that guttation from wild blueberry plants has higher concentrations of both carbohydrates and proteins than that from domesticated blueberry plants. In addition, we conducted studies to evaluate the effects of guttation from wild and domesticated blueberries on the longevity and egg load of insects from different trophic levels: an herbivore (*Drosophila suzukii*), a parasitic wasp (*Trichopria drosophilae*), and a predator (*Chrysoperla carnea*). We also conducted behavioral studies to compare the preference of these insects to guttation from wild and domesticated blueberries. These studies aim at elucidating the consequences of domestication on plant traits, such as guttation, and their effects on tri-trophic level interactions.

Keywords: *Plant-derived foods; highbush blueberry; insect fitness*

Abstract Number: 44

Consequences of plant domestication for resistance against insect herbivores: Case studies

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Plant domestication has generally resulted in decreased chemical and physical defense in crop plants compared to their wild ancestors. A reduction in plant defensive traits is often expected to result in increased performance of herbivorous insects. However, recent studies have shown that this pattern is not ubiquitous. We have examined this by comparing insect behavior and performance on crop plants and their wild ancestors cooccurring in Mesoamerica. I will present results from previous and ongoing research on wild and cultivated plants of beans in the genus *Phaseolus*, corn, squash, cotton and chili peppers.

Wild and cultivated plants were characterized for their concentration of chemical defenses and in parallel we conducted laboratory assays to compare insect performance. In field experiments in Mexico we further studied the plants' susceptibility to generalist and specialist herbivores. Results showed that with the exception of certain varieties of chili pepper, domestication has overall resulted in reduced chemical defense in cultivated plants. However, a reduction in defensive compounds did not always result in increased herbivore performance, nor did it always affect plant susceptibility in the field. The contrasting effects of domestication among different crops can be explained by the purpose of domestication (i.e. ornamental vs. consumption in squash), the organ targeted during the domestication process (i.e. seeds vs. leaves in beans) and the herbivore feeding habit (generalist vs. specialist). These examples highlight the importance of *in situ* studies using wild and cultivated plant systems not only to test ecological and evolutionary hypotheses, but also to provide insights that can be used to develop novel strategies for sustainable agriculture.

Keywords: *Wild and cultivated plants; resistance; plant–herbivore interactions*

Abstract Number: 79

Defensive and nutritional traits shape the resistance of wild and cultivated tomato against *Tuta absoluta*

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Plant domestication process has selected desirable agronomic attributes that can both intentionally and unintentionally compromise other important traits, such as plant defense and nutritional value. However, how domestication can affect the defensive and nutritional traits of plant organs not exposed to selection and the consequent interactions with specialist herbivores remains unclear. Here, we hypothesized that the modern cultivated tomato has reduced levels of constitutive defense and increased levels of nutritional content compared with its wild relatives, and such differences affect the resistance of the South American tomato pinworm, *Tuta absoluta*, a devastating insect pest that co-evolved with tomato. To test this hypothesis, we compared plant volatile emissions, leaf defensive (glandular and non-glandular trichome density, and total phenolic content), and nutritional traits (leaf nitrogen content) among the cultivated tomato *Solanum lycopersicum* and its wild relatives *S. pennellii* and *S. habrochaites*. We also determined the attraction and ovipositional preference of female moths and larval performance on cultivated and wild tomatoes. Volatile emissions were qualitatively and quantitatively different among the cultivated and wild species. Glandular trichome density and total phenolics were lower in *S. lycopersicum*. In contrast, this last species had a greater non-glandular trichome density and leaf nitrogen content. Female moths were more attracted and consistently laid more eggs on the cultivated *S. lycopersicum*. Larvae fed on *S. lycopersicum* leaves had a better performance reaching shorter larval developmental times and increasing the pupal weight compared to those fed on wild tomatoes. Overall, our findings document that agronomic selection for enhanced fruit quality and yields has altered the defensive and nutritional traits in tomato plants, affecting their resistance to *T. absoluta*.

Keywords: *Chemical defences; plant domestication; specialist herbivore*

Abstract Number: 126

Dysbiosis by domestication: Evidence of microbiome perturbations caused by crop domestication in maize

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Plants have evolved and streamlined the process of microbiome selection within and around them to enable collaboration with beneficial microorganisms that assist plants in nutrient acquisition and herbivory defense. Unlike wild plant species, crop species were modified through domestication, geographic spread following domestication, and modern breeding. In particular, crops have been bred to maximize yield in resource-rich agricultural habitats, while crop wild ancestors are selected for survival and reproduction in marginal habitats. Moreover, modern breeding has rendered commercial crop cultivars, including of maize (*Zea mays mays*), inherently dependent on intensive use of fertilizers and insecticides. Separately, abundant research has shown that the wild relatives of maize, the teosintes, are more resistant to pest injury than modern maize cultivars. Here we examined the rhizosphere and phyllosphere microbiota of a suite of teosinte and maize accessions spanning on one hand the evolution of maize from perennial teosinte (*Zea diploperennis*) to Balsas teosinte (*Zea mays parviglumis*) to maize landraces, and elite maize inbreds, and on the other hand the crop's spread from its center of domestication in Mexico to the US Corn Belt. We show for the first time that domestication – but not northward spread and breeding – dramatically affected the maize-phyllosphere microbiota. We found that the microbial community structure in the teosintes is highly stringent and regulated, while in elite maize inbreds it was highly variable, even among replicates of individual inbreds. Our results support the *Anna Karenina* principle in microbial dysbiosis, marking the first evidence of dysbiosis caused by plant domestication. Additionally, we will present the biotechnological potential of using these microbiome data to develop modern crop biopesticides. In that context, we will also present our preliminary findings demonstrating the bioprospecting value of phyllosphere microbiome transplantation from teosinte to modern maize cultivars.

Keywords: *Maize; teosinte; microbiome*

Abstract Number: 209

Induced plant defenses in cultivated and wild tomato

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A reduction in plant resistance against insect herbivores is generally expected in domesticated crop species compared to their wild species. In the present study, the inducibility of several plant defense mechanisms (e.g. defensive chemicals, trichomes, plant volatiles) were investigated, and the performance and preference of the herbivore *Helicoverpa zea* were measured in three different tomato genotypes; a) wild tomato, *Solanum pimpinellifolium* L. (accession LA 2093), b) cherry tomato, *S. lycopersicum* L. var. cerasiforme (wild cherry tomato), and c) cultivated tomato, *S. lycopersicum* L. var. Better Boy). In general, induced defenses were greater in the cultivated tomato while constitutive defenses were greatest in the wild species/genotypes. The role of the salivary effector glucose oxidase in mediating these interactions will be discussed.

Keywords: *Defense proteins; volatiles; saliva*

Abstract Number: 189

Implication of crop domestication on plant-pest interactions

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Wild plants have evolved intricate ways of defending themselves from insect herbivores, over several million years of plant-insect interaction and coevolution. While wild plants in natural ecosystems are under natural selection for the ability to defend themselves, domesticated crops have been subjected to artificial selection, often for yield in a pesticide treated background. These crop plants are vulnerable to attack by adapted insect herbivores, which become pests in this context. Some plant defences are constitutively expressed, whereas others are induced after exposure to herbivore feeding or oviposition. In the latter case, plants often emit herbivore induced plant volatiles (HIPVs) which mediate tritrophic interactions to recruit natural enemies for protection against herbivory. Emission of HIPVs that attract natural enemies early on at the egg-laying stage of herbivore attack could provide timely biological control of pests and deter subsequent infestation by the pest. Our very recent phenotypic and genotypic data, using maize as an example, shows that herbivore egg induced volatile emission is rare in mainstream maize cultivars but common in wild plants or less domesticated landraces. There is considerable potential to improve crop defences against insect attack by learning from nature and introgressing traits from crop wild relatives or landraces.

Keywords: *Crop domestication, plant-insect interactions, plant defense*

SYMPOSIUM 13: MUTUALISM AND MULTITROPHIC INTERACTIONS

Yeast and fruitfly mutualism

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The spotted wing drosophila *Drosophila suzukii* develops in ripening fruit, unlike other drosophilids that feed on overripe fruit, and is associated with the yeast *Hanseniaspora uvarum*. We hypothesized that *D. suzukii* and *H. uvarum* engage in niche construction leading to a mutualistic relation, facilitating the exploitation of fruit as larval substrate. We show that *H. uvarum* proliferates on both ripe and on unripe raspberries, mediates attraction of *D. suzukii* larvae and adult flies, enhances egg-laying and is a sufficient food substrate to support larval development. Moreover, *H. uvarum* suppresses the antagonistic grey mould, *Botrytis cinerea* in collaboration with *D. suzukii larvae*. *H. uvarum* thus creates favourable conditions for *D. suzukii* larval development. *D. suzukii*, on the other hand, vectors *H. uvarum* to suitable substrates such as raspberries, where larval feeding activity enhances growth of *H. uvarum*. Larval feeding also helps to suppress *B. cinerea*, which otherwise outcompetes *H. uvarum* on raspberry, in the absence of larvae. In conclusion, *H. uvarum* enhances *D. suzukii* larval development on unripe berries, and *D. suzukii* promotes *H. uvarum* dispersal and growth on berries. Hence, yeast and fly modify their shared habitat in reciprocal niche construction and mutual interaction.

Keywords: *Fruitfly; yeast; mutualism*

Abstract Number: 111

Sensing volatiles throughout the body: Geographic and tissue-specific olfactory receptor expression

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Chemical interactions between plant volatiles and insect olfactory receptors (ORs) are essential processes for the survival and reproduction of insects. During the evolution of insects, ORs became a massively diverse gene family because of adaptation to complex and changeable chemical environments. In the case of specialist insect pollinators, it is reasonable to expect adaptation to the detection of signature host plant chemical signals. Our model species, *Ceratosolen fusciceps*, a specialist wasp pollinator of *Ficus racemosa*, likely possesses an OR repertoire that allows it to distinguish fig-specific volatiles in highly variable environments. Using a newly assembled genome-guided transcriptome, we annotated 63 ORs in the species and reconstructed the phylogeny of *Ceratosolen* ORs in conjunction with other hymenopteran species. Expression analysis showed that though ORs were mainly expressed in the antennae, 20 percent were also expressed in non-antennal tissues such as the head, thorax, abdomen, legs, wings, and ovipositor. Specific upregulated expression was observed in OR30C in the head and OR60C in the wings. We identified OR expression from all major body parts of *C. fusciceps*, suggesting novel roles of ORs throughout the body. Further examination of OR expression of *C. fusciceps* in widely separated geographical locations, i.e., south (urban) and northeast (rural) India, revealed distinct OR expression levels in different locations. This discrepancy likely parallels the observed variation in fig volatiles between these regions and provides new insights into the evolution of insect ORs and their expression across geographical locations and tissues. Our study will give insight into unexplored modes of plasticity in their olfactory receptor systems and physiological responses.

Keywords: *Fig wasp; olfactory receptors; non-antennal tissues*

Can intraguild interactions on tomato influence responses of the ectoparasitoid *Bracon nigricans*?

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Understanding the influences of intraguild interactions on the performance of natural enemies is critical to developing effective biocontrol methods for insect pests. This is particularly important for horticultural crops such as tomato, which has a multitude of insect pests. In this study, we investigated the effect of intraguild interactions of two important pests of tomato, the greenhouse whitefly, *Trialeurodes vaporariorum*, and the tomato pinworm, *Phthorimaea absoluta,* on the performance of the pinworm ectoparasitoid, *Bracon nigricans*. In oviposition bioassays, *P. absoluta* exhibited significantly higher oviposition and attraction to tomato plants infested with *T. vaporariorum* than uninfested plants. Interestingly, *T. vaporariorum* infestation decreased *B. nigricans* parasitism on *P. absoluta* larvae and attraction to tomato plants. Chemical analyses (GC-EAD and GC-MS) identified green leaf volatiles (GLVs) and terpenes as important semiochemicals that play an important role in attraction and avoidance behavior of the ectoparasitoid. These results highlight the complex interactions in natural ecosystems and potential avenues to increase the efficacy of natural enemies with semiochemicals.

Keywords: *Herbivore-induced plant volatiles; induced defense; biological control*

SYMPOSIUM 14: CHEMICAL ECOLOGY IN AGRICULTURE

Abstract Number: 133

Fruit spotting bug (*Amblypelta nitida*), on the search for a lure

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Fruit spotting bugs of the genus *Amblypelta* (Hemiptera: Coreidae) are native to Australia, mostly living in forest regions north of about 30 °S. They feed on flowers, fruit and young shoots of many plants and are a significant horticultural pest in eastern and northern Australia. Growers typically try to control infestations with broad-spectrum insecticides which impact beneficial insects as well as the pest. The Queensland Department of Agriculture and Fisheries developed and commercialised a pheromone lure for one species (*A. lutescens lutescens*) which can be used to monitor density and inform timing of targeted spraying. The lure is attractive to adult males and females, as well as nymphs. To date no pheromone has been identified for the other commercially important species (*A. nitida*), although several potential targets have been identified. The research described here aims to find pheromone/s specific to *A. nitida* to develop lures capable of mid- to long-range attraction to monitor populations within a cropping area. We have used SPME headspace aerations and gas chromatography-mass spectrometry to identify compounds from male and female *A. nitida* at various physiological stages, including nymphs and mated and unmated adults. There are striking differences in compounds detected from male and female bugs and between mated and unmated individuals. Detection of the compounds by *A. nitida* was then quantified using GC-electroantennographic detection in conjunction with behavioural assays, to further target key compounds. Promising compounds will be tested using olfactometers, wind tunnels and field testing to determine their suitability as a pheromone lure.

Keywords: *Fruit spotting bug; pheromone; lure*

Abstract Number: 42

Investigating metabolites from host plants of *Halyomorpha halys* for a novel push–pull kill strategy

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The Brown Marmorated Stink Bug (BMSB) *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) is an invasive insect native to eastern Asia and has attained pest status in the past years in most parts of the United States and southern and western Europe. It is a polyphagous pest insect that damages various fruit and vegetable crops by piercing the fruit surface and tissue. Research on semiochemicals that exhibit attractive or repellent effects on stink bug species is useful for developing push–pull strategies. Field monitoring revealed a high abundance of *H. halys* nymphs and adults on certain shrubs. Although they are very different from typical commercial woody host plants, they appear to be very attractive, and their volatile and non-volatile compounds could have attractive or phagostimulating properties for stink bugs. In order to design a new push–pull–kill strategy for BMSB management in organic horticulture, we aim to develop capsules that contain attractive volatiles (push) along with an arrestant that promotes feeding in combination with a biological insecticide or a microbial antagonist (kill component). This method will also include encapsulated repellent scents to drive the insect pests away from the plants and prevent further immigration (pull).

Our first step to identify a potential compound for a capsule-based attract-and-kill strategy was to analyze primary and secondary metabolites in berries from selected shrubs by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Chemical analyses were followed by behavioral assays with adult and nymphal stages of BMSB. Initial results of plant metabolome chemical analysis and bioassays with stink bugs are presented and discussed.

Keywords: *Attract-and-kill; BMSB; metabolites*

Abstract Number: 64

Glycoalkaloid–phytohormone interactions mediate plant response to above- and belowground herbivores

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Plants defend themselves chemically against herbivory through family-specific secondary metabolites and phytohormones. These defenses can manifest both locally and systemically, potentially influencing spatially separated herbivores. While studies have investigated the role of phytohormones in plant-mediated interactions, fewer studies have investigated how variation in family-specific secondary metabolites contributes to systemic herbivory response. Constitutive levels of defense metabolites may alter the threshold at which plants induce a response to herbivory, thus inducing systemic effects. We used growth chamber bioassays to investigate how aboveground herbivory by the Colorado potato beetle (*Leptinotarsa decemlineata*, CPB) and belowground herbivory by the northern root-knot nematode (*Meloidogyne hapla,* RKN) altered phytohormones and glycoalkaloids in roots and shoots of two lines of the wild potato (*Solanum chacoense*). These lines varied in their constitutive levels of chemical defense, particularly in the presence of leptine glycoalkaloids. We also determined how these differences influenced the preference and performance of the CPB. The susceptible wild potato line responded to aboveground damage by CPB through induction of jasmonic acid (JA) and OPDA, a JA precursor. However, when challenged by both RKN and CPB, the susceptible line retained high levels of JA, but not OPDA. Beetles gained more mass after feeding on the susceptible line compared to plants of the resistant line. Belowground, JA, JA-Isoleucine, and OPDA were higher in the resistant line compared to the susceptible line. Our results suggest that variation in the constitutive level of family-specific secondary metabolites, such as glycoalkaloids, in plants provides a partial mechanistic explanation for the outcomes of plant-mediated interactions in the field. Additionally, these findings allow us to predict that herbivores feeding on plants with high levels of constitutive defense will experience fewer changes from spatially separated feeding than herbivores on less resistant plants.

Keywords: *Insect herbivory; root-knot nematode; jasmonic acid*

Abstract Number: 127

Cover crop legacy influences predator preferences via prey quality more so than volatile signaling

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Growing “cover crops” between sets of cash crops can improve a multitude of soil traits. We have recently found that cover crops like pea (leguminous, mycorrhizal), triticale (non-leguminous, mycorrhizal) and radish (non-leguminous, non-mycorrhizal) can also affect direct defence of maize against a notorious pest, the fall armyworm (FAW) (*Spodoptera frugiperda*). In this study we aimed to examine indirect defences that may influence the behaviour of the Asian lady beetle (*Harmonia axyridis*), a common predator.

By assessing the feeding behaviour of predators and herbivory-induced plant volatile profiles, we found that nutritional factors may play a role in predator feeding preference. We hypothesise that plant provisioning, from soil quality can make herbivorous prey more nutritious and influence predator foraging behaviour. Thus, soil history via cover-crop selection can have an impact on tritrophic interactions that may influence biocontrol efficacy.

Keywords: *Cover crops; tritrophic interactions; pest management*

Abstract Number: 129

Responses of the dung beetles *Bubas bison*, *Onitis aygulus*, and *Geotrupes spiniger* to dung volatiles

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Many insects, including dung beetles, rely on odour cues to locate sporadically distributed food resources and mates. However, limited information is available on how dung volatiles regulate various behaviours in dung beetles. To investigate this, we conducted an electroantennography (EAG) study to construct EAG response profiles to selected dung VOCs in three dung beetle species introduced into Australia: *Geotrupes spiniger* (Coleoptera: Geotrupidae), *Bubas bison*, and *Onitis aygulus* (Coleoptera: Scarabaeidae). Preliminary experiments revealed that antennae of all species were sensitive to a mix of six compounds commonly found in livestock dung headspace: phenol, skatole, indole, *p*-cresol, butanone, and butyric acid. In addition to these compounds, dimethyl sulfide, dimethyl disulfide, eucalyptol, and toluene were tested for antennal activity. All compounds evoked measurable responses confirming antennal sensitivity. *Geotrupes spiniger* exhibited significant responses to all the individual compounds compared to the control, whereas *B. bison* and *O. aygulus* only responded to a subset of compounds. Comparing relative EAG amplitudes revealed highly significant responses for *p*-cresol in *G. spiniger* and for skatole in *B. bison*. Pooled EAG data suggest highly significant response differences among the three species and between compounds. Scanning electron microscopy (SEM) revealed presence of three morphological types of antennal sensilla, including sensilla trichodea, sensilla basiconica, and sensilla chaetica, for the first time in these beetle species. Distinct patterns of sensilla distribution were observed in different lamella surfaces of all three species in both sexes, especially for sensilla basiconica in *B. bison* and *G. spiniger*. These results provide new insights into dung beetle olfaction and the potential role of different antennal sensilla.

Keywords: *Olfaction; EAG, SEM*

Novel biopesticide discovery using non-targeted metabolomics and RNAi for lepidopteran pest control

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Lepidopteran pests cause severe crop losses. Synthetic pesticides are injudiciously used to control them, which causes environmental and health hazards. Therefore, the use of safe and eco-friendly botanicals as biopesticides is rapidly increasing. *Spodoptera litura* Fabricius (armyworm) is a polyphagous multi-insecticide-resistant lepidopteran pest that infests >100 plant species, including several crops. We explored secondary metabolite-rich *Solanum melongena* L. (eggplant) as a biopesticide source, and using UPLC-ESI-QTOF-based non-targeted metabolomics of seven differentially armyworm-susceptible eggplant varieties, we identified a candidate metabolite chlorogenic acid (CGA). CGA-spiked artificial diet-fed larvae showed a three-fold mass reduction and two-fold mortality increase compared to control diet-fed larvae. We used a reverse genetics approach to assess CGA's *in planta* insecticidal potential. Nutritional indices revealed that CGA ingestion hampered larval digestion and metabolism, leading to slower larval growth and delayed pupation and eclosion. Eggplants in which CGA biosynthesis was silenced were rendered more susceptible to armyworm infestation; resistance was restored upon CGA complementation. We then discovered the armyworm's counter-adaptation against CGA; first, it cleaved CGA using a carboxylesterase (CE) and then converted it to a non-toxic isomer neochlorogenic acid. After silencing this gene by plant-mediated RNAi (PMRi), we generated a loss-of-function phenotype showing high CGA-susceptibility. Our RNAi formulation developed for foliar application showed a similar effect. Lastly, the field application of CGA did not show an effect on beneficial insects. Thus, CGA is a promising biopesticide candidate for the field trial phase against armyworm. We propose a novel pest management strategy combining plant-based candidate biopesticide(s) and RNAi-based silencing of counter-adaptation genes to combat armyworm and other lepidopteran pests in agronomically important plants.

Keywords: *Biopesticide; pest management, RNA interference (RNAi)*

Spodoptera litura secretes cysteine proteinase and metalloproteinase inhibitors that modulate host immunity

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Effector proteins make herbivore more virulent to the plant by suppressing plant defense at multiple steps. However, no comprehensive study has been undertaken to identify effector proteins secreted from salivary glands of the chewing insect pest, *Spodoptera litura*. We have selected *S. litura* and *Arabidopsis thaliana* as model insect–plant interactions for our study. We hypothesize that generalist insects like *Spodoptera litura* are able to parasitize their plant hosts by evading the plant immune system, and effectors proteins, secreted by the insects into plant cells, are responsible for this ability. We further hypothesize that these effectors are responsible for attenuating plant defense by modifying or hijacking defense-responsive genes at transcriptional level signaling complexes. We have used a combination of next generation sequencing (NGS), functional genomics, proteomics and reverse genetics to identify and analyse effectors from *Spodoptera* and their targets in plants. Based on *de novo* transcriptome data in *S. litura*, 808 and 267 proteins from the head and salivary gland respectively were predicted as potential secreted proteins with possible effector functions. We have functionally characterized the role of two potential effector proteins, Inducible metalloproteinase inhibitor protein-like (*Sl*-TIL) and cysteine proteinase like (*Sl*-CPL) from *S. litura*. *Sl-TIL* contains TIL domains (trypsin inhibitor-like cysteine-rich domains), which form several disulphide bonds and keep the protein stable under adverse conditions. The *Sl-CPL* contains cathepsin propeptide inhibitor domain (I29) domains and cysteine-type peptidase activity. *Sl*-CPL functions to elicit plant defense mechanisms and does not suppress them as we predicted. *Sl*-TIL shows effector-like activity in Arabidopsis as it increases *S. litura* feeding, when overexpressed in plants. Both the secreted proteins show diverse roles in host defense modulation and act on diverse targets in host plants.

Keywords: *Effector proteins; insect–plant interaction; plant immunity*

Abstract Number: 156

Chemical ecology of ear-feeding insect pests of maize

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Dynamics of key insect pests of maize was recorded during *kharif* seasons of 2018–2021 in a long-term experiment going on at a fixed site in Delhi since 2008. In reproductive stage maize, tassel infestation by *Chilo partellus* was observed during 2018 (6.88±2.48%) and 2019 (5.53±1%) and by *Spodoptera frugiperda* during 2019 (28.14±1.81%), 2020 (71.52±6.28%) and 2021(70.24±5.75%). Ear infestation by *Mythimna loreyi* was highest during 2018 (27.03±2.14%) and it declined gradually to 10.7±2.5% in 2021. Similarly, ear infestation by *Helicoverpa armigera* was highest during 2018 (9.8±2.68%) and lowest during 2021(1.26±1.09%). Ear infestation by *Spodoptera frugiperda* was 33.3±3.09% during 2019 and 65.15±7.16% and 66.88±9.07% during 2020 and 2021 respectively, revealing its clear dominance over other species since its invasion for the first time during July 2019 at Delhi.

Being an emerging pest in maize, *H. armigera* has been monitored by pheromone trap catches since 2015 and we found its clear preference for anthesis-silking stage maize, and also its drastic decline post-invasion of *S. frugiperda*. Upon detection of *S. frugiperda* during 2018 in Peninsular India, pheromone traps were deployed all over India and it serendipitously revealed its high attractiveness to the native *M. loreyi*. The inter-species attraction was confirmed by male and female moths using two-different set-ups and also by electroantennograms. Observations revealed that *M. loreyi* responded to the blend of FAW pheromone consisting of *Z*-9 tetradecenyl acetate, *Z*-11 hexadecenyl acetate, and *Z*-7 dodecenyl acetate. When tested as individual compounds, the response was 60 % higher for *Z*-9 tetradecenyl acetate as compared to other minor compounds, revealing interesting facts about the role of chemical ecology in evolution. Factors that may play a role in the decline in trap catch of *H. armigera* and *M. loreyi* over the years could include altered host plant volatiles, difficulty in mate finding due to similarity in pheromone composition, and cannibalism among caterpillars which need further investigation.

Keywords: *Maize;* Mythimna loreyi*;* Spodoptera frugiperda

Abstract Number: 188

Cover crop legacy of soil affects maize resistance to insects and fungal pathogens asymmetrically

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Cover crops are known to provide several ecosystem services to organic cropping systems. The direct benefits of using leguminous cover crops such as pea or mycorrhizal species such as cereal rye or oats to enhance soil fertility is well known. However, little is known about the direct impact of different cover crop species on pest suppression in the subsequent cash crop. In our work, we show that mycorrhizal cover crop species such as triticale can increase resistance to fall armyworm herbivory in next generation maize when compared to a non-mycorrhizal cover crop such as radish. However, maize resistance to the fungal pathogen *Fusarium verticillioides* was enhanced in plants that were grown in soil with radish as cover crop compared to triticale. This suggests that cover crop legacy of soil affects host plant resistance to insects and pathogens asymmetrically. Quantitative proteomic analyses of maize plants grown in soil with different cover crop legacies showed that plants grown in soil with triticale as cover crop induced the jasmonic acid (JA) biosynthetic precursor *lipoxygenase6*. Therefore, maize plants grown in soil legacy of triticale can induce host plant resistance (HPR) downstream of jasmonic acid to counter caterpillar herbivory. Since it is already known that HPR against caterpillars can also be induced by caterpillar oral secretion or jasmonic acid (JA), we applied both these elicitors to maize plants grown in soil with radish as cover crop in an attempt to rescue their susceptibility to chewing herbivores. Notably, maize plants grown in soil with radish as cover crop were unable to induce responses induced by caterpillar oral secretion or by jasmonic acid. Therefore, perception of herbivory and ability of plants to induce defense responses to insects is directly correlated to cover crop soil legacy. Our work also brings us a step closer towards understanding the molecular mechanisms that are responsible for plant interactions with aboveground pests as a direct correlation of successional soil legacies due to cover cropping in agricultural systems.

Keywords: *Cover crop; fall armyworm;* Fusarium verticillioides

SYMPOSIUM 15: CHEMICAL COMMUNICATION

Impacts of ocean acidification on chemical communications in the marine worm *Platynereis dumerilii*

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One important part of climate change is ocean acidification caused by the increase of atmospheric CO_2 since the industrial revolution, lowering the pH from 8.2 to 8.07. By the year 2100, a pH of 7.7 is predicted with increased fluctuations of pH during the day. So far only a few studies have investigated the impact of OA on chemical communication, which directly affects the survival of species (detection of prey/predator, mating partner), causing changes in the marine food webs, and marine communities. This study focuses on the globally distributed non-calcifying model species *Platynereis dumerilii* and on the potential effect of a lower pH on the chemical communication used in their mating behavior. It is known that *P. dumerilii* males and females use pheromones, such as 5-methyl-3-heptanone, uric acid, and L-ovothiol-A, to induce rapid swim speed or to trigger the release of their gametes. Here we aim to identify the receptors of these pheromones in *P. dumerilii* and determine if a lower pH impacts their pheromones and/or receptors. On one hand, we tested if a lower pH impacts the response (swim speed) to the pheromones with behavioral assays. On the other hand, we identified candidate genes for the receptors of the pheromones coding for G protein-coupled receptors (GPCRs) using comparative transcriptomics. With gene trees and deorphanization assays, we found a few promising candidates for which we will perform knock-out experiments. Once we validate the function of these genes, we will also be able to determine with computational modelling if such receptors might be impacted by a lower pH and if *Platynereis* populations living in more acidic environments use different receptors. This study helps to better predict how ocean acidification impacts marine communities by modifying their chemical communication, whether acclimation and adaptation occur and at what costs.

Keywords: *Pheromones; ocean acidification; GPCRs*

Abstract Number: 28

Deciphering chemical communication in the diving beetle *Rhantus suturalis*

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Chemical senses (taste and smell) play a key role in the adaptation of species to their environment, especially in insects. Changes in the sensory equipment of insects that have evolved from a terrestrial to an aquatic environment have long aroused the curiosity of scientists, but the mechanisms of sensory perception have never been studied in aquatic insects. My postdoctoral project aims at addressing this question in the diving beetle *Rhantus suturalis*, a common species in Western Europe ponds and an important predator of mosquito larvae. Chemicals from ecologically relevant sources were identified using physicochemical analyses (GC-MS) and their detection by the beetle's antennae was verified with electro-antennogram (EAG) technique, providing first insights on the olfactory capacities. Finally, I try to link electrophysiological responses to behavior, and thus determine the role of these compounds in this predatory beetle.

Transcriptomic studies have revealed a significant number of chemosensory receptor families expressed in the sensory organs of *R. suturalis*. Therefore, studying the function of these genes would provide key information on the properties of receptors tuned to specific compounds in aquatic environments. I am currently working on the functional characterization of these receptors using heterologous expression in *Xenopus* oocytes and two-electrode-voltage clamp (TEVC).

This project will provide essential information on prey detection in aquatic insects, which can be transposable to other species for comparative studies. Better assessing the degree of prey selectivity in aquatic beetles can contribute to the development of sustainable mosquito biocontrol methods.

Keywords: *Aquatic insects; predatory beetles; mosquito control*

Abstract Number: 12

Use of non-targeted metabolomics to detect trace pheromone components – a spider case study

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Most pheromones to date have been identified in insects, in part because insect antennae can help locate trace pheromones in complex extracts. In pheromone studies on taxa that lack antennae, such as spiders, pheromone components are detected by searching for compounds that are predominantly present in extracts of the signalling sex. Using the triangulate cobweb spider *Steatoda triangulosa* as a model species, we tested whether the software 'XCMS Online' for non-targeted metabolomics can help locate trace pheromone components. Using gas chromatography-mass spectrometry and high-performance liquid chromatography-mass spectrometry to analyse pheromone-containing web extracts of female spiders by visually comparing total ion chromatograms (targeted metabolomics), we found the major contact pheromone component of female *S. triangulosa*. Using 'XCMS Online' for non-targeted metabolomics of web extracts, we found two additional minor contact pheromone components. A synthetic blend of the three contact pheromone components, but not the major contact pheromone component on its own, elicited courtship by spider males as effectively as female web extract. Interestingly, the mate-attracting pheromone components are breakdown products of the courtship-inducing contact pheromone components. Our findings provide incentive to use non-targeted metabolomics such as 'XCMS Online' as an advanced analytical tool in chemical ecology research.

Keywords: *Metabolomics; spider pheromone; XCMS online*

Abstract Number: 98

Controlled release matrix for delivery of pheromone of tomato pinworm, *Tuta absoluta*

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The South American tomato moth, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera), is a key pest of tomato, *Solanum lycopersicum*, in tomato-growing countries across the globe. The female sex pheromone of *T. absoluta* comprises (3*E*, 8*Z*, 11*Z*)-3, 8, 11-tetradecatrien-l-yl acetate and (3*E*, 8*Z*)-3, 8- tetradecadien-l-yl acetate (9:1). Pheromone loaded in rubber septa dispensers are being used @ 30 per ha to trap adult males. It has a high release rate of pheromone and warrants frequent replacement. This adds to the cost of labour and chemistry. Nanoporous materials are a novel carrier/dispenser for volatile signaling molecules with controlled spatiotemporal release rates. Dispensers made of mesoporous sieves with ordered pore channels were developed for loading the *T. absoluta* pheromone. Characterization by field scanning electron microscopy (FESEM) and X-ray diffraction (XRD) confirmed the ordered structure of the pores on the matrix. Pheromone when loaded in the nanomatrix showed delayed dissipation as compared to pheromone alone when assayed by thermal gravity analysis (TGA). Fourier transform infrared (FT-IR) measurements confirmed the presence of pheromone in the nanomatrix. Entrapped pheromone in the nanomatrix revealed controlled release of pheromone as compared to release from rubber septa. The physiological response in olfactory receptor neurons of the male moths to the pheromone released from nanomatrix was ascertained. Field test of pheromone loaded in nanomatirx captured more moths than unbaited traps. On the longevity of pheromone lures, the commercial lure containing 3 mg pheromone was exhausted in 20–30 days, whilst pheromone loaded into nanomatrix was effective for 45–60 days.

Keywords: *Nanomatrix; pheromone;* Tuta absoluta

Red palm weevil odorant receptor detects palm tree-emitted volatile esters and mediates host selection

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The red palm weevil (Asian palm weevil), *Rhynchophorus ferrugineus* (Olivier), is primarily adapted to palm trees and causes destructive pest attacks threatening sustainable palm cultivations worldwide. The host plant selection of this weevil is mainly attributed to the functional specialization of its odorant receptors (ORs) that detect palm-derived volatiles. We inferred how palm weevils detect host plants by deorphanizing an OR tuned to natural palm-emitted odors. We identified several palm ester volatiles as robust ligands for this OR. We demonstrated that including these palm esters in pheromone-based mass-trapping had a synergistic effect on pheromones, resulting in significantly increased weevil catches. Thus, we revealed that insect OR deorphanization could identify novel behaviorally active volatiles (reverse chemical ecology) with synergistic effects on the pheromone to be included in pest management. Our results identified a key OR that could be used further to design receptor antagonists and blockers that interfere with the weevil host-searching associated behavior with sustainable pest management applications.

Keywords: *Chemical communication; odorant receptor; deorphanization*

Abstract Number: 155

Odor imaging reveals congeneric herbivores' differential olfactory perceptions of congeneric hosts

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Identifying hostplants amidst mixed natural vegetation is challenging for foraging insects due to the complex sensory background they must navigate. The process primarily involves olfactory cues from the plant's volatile chemical repertoire. Researchers have mainly studied hostplant identification using a single hostplant species; however, natural ecosystems frequently harbour co-occurring closely-related plant species, often with similar chemical repertoires. How insects resolve these types of complex olfactory cues is not understood. We investigated this using four specialist *Chiridopsis* spp. beetle folivores exhibiting varying diet breadths within five commonly co-occurring *Ipomoea* spp. hostplants. In this sympatric system from the Indian Western Ghats forests, we experimentally established beetles' monophagous, biphagous, and oligophagous habits comprising strict preferences. We used GC-MS/-FID and SPME headspace analyses to characterize leaf odour blends of the five *Ipomoea* spp., and identified putative attractants and repellents for each *Chiridopsis* sp. using multivariate statistics. We confirmed these compounds' EAG-activity and behaviourally ascertained their attractant/repellent natures. Beetles responded to these compounds only when delivered via hostplant odour blends and not when provided singly or via non-host odour blends. We infer that these semiochemicals' attractant, repellent, or neutral characters are linked with the hostplant's complete odour blend, i.e. the matrix. Additionally, monophagous and biphagous species responded sharply to repellents, whereas oligophagous species responded sharply to attractants. We integrated the multi-source data to visualize this in-flight odour perception by representing odour as colour variations or 'odour images.' This novel tool revealed how beetles resolve closely-related hostplant species based on their proportions of attractants and repellents. Additionally, it showed how these closely-related beetles have different olfactory perceptions of the same hostplant, suggesting they have evolved to recognize the same odour using different components. Our work demonstrates that the hostplant's odour blend matrix is crucial; beetles do not respond to attractants/repellents without it. Together, the closely-related plant species form an ideal system to understand how insects perceive subtle differences between hosts and non-host cues in nature. This investigation also underlines the relevance of studying entire odour blends over individual compounds.

Keywords: *Host identification; volatile organic compounds; odour imaging*

Olfaction at the interface of host–microbe interactions

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Recognition of pathogens in a timely manner is the keystone for protective immune response in eukaryotes. In larger animals, the innate immune system utilizes a number of sensors called pattern recognition receptors (PRRs) to sense pathogen-associated molecular patterns (PAMPs) or endogenous damage-associated molecular patterns (DAMPs). The PRRs could be membrane bound such as *toll*-like receptors or cytosolic such as *nod*-like receptors. Several small invertebrates such as *Caenorhabditis elegans* lack many classical PRRs. However, they have very effective and directed immune response to various pathogens. We hypothesize that such animals likely have non-canonical PRRs. We utilized *C. elegans*, a bacterivore, to understand if the worm utilizes non-immune cells for pattern recognition during infection. We find that sensory neurons regulate survival to broad classes of pathogens, i.e. Gram negative bacteria, Gram positive bacteria and pathogenic yeast. Olfactory neurons of worms show specific defects in sensing the pathogenic bacterium *Pseudomonas aeruginosa*. We find that a volatile, 1-undecene, produced by the bacterium, induces immune response in *C. elegans* via olfactory neurons. Other pathogenic bacteria produce distinct volatiles that act as PAMPs for *C. elegans*.

Keywords: C. elegans*; PAMPS, PRRs*

SYMPOSIUM 16: INSECT CHEMICAL ECOLOGY

Robust search templates that mitigate uncertain worlds

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Solitary generalist insects need to identify food innately early in their lives. This requires an innate search template that is specific enough to discriminate objects, yet general enough for the diversity of objects relevant to that species. The solitary generalist hoverfly pollinator *Eristalinus aeneus* is one such species. Hoverflies are important pollinators that visit at least 72% of global food crops. They are robust pollinators and resilient to changes in land pattern usage.

We studied the innate floral search template of *E. aeneus* by sequentially perturbing an innately attractive floral object and conducting choice assays. We found that the innate floral search template in *E. aeneus* is a product of contextual integration of a small number of olfactory and visual cues. The template includes broad, plant-based olfactory cues and visual cues (radial symmetry and high spectral reflectance in the 500–700 nm range). Such a template is parsimonious yet robust for the broad ranges of food options available to a hoverfly.

Using this understanding of the innate floral search template of hoverflies, we built an image classifier to sort flowers from non-floral objects based on the features mentioned above. This classifier has high accuracy, is faster than other tested CNNs (convolutional neural networks) for floral image classification, and is robust regardless of the size of training datasets.

Finally, we found that hoverflies can even learn to avoid innately attractive floral models when they repeatedly encounter quinine (an aversive stimulus) on them. Further, hoverflies retain this avoidance for several days. Additionally, they quickly learn to associate neutral objects with food, after less than ten exposures to a neutral object coupled with 10% sugar. However, this appetitive learning is also unlearned quickly within 48–72 hours of ceasing the sugar rewards. This implies that hoverflies could balance opportunity costs in a dynamic vegetation community using long-term memory and avoidance of suboptimal innately recognized food sources coupled with quick adaptation to novel short-lived rewards. Our study highlights how hoverflies efficiently forage in uncertain environments with multitudes of sensory cues.

Keywords: *Search templates; hoverflies*

Abstract Number: 50

Horizontal gene transfer (HGT) contributes to the evolution of *Bemisia tabaci*

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Growing evidence indicates that HGT events have shaped the adaptive evolution of herbivory in arthropods. In this study, we selected five HGT genes that *B. tabaci* may have acquired from plants and bacteria. These HGT genes are categorized based on how their enzyme products contribute to (1) penetrating and digesting plant cell walls, (2) assimilating plant nutrients, and (3) overcoming pathogens, natural enemies, and abiotic stress. To predict the functionality of the selected HGT genes, we utilized a combination of gene silencing, behavioral, metabolomics, and genomics approaches. We describe the phylogenetic relationships for each of the selected HGTs between *B. tabaci* species, and representative species of plants, bacteria, animals, fungi, and archaea. The genomic characterization, metabolomics, and expression patterns during development in the *B. tabaci* complex are also described. HGT genes may have conferred on *B. tabaci* the ability to feed on a large spectrum of hosts. We propose that the HGT candidate genes can be targeted to improve current control strategies against whiteflies.

Keywords: *Horizontal gene transfer;* Bemisia tabaci*; plant secondary metabolites*

Abstract Number: 71

Sensory ecology of Himalayan pollinators in the context of environmental change

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Plant–pollinator interactions are a classic co-evolutionary system that has evolved over millions of years. How these relationships are impacted by changing environmental conditions is an important topic of study given the current rapid changes caused by anthropogenic impacts on land use and climate worldwide. Altitudinal shifts provide a system to study rapid changes in abiotic and biotic conditions in a small geographic area. The Himalayas are not only an excellent altitudinal system to understand changing environments, but they are also one of the most susceptible environments for climate change on the planet. Insect pollinators are known to use various visual and olfactory cues to locate flowers. Recent evidence from the Sikkim Himalayas shows that conspecific flowers emit qualitatively and quantitatively different floral volatiles at different elevations, but still receive pollinator visits at all elevations. Using 3-D printed floral models containing synthetic odour blends, we performed behavioural assays at the same sites with wild pollinators to assess their preferences towards these variable floral odour profiles in the context of visual cues. These were repeated in different elevations, seasons, and contexts. We show that wild pollinators show different preferences towards odours across elevations and seasons. However, they do not show preferences on encountering these odours in a novel context. Further, we show that while visual cues are sufficient to receive visitations, presence of certain chemicals alters preference to the floral models. These observations suggest important boundary conditions for plant–pollinator relationships that help us understand how insect pollinators might adapt to climate and environmental change.

Keywords: *Volatile organic compounds; altitudinal variation; behaviour*

Abstract Number: 87

Finding parallels: latitudinal and altitudinal differences in cuticular hydrocarbons

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The hard cuticle of arthropods has contributed substantially to their evolutionary success on this planet. Cuticular hydrocarbons (CHCs) are key constituents of this hard layer. In insects, CHCs are involved in stress handling as well as communication. There are several studies establishing a relationship between desiccation tolerance and CHCs. Latitudinal clines have been observed for desiccation tolerance and CHCs and their associations. However, a possible link between desiccation tolerance and CHC profiles had remained unexplored in altitudinal populations of *Drosophila*. We extracted CHCs from three altitudinal populations of *Drosophila melanogaster* from the Western Himalayas (760–1500m asl). Besides the characterization of altitudinal differences in CHCs, additional experiments were done to understand the rapid changes in CHCs. It was expected to find parallel patterns as seen along latitudinal scales. This study discovered significant differences in CHC profiles along varying elevations. Besides this, the study also revealed significant differences in CHC profiles between control and 8-hour desiccated flies. Collectively this study found an altitudinal cline in CHCs and evidence for rapid changes in CHCs under drought conditions.

Keywords: *Cuticular hydrocarbons; altitude;* Drosophila

Abstract Number: 99

The role of toxic quinolizidine alkaloids from *Genista* plants (Fabaceae) in aphid–plant interactions

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The pea aphid (*Acyrthosiphon pisum*), native to legume plants, comprises at least 15 genetically different biotypes, each adapted to just one or a very few different host plants. Only on its respective native host plant, an aphid biotype can perform well. One of these biotypes is specialized on *Genista* plants, known to contain quinolizidine alkaloids (QAs). These compounds stimulate and paralyze the central nervous system of animals and thus can ultimately lead to their death. Hence, we wanted to know whether QAs are responsible for the death of non-native biotypes.

We identified the QAs of *Genista tinctoria* and investigated the compounds' distribution within the aphid–plant system. Afterwards, the toxicity of some of these compounds for pea aphid biotypes native and not native to *Genista tinctoria* was examined.

Eight QAs were found in *G. tinctoria* extracts, one of which being new for *Genista* species and one previously unknown. The QAs showed an organ- and cell-type-specific distribution. Only three of these compounds were found within *G. tinctoria* phloem sap. These three have also been observed in the hemolymph of aphids, as well as in their honeydew. Some QAs are highly toxic, but only to pea aphid biotypes not native to *G. tinctoria*. This indicates a large degree of adaption for the pea aphid biotype native to *G. tinctoria* towards its native host plant.

Keywords: *Plant–aphid interaction; alkaloids; plant chemical defense*

Abstract Number: 166

Variation in CHC profiles with elevation in *Bombus haemorrhoidalis* in the eastern Himalayas

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A few years ago, we started to study the diversity of bumble bee species in the eastern Himalayas (Arunachal Pradesh) which comprise two of the three biodiversity hotspots in India. Bumble bee species in this area occur from elevation as low as 150 m to 4403 m above sea level, and the bumble bee community is characterized by a high degree of Muellerian mimicry. In this study, we were interested in the intra- and inter-species variation of cuticular hydrocarbons (CHCs) along the elevational gradient. CHCs are secreted into the outermost layer of insect cuticle and function as desiccation barrier and short-range chemical communication signals.

We collected a total of 334 worker CHC profiles from field sites at 25 elevations. We used DNA barcoding to confirm the species of the worker samples. Our collection comprised worker samples from 14 bumble bee species (*B. haemorrhoidalis*, *B. breviceps*, *B. albopleuralis*, *B. sikkim*, *B. pressus*, *B. festivus*, *B. abnormis*, *B. parthenius*, *B. luteipes*, *B. mirus*, *B. miniatus*, *B. prshewalskyi*, *B. lemniscatus*, *B. eximius*). Among these species only *B. haemorrhoidalis* was found from low to high elevation. Thus, we used the CHC extracts of *B. haemorrhoidalis* workers – 92 samples from 11 different elevations sites from 941 m – 2872 m above sea level to investigate whether the whole CHC profiles change with elevation. CHC profiles were analysed with gas chromatography and mass spectrometry (GC-MS), and CHC components were identified via compound specific mass spectra and retention indices. The individual CHC profiles were compared using non-metric multidimensional scaling (NMDS). The CHC profiles of *B. haemorrhoidalis* workers from different elevations clustered separately.

Keywords: *Himalayas; bumble bees; cuticular hydrocarbons*

Abstract Number: 175

Decoding the genetic and chemical basis of universal information carriers in insect communication

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As the earliest and most wide-spread form of communication, chemical signaling has permeated through all known taxa of life and is particularly prevalent in insects. Cuticular hydrocarbons (CHCs) are an excellent example for this, as they have been demonstrated to encode and convey a vast array of differential chemical information, from sex pheromones to nestmate recognition. However, it has been notoriously difficult to unravel how exactly biologically relevant information is encoded and conveyed in complex CHC profiles. Several studies from my current research now collectively show and single out the hitherto little investigated impact of differential methyl-branched CHCs as the main mediators of biologically relevant information as diverse as social immunity and sexual attractiveness. Focusing mainly on the parasitoid wasp model system *Nasonia*, we also investigated the genetic background of primarily methyl-branched CHC variation, discovering multiple genomic “hotspots” governing primarily species-specific methyl-branched CHC variation. Intriguingly, these hotspots coincide spatially with the genomic location of various CHC biosynthesis candidate genes. Further narrowing down these promising targets, we were able to characterize the first hymenopteran gene impacting both up- and down-regulation of distinct methyl-branching patterns as well as sexual attractiveness of female wasps. Transcending the demonstrated impact on sexual signaling, these findings also substantially advance our general knowledge on the so far little investigated genetic underpinnings of methyl-branched alkane variation. This also sheds light on how genetic information can be translated into biologically relevant chemical information, governed by a comparably simple genetic basis.

Keywords: *Sex pheromones; methyl-branched alkanes; parasitoid wasps*

Abstract Number: 184

The impact of heat shock during pupal development on pheromone-mediated mating in *Mythimna unipuncta*

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Both beneficial and pestiferous insects will be impacted by the increased frequency of extreme weather events associated with climate change, with subsequent consequences for pest management programmes. Previous experiments examining the effect of heat stress during different stages of pupal development in the true armyworm, *Mythimna unipuncta,* found that regardless of the sex treated there was a reduced incidence of mating, as well as marked decline in the number of total and fertile eggs produced, especially where heat stress occurred early in metamorphosis. While there is some evidence of direct effects on eggs and sperm, the results suggest there were effects on pheromone mediated mating. We will present the results of ongoing experiments comparing the female calling behaviour, as well as the titers of both male and female pheromones, of individuals exposed to heat stress at three different times during early pupal development and control moths.

Keywords: *Pheromone-mediated mating; heat shock; climate change*

p-Coumaroyl anthocyanins reduce petal visibility to bees in the bird-pollinated *Camellia japonica*

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Red coloration predominates among ornithophilous flowers, which has been associated with pollination syndrome. In the sect. *Camellia*, the snow camellia (*C. rusticana*) and the Japanese camellia (*C. japonica*) bear red flowers and recruit different pollinators; the former is entomophilous (bees, flies, beetles), while the latter is ornithophilous (passerines). *C. japonica* is considered to have been speciated from a common ancestor later than *C. rusticana* accompanying a pollinator shift from insects to birds. This study explored factors underlying the pollinator difference in these 'red' camellias. We previously reported the selective visitation of bees towards *C. rusticana* before accessing rewards in the two-choice assay between the two species, indicating that bees chose *C. rusticana* by visual and/or olfactory cues. A color difference was found in the reflectance spectral analyses, considering the visible range of pollinators. The petal of *C. rusticana* reflects UV + red, whereas that of *C. japonica* reflects just red. Using the reflectance spectra, visual modeling was conducted to show that *C. rusticana* is more conspicuous to bees than *C. japonica*, due to the UV-reflection. The petals of *C. japonica* exhibited crucially low chromatic contrast against a leaf background to bees, indicating them to be almost indistinguishable. These result suggested differences in the petal constituents affecting UV-reflective properties. In this study, the petal constituents of the two camellias were examined to determine compounds responsible for the difference in UV-reflecting properties. UV-Vis absorption spectra of the petal extract showed a much higher UV absorption in *C. japonica* than in *C. rusticana*, suggesting that the non-reflective property of UV in the petals of *C. japonica* is attributed to the UV-absorption by chemical constituents in the petals. HPLC-PDA analyses of extracts from the petals of two species detected two peaks specific to *C. japonica*; they were isolated and identified as anthocyanins acylated with a *p*-coumaroyl moiety. Major anthocyanins detected in the petal extract of *C. rusticana* did not have a *p*-coumaroyl moiety. In *C. japonica* petals, the *p*-coumaroyl acylation of anthocyanins reduces UV-reflectance, thereby reducing visibility to bees.

Keywords: Camellia*; anthocyanin; bee*

SYMPOSIUM 17: CHEMICAL ECOLOGY AND PEST CONTROL

Linolenic acid in oral secretion of *Spodoptera litura* induces defense responses in *Arabidopsis*

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Plants have multiple mechanisms to perceive herbivory and activate its defense responses. Plants identify different organic molecules in the oral secretions (OS) of the insects and commence molecular sensing to induce their defense mechanism. These herbivore derived agents, collectively known as Herbivore Associated Molecular Patterns (HAMPs) or elicitors, have significant potential to elicit the plant's defense system. Hitherto, a few numbers of herbivore derived elicitors have been reported such as volicitin and other fatty acid-amino acid conjugates (FACs), inceptin, caeliferins and bruchins. However, most of their activities are hos- specific and none has been reported yet in *Arabidopsis*. We applied an untargeted metabolomics-based metabolite biomarker identification approach to identify elicitor molecules in the OS of *Spodoptera litura* and identified probable elicitors. Further, for validation of the biomarker identification data, we conducted bioassay-guided solid phase extraction (SPE) column fractionation followed by high-performance liquid chromatography (HPLC)-based fractionation of OS to select the most active fraction. We selected the active HPLC fraction showing maximum cytosolic Ca^{2+} elevation, reactive oxygen species (ROS) signal and jasmonate-responsive gene expression. This active fraction was analyzed through liquid chromatography-mass spectrometry which revealed that the fraction contains α-linolenic acid as a dominant molecule, which was also identified as a biomarker. Confirmation of α-linolenic acid was done by LC-MS/MS followed by comparison with the MS/MS spectrum of the authentic standard. For functional confirmation, we tested α-linolenic acid as a potential elicitor and observed that it significantly induced early defense responses such as cytosolic Ca^{2+} signalling, jasmonate elevation and jasmonate-responsive gene expression in *Arabidopsis*. Thus, our work establishes that α-linolenic acid content in *S. litura*'s OS induces defense responses during herbivory perception in *Arabidopsis*.

Keywords: *Plant–insect interaction; elicitor; plant defense*

Abstract Number: 152

The red palm weevil chemical ecology in the OMICS and post-genomic eras

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The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) is the most destructive and invasive insect pest of palm trees worldwide. The RPW has a native distribution in Southeast Asia and its invaded ranges include almost all Asian and Middle Eastern countries, the Mediterranean, and North Africa, causing widespread mortality of palms. As valuable resources to understand the RPW's important traits of life, two genomes have been published recently, releasing a plethora of gene candidates for further functional studies. However, such functional investigations require perfectly annotated genes since slight variations in protein-coding sequences can lead to protein misfunction or inefficient gene editing. In addition, automatic annotation could miss important genes, especially for poorly conserved genes, or highlight artefactual duplications or expansions through heterozygosity. To fulfill this gap, we have sequenced a third RPW genome using Illumina and PacBio, generated multiple RNAseq data from key tissues, and manually curated by experts gene families involved in important functions, including immunity, detoxification, digestion, and chemosensation. As key features of the RPW interactions with conspecifics and hosts, we looked in detail at chemosensory genes, especially chemosensory receptors (odorant receptors, ORs; gustatory receptors, GRs; ionotropic receptors, IRs). Manual curation allowed us to annotate an impressive number of 74 IRs, the highest number of IRs described in Coleoptera so far. IRs are mainly involved in detecting acids and fermentation products; such expansion could be related to the RPW lifestyle in close interaction with its host within fermented tissues. We identified 37 GRs, less than the previous annotation, and extended the previously described repertoire of ORs. We demonstrated tandem duplication of key ORs, such as the aggregation pheromone receptor, and suggest that their function could be addressed by heterologous expression in *Drosophila* and extensive screenings of volatile organic compounds, proposing a scenario for OR functional specialization.

Our collection of curated genes constitutes a valuable resource for such functional characterization, and our functional data pinpoint interesting volatiles to be included in olfactory-based RPW control strategies.

Keywords: Rhynchophorus ferrugineus*; chemosensory receptors; deorphanization*

Characterization of volatile compounds of *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae)

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Ants have begun to gain more interest as pest control agents, in particular, studies on weaver ants (*Oecophylla smaragdina*) have created lot of interest in Asia among organic farmers. Research on the chemical profile of *O. smaragdina* is limited. In the present study, identification of volatile chemicals were done in hree body sections *viz.,* head, thorax and abdomen of major and minor workers using GC-MS (modified after Igwe and Eze, 2015). The extract was analyzed with Perkin Elmer Clarus SQ8C (PerkinElmer, Waltham, USA) gas chromatograph coupled with an unique Clarifi™ detector. In the head, naphthalene (61%) followed by oleic Acid and triphenylphosphine oxide were found as major components. Biochemical constituents of the thorax were reported for the first time. Isoquinoline (43.2%) was present as the major component in thorax followed by cis-13-octadecenoic acid (29.0%) and n-hexadecenoic acid (Palmitic acid) (5.5%). Napthalene (23.4%) was the major component in the abdomen which was followed by hexanediolic acid, bis (2-ethyl hexyl) ester (2.1%); 1-(H)- Ascorbic acid 2,6 – dihexadecanoate (1.7%) and tetra decyl phosphonate (1.6%).

1. Igwe OU, Eze PN (2015). Chemical analyses of volatile compounds from cuticular and non-cuticular abdominal glands of African weaver ants (*Oecophylla longinoda*). International Journal of Chemical Sciences 5: 304-312.

Keywords: Oecophylla smaragdina*; volatile chemicals; pest management*

Silencing of target genes via ingestion of dsRNA affects development and survival of fall armyworm

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Fall armyworm (FAW), *Spodoptera frugiperda,* is a polyphagous generalist insect herbivore that can feed on more than 100 plant species, with a strong preference for maize. Effective control is challenging with the pest exhibiting resistance to different synthetic pesticides across various groups. *Spodoptera* ABC transporters have evolved the capability to detoxify *Bt* toxins, thus conferring resistance to transgenics. RNA interference (RNAi) is a sequence-specific down-regulation in the expression of a particular gene, induced by double-stranded RNA (dsRNA). Feeding of dsRNA has been proven successful against lepidopteran pests, establishing an additional alternative tool to control insect pests. To tap the potential of RNAi against FAW, genes coding for *Coatomer Subunit alpha* (*COPα*), *Coatomer Subunit beta (COPβ), Chitin Synthase B* (*CHSB*), and *Glutathione-S-transferase* (*GST*) have been targeted through an exogenous delivery of dsRNA. The introduction of dsRNA through artificial diet caused “Half ecdysis” and “Black body” type lethal phenotypes and a significant reduction in larval body weight for all the target genes. Analysis of transcript levels by quantitative RT-PCR revealed that larval mortality was caused due to the high suppression of genetic targets by RNAi. In addition, abnormalities in pupal-adult and adult stage were also found, suggesting RNAi effect at late developmental stages. These findings strongly support the use of *SfCOPα*, *SfCOPβ*, *SfCHSB* and *SfGST* for RNAi targets, as they play a critical role in FAW growth and development. Further study on the potential use of dsRNA for foliar application, production of chimeric dsRNA and functional studies on insect effectors are on-going.

Keywords: *RNAi; fall armyworm; herbivory*

Abstract Number: 29

Estragole, a potential attractant of the winged melon aphid, *Aphis gossypii*

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Aphids are sap-sucking insect pests of economic importance. They exhibit polyphenism and can produce two kinds of morphotypes: winged and wingless morphs. While wingless morphs can be controlled by insecticides, winged morphs are a challenge as they can fly. Although coloured sticky traps are used to control and monitor winged aphids, only a small population is trapped, making sticky traps less effective in controlling aphids. Previous studies have shown that fragrant flowers and essential oils applied to sticky traps increased trap catches of sap-sucking insects like whiteflies and thrips. Considering this analogy, we tested few selected essential oils for their attractiveness to winged melon aphids in potato fields. In preliminary field assays, selected essential oils with yellow or colourless sticky traps attracted significantly more winged *A. gossypii* compared to the controls. However, the combination of yellow traps with essential oils attracted more winged *A. gossypii,* with ~2–3-fold increase in trap catches, compared to colourless traps with essential oils. In a multi-cycle two year-study, yellow sticky traps with basil oil attracted winged *A. gossypii* more consistently than yellow sticky traps with lavender, geranium or tea tree oils. In GC-EAD studies, winged *A. gossypii*'s antenna responded consistently to a single component, estragole, in basil oil. In 4-arm olfactometer assays with estragole, winged melon aphids spent significantly more time in the treatment arm of the olfactometer compared to the control arm, validating estragole's attractive nature. Further, yellow sticky traps with pure estragole, in potato fields, attracted similar number of winged *A. gossypii* as yellow sticky traps with basil oil (control). Our findings demonstrate the potential of using basil oil as a potential attractant to improve the efficacy of sticky traps in monitoring and control of winged aphids.

Keywords: Aphis gossypii*; attractant; estragole*

SYMPOSIUM 18: INTRA- AND INTERSPECIFIC CHEMICAL COMMUNICATION OF BARK BEETLES AS FOREST PESTS IN GLOBAL CHANGE

Abstract Number: 58

Ophiostomatoid fungi synergise attraction of *Ips typographus* to its aggregation pheromone in the field

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The Eurasian spruce bark beetle, *Ips typographus*, is a devastating pest for Norway spruce in Europe. Bark beetles interact with complex olfactory cues originating from their environment, where the most powerful is the aggregation pheromone. *I. typographus* perceives VOC signals produced by multiple other organisms during their life cycle. Besides the host trees, beetles can smell their symbiotic ophiostomatoid fungi supporting their successful colonisation and development. It was recently shown that *I. typographus* were attracted to VOCs of *Grosmannia penicillata*, *Endoconidiophora polonica* and *Leptographium europhioides* in a short-distance assay. In the fungal VOC emissions, were present the most active fusel alcohols and their acetates. In this study, we tested the relevance of fungal VOCs to dispersing *I. typographus* using a long-distance field trapping experiment. Live cultures of fungi grown on potato dextrose agar or the dispensers containing synthetic fusel alcohols and acetates were combined with beetle pheromone in traps, and catches were compared. Consequently, the composition of VOCs emitted by tested fungal dispensers was analysed. Our results showed that fungal culture synergistically increased the attraction of beetles to pheromones in traps and the dose range of active fungal VOCs depended on fungal species. A low dose of *E. polonica* combined with the pheromone significantly increased trapping efficiency, while *L. europhioides* in a high dose caused the same effect. The synthetic mix of fusel alcohols and acetates in an equal ratio improved the catches to pheromone only at a low dose (1 mg/day). VOC analysis of tested fungi dispensers revealed that three fungi have distinct volatile profiles and differ quantitatively and qualitatively. This is the first study to comprehensively show that VOCs from symbiotic fungi of bark beetles can increase the attraction of dispersing beetles to their pheromones in a long-distance field trapping assay.

Keywords: *Eurasian spruce bark beetle;* Endoconidiophora polonica*;* Leptographium europhioides

Abstract Number: 125

Finding friends in the forest: Volatiles of bark beetle fungi help maintain symbiosis with beetles

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Global warming has been increasing the frequency and intensity of bark beetle outbreaks throughout the world by accelerating beetle life cycles and decreasing host tree resistance. For successful attack on living trees, bark beetles rely on mass attacks to overcome tree defenses and the presence of free-living fungal symbionts of the Ascomycota that are said to contribute to beetle nutrition. While the chemical basis of aggregation for mass attacks has been well elucidated, we know little about the role of chemical communication in maintaining the fungal symbiosis. Yet, each generation of beetles and fungi must find each other anew.

We have shown that fungi associated with the Eurasian spruce bark beetle (*Ips typographus*) produce a range of aliphatic and aromatic esters and alcohols as well as terpenes when growing on spruce bark-amended medium. In addition, we determined that some fungi can covert monoterpenes of spruce resin to oxidized products. Single-sensillum recordings from bark beetle antennae showed that beetles detect certain fungal volatiles and monoterpene metabolites, and possess olfactory sensory neurons specialized for these compounds. Blends of volatiles attracted beetles in olfactometer experiments, and beetles discriminated between symbionts and non-symbionts on the basis of their odour profiles. Our results suggest that fungal volatiles help beetles to choose their symbionts in host tree galleries, which they carry with them to their next host. Individual volatiles may provide information about the fungal species, its nutritional status and its ability to detoxify host tree defenses. Upon arriving at a new host, fungal volatiles may aid beetles in assessing the presence of the fungus and the defensive status of the tree. In this way, chemistry appears to mediate one more aspect of bark beetle life history.

Keywords: *Monoterpenes; Ascomycetes; olfactometer*

Abstract Number: 164

Chemistry of symbiosis: Volatiles mediate interactions between conifer bark beetles and their fungal associates

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Many insects exhibit symbioses with other organisms through signaling compounds, which drive the evolutionary maintenance of mutualistic associations. Bark beetles are associated with a diverse species of symbiotic fungi that are vital for the successful colonization and development of these insects in their host trees. Fungi can mobilize/supplement nutrients for beetles by growing in the bark and wood and also be able to detoxify the defense chemicals of trees which are toxic to beetles.

We investigated if volatile organic compounds emitted from different fungal symbionts could act as cues for bark beetles to recognize and distinguish their microbial community. Fungi associated with bark beetles emit a variety of volatiles comprised of simple aliphatic, aromatic alcohols and their esters, benzenoids, mono-, and sesquiterpenes. Using the Eurasian spruce bark beetle, *Ips typographus,* and its fungal symbionts, we showed that bark beetles attracted fungi in olfactometer assays and beetles preferred to tunnel on diets colonized by beneficial symbionts but not by commensals and pathogens. Testing these fungal compounds on beetle antennae using single sensillum recording revealed that beetles could detect many fungal volatiles and possess several new olfactory sensory neuron classes specialized in detecting the fungal volatiles. Finally, we showed that a synthetic blend of fungal volatiles attracted bark beetles in olfactometer assays. These findings indicate that volatile compounds produced by fungi may 1) help beetles to evaluate the defense status of the tree under attack, 2) aid beetles in coordinating mass attacks, and 3) act as recognition cues for bark beetles to maintain specific microbial communities.

Keywords: *Bark beetles; fungal symbioses; volatiles*

Functional characterization of a gene from gut tissue of the bark beetle, *Ips typographus*

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Bark beetle devasting effect on conifer vegetation is known in many parts of the world. Certain bark beetle species were identified and studied exclusively on European and Asian continents. One of the key pests in the Eurasian region is *Ips typographus*, a specialist in spruce forests. Pheromone-mediated coordination is a vital factor for the pests to overcome the tree defence mechanism. Over a decade, screening the gene families involved in the pheromone biosynthesis of many bark beetles has yielded insightful research, that consider eco-friendly pest management strategies. In *I. typographus*, recent findings have provided key genes for pheromone biosynthesis in the respective life stages of the beetle. Genes from both *de novo* and precursor-mediated biosynthesis were identified from the gut tissue of *I. typographus*. Exclusively, mevalonate pathway genes were identified as highly expressed genes in the key pheromone-producing male beetles. One of the candidate genes, *isoprenoid-di-phosphate synthase* (IPDS) was identified and proposed for 2-methyl-3-buten-2-ol, an aggregation pheromone of *I. typographus*. Though this gene was known for similar hemiterpene alcohol synthesis in trees, the functional validation of this gene for the beetle is necessary. We standardised the functional characterisation of the IPDS gene in a bacterial expression system using the gateway pDEST17 and pET100D cloning protocol. An added RNA interference of the gene was also established to understand the functional importance of the identified gene. This study produces further knowledge on the identified gene of *I. typographus* gut tissue.

Keywords: Ips typographus*; pheromone biosynthesis; gene characterisation*

POSTER ABSTRACTS

SYMPOSIUM 10: CHEMICAL AND MOLECULAR ECOLOGY OF PLANT–HERBIVORE–NATURAL ENEMY INTERACTIONS

Abstract Number: 53

Potential drivers of chemodiversity in *Solanum dulcamara*

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Plant chemodiversity is an important dimension of biodiversity. Intraspecific variation in plant chemical composition is assumed to be driven by adaptation to (a)biotic factors that vary in space and time. Yet, the raison d'être of plant chemodiversity is still a matter of debate. Therefore, we investigate how chemodiversity of natural plant populations relates to environmental factors in *Solanum dulcamara*, a widely distributed plant in the northern hemisphere displaying high phenotypic plasticity.

Native *S. dulcamara* populations growing in different habitats were investigated for (a)biotic factors in relation to leaf chemistry for a representative number of genotypes. Abiotic factors were evaluated using indicator values[1] of plant species in the surrounding vegetation, which provided an integrative measure of these factors over time. Chemodiversity was quantified by analysing leaf chemistry at three time points over a growth season with an untargeted metabolomics approach and different targeted analyses for major classes of defence-related compounds. At mid-season, herbivore community and leaf damage assessments were carried out on the same genotypes.

We found considerable variation in leaf chemistry over the growth season as well as within and between populations. Chemodiversity of plant individuals (α-chemodiversity) was highest at early season and inter-individual variation in chemodiversity was higher in all populations at the end of season. Of the abiotic factors, soil humidity and light were the most variable between populations. Strong correlations indicated that α- and γ-chemodiversity (at population level) of phenolics are higher in drier habitats with higher species numbers of plants and herbivores than in wet rather species-poor habitats. Overall, leaf damage patterns and herbivore scores on *S. dulcamara* individuals indicated that herbivory in wet habitats was dominated by few mostly specialised herbivores, while herbivory by generalists dominated in drier habitats. Our results suggest that complex ecological communities may promote plant chemodiversity. Further studies are on-going to disentangle the contributions of genotypic and phenotypic variation to chemodiversity in natural *S. dulcamara* populations and to test the consequences of chemodiversity in different herbivore communities.

[1] Ellenberg H, Weber HE, Düll R, Wirth V, Werner W (2001) Zeigerwerte von Pflanzen in Mitteleuropa 3.Ed. Erich Goltze GmbH, Göttingen

Keywords: *Plant secondary metabolites; intraspecific variation*

Understanding the influence of tansy chemodiversity on pollinators and florivores across scales

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Chemodiversity refers to the diversity of chemical compounds, particularly specialized metabolites, in living organisms. This diversity may play an important role in interactions between plants and various antagonists and mutualists, however, little is known about its role in flower–insect interactions, including different functional groups such as pollinators and floral herbivores or 'florivores'. The common tansy, *Tanacetum vulgare* L. (Asteraceae), produces many different chemotypes, characterised by distinct leaf terpenoid profiles, which can co-occur within populations. We investigated the role of tansy chemotypes on preferences of pollinators as well as florivores in field or laboratory settings. In a field setting, plants from five different chemotypes were grown in 60 groups (=plots) of five plants each. Homogeneous plots contained plants of a single chemotype, while heterogeneous plots had plants of all five chemotypes; flower visitors were monitored in two consecutive years. In the laboratory, we studied the preference of representative pollinator (*Bombus terrestris*) and florivore (*Olibrus* spp.) insects towards three chemotypes in paired choice tests. In the field setting, chemodiversity on the plot level was found to be important for pollinator but not florivore visitation. Certain chemotypes showed interactive effects with plot type, exerting a stronger influence on pollinator visitors when growing in homogeneous plots. In the laboratory, both pollinators and florivores showed preferences for certain chemotypes. Terpene composition of flowerheads and pollen as well as nutrients in pollen were determined, revealing differences among the chemotypes. In summary, our results demonstrate that chemodiversity at both the plant and plot level has distinct impacts on floral insect visitors of different functional groups.

Keywords: *Chemodiversity; florivory; pollination*

Abstract Number: 213

Exploring role of plant chemical defenses in shaping plant communities in human-modified landscapes

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Herbivorous insects play a key role in shaping local plant diversity and composition. Recent studies suggest that plant defences, particularly secondary metabolites, play a significant role in shaping plant communities and promoting species co-existence. Furthermore, a higher diversity of defence chemicals in the neighbourhood decreases herbivory and improves plant survival. While evidence for plant chemical defences shaping plant community is increasing, it remains unexplored in the context of influencing plant community dynamics in human-modified landscapes.

In forest fragments, edges typically have lower plant diversity, dominated by fast-growing species. Fast-growing species trade-off defence for growth (growth–defence trade-off hypothesis) and produce lower diversity and lower quantity of defence chemicals and vice versa.

In this study, we will examine how leaf chemical diversity and composition change across an edge-to-interior gradient in the Western Ghats (India), a biodiversity hotspot. Additionally, we examine how seedling herbivory rates and survival change with neighbourhood chemical diversity along an edge-to-interior gradient.

In 134 1 x 1 m plots along an edge-to-interior gradient, we identified, tagged, and monitored herbivory rates, growth and survival biannually for seedlings. For each species found in the plots, we have collected leaves from seedlings outside but around the plots and extracted total phenols and alkaloids to analyse investment in defence using HPLC. We will then explore the distribution of defence chemicals in the edge and interior seedling community (using non-metric multidimensional scaling) and use structural equations models to evaluate the influence of neighbourhood chemical diversity on seedling herbivory rates and survival in edge vs. interior.

We expect to find lower diversity and quantity of defence chemicals in edges compared to forest interiors, with herbivory rates increasing with declining chemical diversity in a neighbourhood. However, in the high-light conditions of the edge, fast-growing seedlings will be able to overcome herbivory and survive. In contrast, in the darker understory of forest interiors, if neighbourhood chemical diversity is low, herbivory rates will increase but seedlings will not be able to overcome the damage.

Keywords: *Plant–insect herbivore interactions; plant community ecology; forest fragmentation*

SYMPOSIUM 11: CHEMICAL ECOLOGY OF VENOMS AND POISONS

Abstract Number: 216

To eat or not to eat: Evolution of toad toxin-resistance in Indian snakes

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The coevolution of predators and their prey is often shaped by an ever-escalating arms race, wherein one tries to gain an advantage over the other. One such example is the arms race between the poisonous bufonid toads and their reptilian predators. Bufonid toads secrete bufadienolides that block the Na^+/K^+-ATPase pump, resulting in lethal cardiotoxicity. Only a handful of organisms have evolved the ability to feed on such extremely toxic toads. Certain snakes, for example, have developed resistance to bufonid toxins by acquiring specific mutations (111th and 122nd positions) in the α-subunit of the Na^+/K^+-ATPase. It has been shown that identical substitutions, and the consequent emergence of resistance to cardiac glycosides, has independently evolved across the animal kingdom, at least 15 times. Therefore, the evolution of toad toxin resistance is a remarkable example to understand the predictable nature of evolution, as well as the origin of adaptive traits. While this phenomenon is well-documented in squamate reptiles worldwide, our understanding of the prevalence of toad toxin resistance in Indian snakes and the underlying mechanisms of resistance remain unexplored.

To bridge this knowledge gap, we investigated the emergence of toad toxin resistance in Indian snakes by sequencing the α-subunit of Na^+/K^+-ATPase from diverse species. We have examined the molecular mechanisms underlying the interactions between the toad toxin and its target receptor using molecular docking and simulations. These investigations have led to the identification of various residues that are involved in underpinning these interactions. We demonstrate that by incorporating charged residues at the toxin interaction site, the resistant lineages prevent the effective binding of bufadienolides to their Na^+/K^+-ATPase. Considering that only organisms with resistant substitutions can exhibit bufophagy, our findings also provide fascinating insights into the feeding ecology of many Indian snakes. In contrast to our current understanding, we show that the rarely documented genotype consisting of resistant and susceptible substitutions (i.e., L111 and G120) may offer intermediary resistance. Thus, our findings provide novel insights into the emergence of toad toxin resistance in snakes and the complex molecular dynamics that underpin this adaptation.

Keywords: *Bufadienolides; toxin resistance; Na+/K+-ATPase*

Abstract Number: 217

The primordial knot: the deep-rooted origin of the disulfide-rich spider venom toxins

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Spider venoms are a complex concoction of enzymes, polyamines, inorganic salts and disulfide-rich peptides (DRPs). Although DRPs are widely distributed and abundant, their evolutionary origin has remained elusive. This knowledge gap stems from the extensive molecular divergence of DRPs and a lack of sequence and structural data from diverse lineages. By evaluating DRPs under a comprehensive phylogenetic, structural and evolutionary framework, we have not only identified over 70 novel spider toxin superfamilies but also provide the first evidence for their common origin. We trace the origin of these toxin superfamilies to a primordial knot – the 'Adi Shakti' – nearly ~375 MYA in the common ancestor of Araneomorphae and Mygalomorphae. As these lineages constitute over 50% of the extant spiders, our findings provide fascinating insights into the early evolution and diversification of the spider venom arsenal. Reliance on a single molecular toxin scaffold by nearly all spiders is in complete contrast to most other venomous animals that have recruited into their venoms diverse toxins with independent origins. Moreover, by comparatively evaluating araneomorph and mygalomorph spiders that differentially depend on their ability to secrete silk for prey capture, we highlight the prominent role of predatory strategies in driving the evolution of spider venom.

Keywords: *Disulphide-rich peptides; venom evolution, toxin superfamily*

Abstract Number: 218

Comparison of venom composition across groups showing temporal polyethism in *Apis cerana*

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Venoms of different organisms exhibit huge compositional differences even at an intra-population level. Ontological variation in venom is usually attributed to offensive venom complexes used by snakes, spiders, scorpions, etc. The eusocial insects belonging to Hymenoptera (honeybees, wasps, and ants) usually use venom as a defensive weapon to save the colony from invading predators. Honeybee workers show temporal polyethism and can be further divided into castes: nurses, guards, and foragers (younger to older). This ontogenetic change in behaviour reflects in their defensive duties as well. Guards are usually the first line of defence, while the nurses are usually confined to the cells deep in the hive. Foragers might encounter much more self-defensive situations than others. We assessed the difference in the venom of these groups in *Apis cerana* based on discrete behavioural cues. Putative venom profiles of each caste are generated from their transcriptomes, and they look extremely similar. More than 90% of the transcripts in all three castes is melittin, a pain-inducing factor. Icarapin was the next major component in all three profiles, with no significant difference across the castes. Other common honeybee allergens and venom components constitute the rest of the profile. These results overall indicate that there is little to no difference in the venom of *Apis cerana* based on temporal polyethism. It is interesting that *Apis cerana* develops a fairly complex and effective venom composition in the very early stages of its life. Here, all three castes of bees heavily focus on making a painful mixture as their defensive measure. The presence of common allergens and pain-inducing factors also suggests a generalised venom strategy for defence.

Keywords: *Honeybee venom; temporal polyethism; transcriptomics*

SYMPOSIUM 13: MUTUALISM AND MULTITROPHIC INTERACTIONS

Abstract Number: 37

Inter-varietal chemical variations of the pollinator attraction signal in the Mediterranean fig tree

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For thousands of years, humans have domesticated different plants by selecting for particular characters, often affecting less-known traits, including the volatile organic compounds (VOCs) emitted by these plants for defense or reproduction. The fig tree *Ficus carica* has a very wide range of varieties in the Mediterranean region and is selected for its traits affecting fruits, including pollination, but the effect of human-driven diversification on the VOCs emitted by the receptive figs to attract their pollinator (*Blastophaga psenes*) is not known. In the present study, VOCs from receptive figs of eight varieties in northern Morocco, were collected at different times within the manual pollination period and analyzed by gas chromatography-mass spectrometry. Genetic analyses using microsatellite loci were performed on the same varieties. Despite strong inter-varietal differences in the quantity and relative proportions of all VOCs, the relative proportions of the four attractive VOCs showed limited variation among varieties. There was no significant correlation between genetic markers and chemical profiles of the different varieties. While diversification driven by humans has led to differences between varieties in VOC profiles, this paper suggests that throughout the process of domestication and varietal diversification, stabilizing selection has maintained a strong signal favoring pollinator attraction.

Keywords: *Volatile organic compounds (VOCs); mutualism; domestication*

Abstract Number: 48

Plant volatile impacts on pollination services are dependent on spatial scale

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Pollination services are likely influenced by landscape composition in agroecosystems. Cranberry (*Vaccinium macrocarpon*) is a crop native to North America that depends on these services to maximize yield. Natural habitats surrounding cranberry farms can provide alternative resources and nesting sites for native pollinators. In addition, flowers emit volatile organic compounds, such as methyl salicylate (MeSA), which can attract pollinators. By utilizing MeSA in the field, growers can potentially increase pollination services provided by naturally occurring pollinators. In this study, 25 sites across three commercial cranberry farms and five cultivars were georeferenced; these farms account for about 70% of the total production acreage in New Jersey (USA). The study had a factorial design with each site containing 2 open and 2 closed (pollinator-exclusion) cages either baited or not with MeSA; all cages were set up before bloom. Landscape composition was quantified within 100 m, 250 m, 500 m, and 1500 m buffers, and was determined through the most recent land-use map provided by the New Jersey Department of Environmental Protection. To calculate pollination services, all berries within cages were counted and weighed and then an average fruit volume was measured based on 10 random berries per sample. Results showed that both pollination and MeSA increase cranberry yield. Moreover, the effect of MeSA on pollination services was influenced by landscape complexity; however, this effect was dependent on the spatial scale such that pollination services increased with higher landscape complexity only at the 1.5 km radius buffer zone. This study is the first to investigate the landscape-level effects of a plant volatile, such as MeSA, on pollination services.

Keywords: *Landscape complexity; ecosystem services; methyl salicylate*

Source–sink relationships within mutualisms: Role of plant hormones in resource allocation

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Most mutualistic interactions involve trading resources in exchange for other resources or for services. In mutualisms involving plants, the partner accessing these resources, e.g, mutualistic symbionts, are usually resource sinks of their partners, the hosts. These interactions can therefore be viewed as source–sink relationships. Sinks often have the ability to manipulate source–sink exchanges, resulting in altered allocation patterns. It is thus important to address how resource exchange is regulated in mutualisms involving resource sinks. Translocation of photosynthates to sinks is controlled via signalling through plant hormones. Measuring plant hormones thus informs us about source–sink relationships within these mutualisms. In the interaction between fig trees and their pollinator wasps, seeds of the fig tree and offspring of pollinator wasps develop together within closed inflorescences called fig syconia. Pollinator offspring develop within galled uniovulate flowers. Insect galls in plants are strong resource sinks and they release different phytohormones that aid in acquiring resources for their development. Mutualism between the fig tree and their pollinator wasps might therefore be regulated by plant hormones of insect pollinator or plant origin. We investigated the relative concentrations of growth hormones released by the seeds, pollinator galls and the wall of the fig syconium. Experimental treatments included syconia containing only seeds, only pollinator galls, and those with both seeds and galls. These syconia were harvested at different stages of their development and their occupants were separated to analyse their growth hormone levels. We also conducted hormone analysis using naturally developed and unmanipulated syconia by separating seeds and pollinator galls within them. Preliminary results show that, compared to the wall tissue, galls contained significantly higher concentrations of the cytokinin zeatin and indole acetic acid (IAA). There was no significant difference between hormone concentrations of seeds and galls suggesting that galls are as competent at acquiring resources as seeds. Individual galls and their contents have higher biomass than individual seeds; it is possible that galls are better than seeds at garnering plant resources. Our study highlights the importance of viewing mutualisms as source–sink relationships to better understand the mechanisms of exchange between the partners.

Keywords: *Mutualism; source–sink relationship; plant hormones*

Reciprocal antagonism between a plant virus and rhizobacterium on plant–insect vector interactions

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Plant-associated soil bacteria can have strong effects on aboveground interactions between plants and other organisms, including via the induction of systemic resistance against pathogens and their insect vectors. Meanwhile, vector-borne pathogens can also alter host-plant traits, frequently in ways that increase insect vector attraction and facilitate disease transmission. Yet, despite the likely frequent co-occurrence of these microbial symbionts on the same host plants and despite the evidence of their diverse effects on plant and insect traits, few studies to date have explored the effects mutualistic and antagonistic microbial symbionts on host-vector interactions. We explored how the presence nodule-forming rhizobacteria (*Rhizobium leguminosarum*) and a plant virus (pea enation mosaic virus) influenced interactions between pea aphids (*Acyrthosiphon pisum*) and their host plants (*Vicia faba*). In addition to examining effects of these symbionts on plant and aphid traits, we also assessed their effects on host plant chemistry to gain insight into the potential mechanisms by which these symbionts can influence interactions between host plant and aphid vectors.

Our results indicate a reciprocal antagonism between the rhizobacteria and the virus–vector association, including via opposite effects on host plants and aphid vectors. In the presence of rhizobacteria, plants had enhanced biomass and aphid vectors had decreased reproductive rate, while these effects were reversed in the presence of the virus and generally intermediate when both rhizobacteria and virus were present. Subsequent hormone analysis with liquid chromatography revealed that plants associated with the rhizobacteria had elevated levels of anti-herbivore defenses even in the presence of the virus, including levels of 12-oxophytodienoic and jasmonic acid. In contrast, preliminary metabolomic analysis with gas-chromatography indicate that, regardless of the rhizobacteria presence, plants infected with the virus had enhanced availability of key resources for aphid vectors, in particular amino acids. Altogether, these results document conflicting effects of mutualistic and antagonist microbial symbionts on host plant chemistry, and ultimately on host–vector interactions. Moreover, these findings suggest potential implications of rhizobacterial symbionts on the transmission of plant diseases and management of insect vectors.

Keywords: *Multitrophic interactions; beneficial plant microbes; vector-borne pathogens*

Abstract Number: 148

The role of the endosymbiont *Regiella insecticola* in pea aphid performance and plant defence response

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The pea aphid (*Acyrthosiphon pisum*), native to legume plants, comprises at least 15 genetically different biotypes, each adapted to just one or a very few different host plants. An aphid biotype can only perform well on its respective native host plant. However, all biotypes can also perform on the universal host plant *Vicia faba*. As the majority of insects, the pea aphid harbours endosymbionts. The obligate endosymbiont *Buchnera aphidicola* provides the aphid with essential amino acids which are absent in the phloem sap. Many pea aphids also harbour one or sometimes even two facultative endosymbionts whose role is often still under investigation.

One of the pea aphid biotypes, native to *Trifolium pratense*, is predominantly associated with the facultative endosymbiont *Regiella insecticola*. Even though it is known that *R. insecticola* provides protection against entomopathogenic fungi, the reason for the predominant association with pea aphids from the *Trifolium* biotype is unknown. Thus, we investigated whether pea aphids from the *Trifolium* biotype differ in their performance depending on whether they harbour *R. insecticola* or not. Whereas the performance of the pea aphids on its native host plant *T. pratense* was not influenced by the presence of *R. insecticola*, pea aphids bearing *R. insecticola* showed a reduced fecundity and a higher mortality on the universal host plant *V. faba*. We also investigated the plant response towards pea aphids with and without *R. insecticola* to see whether plant defence mechanisms are responsible for the different aphid performance. However, the plant response towards aphids with and without *R. insecticola* differed only marginally. Thus, different plant defence mechanisms are not likely to play a major role.

Keywords: Regiella insecticola*; plant defence response*

SYMPOSIUM 14: CHEMICAL ECOLOGY IN AGRICULTURE

Abstract Number: 135

Preference and performance of *Plutella xylostella* on different mustard varieties

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Host plant selection by female adult insects is crucial for larval survival and fitness. Studying adult preference is therefore important to understand insect adaptation strategies as well as combat pest prevalence towards sustainable agriculture. Host plant physiology, chemistry and larval performance are the key factors that influence this preference in addition to the presence of enemy-free space. Here, oviposition preference of the specialist herbivore, *Plutella xylostella*, was examined on eight different Indian mustard varieties to estimate the impact of various parameters such as trichome density, glucosinolate (GSL) and other metabolites on insect choice. The larval growth and fitness were also measured. Though total GSL content and other metabolites like flavonoids, phenolics and antioxidants varied significantly between varieties, it did not affect insect choice. The adult preference varied significantly but it did not affect the larval growth or development. Interestingly, the cellular immunity of *P. xylostella* larvae showed a strong positive correlation with adult choice. A similar correlation was observed in our previous study on the preference and performance of *P. xylostella* on two different *Brassica* species, where the enhanced larval immunity provided better survival against natural enemies of the herbivore (Ghosh et al., 2022). In summary, our results highlight that multiple factors influence oviposition choice and a multipronged approach is essential to fully understand this complex decision-making process in insects.

Keywords: *Oviposition preference; secondary metabolite; cellular immunity*

Abstract Number: 176

Epigenetic regulation of combined heat and salinity stress responses of rice

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Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. However, the production potential of modern cultivars has remained stagnant. Increasing rice production in different rice-growing ecosystems is imperative to feed the increasing world population. The climatic changes and challenging environmental dynamics have added new challenges to crop sustainability and food security for the current and upcoming generations. Due to global warming and increasing sea levels, there is a gradual increase in soil salinity and atmospheric temperature, especially around coastal regions, the home to the well-explored coastal rice fields. Combined heat and salinity stresses imply additive stress impacts and are projected to drastically reduce the yield and quality of rice harvests. Therefore, understanding and exploring the in-depth mechanistic insights (especially epigenetic plasticity) of rice responses and adaptations to these combined stressors is the need of the hour. However, historically, studies are primarily focused on either of these single abiotic stresses though in nature plants rarely face single stress. In this study, two Indica rice cultivars with contrasting responses to heat+salinity stress were selected based on their phenotypic and biochemical responses. To decipher the transcriptomic behavior of these cultivars under combined stress conditions, the gene expression patterns of key heat and salinity stress-responsive genes *OsNHX*, *OsHSP18*, *OsT6PS8* and *OsNR* using qRT-PCR analyses. Besides, the epigenetic response to single and combined stress was examined by analyzing expression patterns of *OsDDM1a*, *OsDDM1b*, *OsRDR1*, *OsMET1* and *OsDRM2*, the genes known to maintain and modify the methylation epimarks on DNA. The results showed interesting and important findings and the differential expression patterns confirm the critical roles of these genes in epigenetic regulations of heat+salinity adaptive responses in rice cultivars. Cultivars showed differential expression levels, highlighting the involvement of these epimarks in the tolerance abilities of rice plants. These epimarks thus have the potential to be explored as targets for engineering heat+salinity stress tolerance in rice and other related crops.

Keywords: *Agriculture; climate change, epigenetics*

Abstract Number: 182

Effects of hemp aphid herbivory in industrial hemp

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Industrial hemp, *Cannabis sativa* L., is a genetically diverse plant that expresses distinct suits of chemical compounds of interest to humans for food, fiber, and medicine. In contrast to forms of *C. sativa* marketed for one specific plant defense compound, tetrahydrocannabinol (THC), industrial hemp has been intentionally bred to be low in THC since this compound is federally regulated in the United States of America. If industrial hemp crops exhibit a concentration of THC over 0.3%, the entire crop must be destroyed. However, little is known about what biotic and abiotic stressors can influence these compounds within the plant.

Insect pests have the potential to alter plant chemistry and transform plant performance through herbivory. In this system, it is possible that these interactions could render the crop unmarketable by increasing the level of the highly regulated THC compound. We evaluated how a specialist aphid, *Phorodon cannabis,* one of the most common pests in hemp crops in the United States, affects plant biomass, photosynthetic potential, and plant defenses. Laboratory experiments were conducted on hemp plants at both the vegetative stage and flowering stages where we compared plants that were either infested with aphids for two weeks or pest aphid-free. We evaluated photosynthetic potential (yield of photosystem II - YII), electron transport rate (ETR), dry weight, and population growth of aphids to assess plant performance and pest success. We also assessed the suite of cannabinoid compounds produced by plants in each treatment and performed phytohormone analyses to evaluate changes plant defenses. The YII and ETR values were not different between infested and non-infested plants at each phenological stage. However, in flowering plants, aphid infestation significantly reduced plant biomass even though the number of aphids was significantly lower compared to vegetative plants. These results indicate that aphid herbivory is not impacting the initial stage of photosynthesis, but aphids are impacting plant biomass – especially when aphids are introduced during the flowering stage. Cannabinoid content and plant defense induction could be contributing to this and the intersection of pest pressure, defensive chemistry, and plant phenological stage will be the foci of our future work.

Keywords: *Industrial hemp; aphids; plant defenses*

Abstract Number: 140

Maize benzoxazinoid exudation in soil follows a diurnal rhythm

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Root exudation of specialized metabolites is a dynamic process that shapes soil properties, plant nutrition, and plant interactions with soil organisms and other plants. While the pivotal roles of root exudates are increasingly acknowledged, the factors that modulate their release remain poorly understood. Here, we characterized the diurnal exudation of the maize specialized metabolites, benzoxazinoids (BXs). BX exudation increased over daytime and decreased during nighttime. The release of DIMBOA-Glc and of DIM2BOA-Glc respectively reached at 6.2 and 2 ug/cm 14 hours after sunrise and decreased to levels below 1.2 ug/cm at night. HDMBOA-Glc exudation rapidly increased to 1.5 ug/cm after sunrise and remained rather constant over daytime, before dropping 15-fold at night. To investigate whether these patterns were modulated by light or by the plant circadian rhythm, we exposed the plants to extended darkness for 12 hours. Preliminary data suggest that BX exudation is mostly modulated by light but should be further confirmed. Understanding exudate patterns and their regulation is critical to better grasp plant–environment interactions at the root–soil interface.

Keywords: *Root exudation; benzoxazinoids; root–soil interface*

SYMPOSIUM 15: CHEMICAL COMMUNICATION

Comingling odours: A preliminary study on background noises in volatile mediated plant signalling

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Plants being sessile, utilize a multitude of specialized metabolites that are essential for communicating with their biotic and abiotic environment. A major class of these metabolites are volatile organic compounds including floral scents emitted in very minute concentrations to attract mutualists or deter antagonists in order to ensure plant fitness. But this volatile-mediated signalling could be disrupted due to various anthropogenic activities resulting in elevated background noises in the smell-scape of the organism.

As part of our ongoing project on the ecology and evolution of floral volatiles in gingers, fieldwork was conducted in the North-East of India, a region facing constant anthropogenic threats. We incorporated the dynamic headspace sampling technique, one of the best methods for the extraction of plant volatiles in their true forms. The entire inflorescence was bagged and a chemical space was created within the bag so that only the plant volatiles will be concentrated. Ambient air acted as a control to check for environmental volatiles.

The study resulted in capturing synthetic chemical compounds which majorly form constituents of anthropogenic volatile emissions from vehicular fumes, paint thinners, soaps and perfumes from the plant headspace along with floral scent molecules. As the literature suggests, these compounds could act as info-disruptors hampering the plant communication system by degrading or transforming plant volatiles and changing floral visitor perception and behaviour, thereby leading to a fitness disadvantage.

Although multiple studies have investigated the impact of pollutants especially heavy metals on aquatic systems and plant–root–plant communication, the extent of air-borne pollutants on plant volatile signalling is still in its infancy and is the need of the hour for ecosystem functioning.

Keywords: *Anthropogenic threats; dynamic headspace sampling; floral scents*

Mating disruption of Pink bollworm, *Pectinophora gossypiella*, using tube-type dispensers in cotton

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The pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelichidae), is one of the most serious pests of cotton. This pest, native in Asia, is now widespread in many cotton-growing countries. The larvae of PBW enter the cotton bolls after hatching, damage seeds and fibers, and reduce cotton yield and quality. Insecticide application against PBW has been used; however, it is sometimes ineffective because pesticides are difficult to reach inside bolls where the pests exist, and due to insecticide resistance. Pheromone-based mating disruption (MD) to control this destructive pest is one of the promising alternative methods.

Female produced sex pheromone of PBW consists of a 1:1 mixture of the *Z,Z*- and *Z,E*-isomers of 7,11-hexadecadien-1-yl acetate, named gossyplure. We synthesize these sex pheromones using two C-8 key building blocks. Coupling reaction of these two C-8 building blocks enables an industrially scalable production method of both active ingredients.

Field MD trials using tube-type dispensers (PB-ROPE L, PB KNOT) have been conducted in several countries including India. The dispensers release both sex pheromones constantly into the field. MD efficacy was demonstrated through suppression of the male trap capture and reduction of crop damage. These results suggested that using this formulation could effectively control PBW and reduce the use of pesticides.

Keywords: *Pink bollworm; tube-type dispensers; mating disruption*

Abstract Number: 88

Search for determinants of pheromone-biosynthetic fatty acyl-CoA reductase specificity

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During the fatty acyl-derived pheromone biosynthesis, insects utilize various tailoring enzymes, including fatty acyl-CoA reductases (FARs, EC 1.2.1.84). The FARs reduce a broad spectrum of substrates, both saturated and unsaturated, to fatty alcohols which can be further converted to fatty aldehydes, acetates or hydrocarbons. The FARs either are very specific for a few substrates or accept a multitude of acyl chains. Taking the abundance and total number of insect species worldwide into account, the FARs have a great potential for their biotechnological application in pheromone-targeted pest management. Some of the sequence features important for enzymatic function have already been described for a few FARs. However, the structural basis of the specificity still poorly understood, mainly due to the complete lack of any experimental FAR structures.

In this project, we combine *in silico* inspection of FAR models predicted by an artificial intelligence (AlphaFold2) with functionally determined specificities reported in literature to indicate the possible structural determinants of enzyme function. In addition, using a model system consisting of pheromone-biosynthetic FARs from common bumble bees (*Bombus lapidarius*, *B. terrestris*) with distinct specificities, we integrate site-directed mutagenesis with reciprocal swapping, to uncover the sequence-based FAR specificity determinants in these enzymes.

The project is supported by RVO project 61388963.

Keywords: *Enzyme substrate prediction*

Courtship behavior of the Saharan sand viper, *Cerastes vipera*, is influenced by a chemical stimulus

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The Saharan sand viper, Cerastes vipera (Linnaeus, 1758), is distributed in all Saharan countries, being confined to sand and dune systems. In Israel, it inhabits the western Negev. This relatively small snake, up to 35 cm, is nocturnal, is active from spring to autumn (April to October) and hibernates during the winter (November to March).

The mating period is relatively short (two weeks) and takes place at the end of April or the beginning of May depending on the climatic conditions that year. In the days before mating, the individuals gather in the high areas of the vertebrae. Typical evidence of this event can be found in the sand.

Before mating, the male looks for the female. When the male recognizes an attractive female, he advances towards her while immediately changes the way he advances. He crosses the side winding trail of the female in a straight crawl contrary to his regular movement throughout the year (side winding). Tracks towards mating as described above, were observed only in the spring during 20 years of observations. Rarely, a male has been observed following the tracks of a female when his tongue (the organ of smell) touches both sides of the female's tracks and then crawls right up to her to mate.

The concentration of testosterone in the male's plasma is measured throughout the year and is found at a high concentration during the mating season in the spring as well as in the fall. During the summer, testosterone concentrations were low. Winter measurements were not conducted.

The findings and observations of the Saharan sand viper's behavior indicate the possibility of the existence of a sex pheromone secreted by the female. This year we will collect volatile substances from the sand tracks of females and males during the mating season in the spring and compare to volatile substances that will be collected in the summer from the sand tracks of both sexes. with the intention of finding compounds unique to females.

Keywords: Cerastes vipera*; behavior; female's tracks*

Abstract Number: 72

Assessing physiological parameters of *Eristalis tenax* across an elevational gradient

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Being ectotherms, insects rely on external abiotic cues to regulate their metabolism and other body functions. These abiotic cues change significantly across elevations in mountains like the Himalayas, thus potentially impacting the physiological parameters of these insects. This variation could also impact the insects' ability to sense and respond to food, mating or oviposition cues. Airborne olfactory cues are particularly impacted by changes in vapor pressure, temperature, and humidity, and these factors in turn could impact how insects detect odor cues. We therefore assessed two parameters of insect pollinators: a) the heart rate and b) the antennal detection and sensitivity towards odour cues from host flowers in their native habitats, as well as how these parameters vary across elevations. We standardised a portable dissection and electroantennography (pEAG) rig to perform these experiments directly *in situ*. We will present the results from these recordings performed on wild individuals of *Eristalis tenax* (Diptera: Syrphidae), a common generalist pollinator, across an elevational gradient in the Himalayas and also on culture-reared individuals in laboratory conditions in Bangalore. These results yield important baseline data of insect physiology and will help in understanding and mitigating the impacts of environmental change on plant-pollinator interactions.

Keywords: Eristalis tenax*; portable electrophysiology; heart rate*

SYMPOSIUM 16: INSECT CHEMICAL ECOLOGY

Abstract Number: 27

Exploring the olfactory neuroecology of *Drosophila busckii*

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Spatial and temporal niches are often co-occupied by multiple animal species. Among drosophilid flies, a number of species have evolved to be human commensals, thereby often dependent on fruits, fungi and vegetables cultivated by humans. Here, we studied the olfactory neuroecology of one of the comparatively less studied human commensals, *Drosophila busckii*, representing the subgenus *Drosophila*. In contrast to the fruit-eating majority, this species is known to utilize rotting vegetables as food and oviposition substrate, thereby gaining a unique niche among drosophilids living with humans. A common feature of all substrates attractive to *D. busckii* is that, when rotting, they emit dimethyl disulfide (DMDS). We found that *D. busckii* is attracted to and oviposit on vegetable and fungal substrates emitting short chain volatile oligosulfides. The flies are also attracted to DMDS itself. To establish the olfactory background to this attraction we screened olfactory sensilla on the antenna of *D. busckii* using single sensillum recording technique. Among the olfactory sensory neurons (OSNs) present in the sensilla, we identified a physiological type narrowly tuned to oligosulfides and specifically to DMDS. DMDS, however, is known for its insecticidal activity and quickly killed most drosophilid flies tested. *D. busckii*, however, has evolved to tolerate high levels of DMDS, likely allowing it to exploit other food sources. Taken together, these features constitute an evolutionary strategy allowing *D. busckii* to utilize unique resources not exploited by competing drosophilids.

Keywords: Drosophila busckii*; short chain oligosulfides; single sensillum recording technique*

Abstract Number: 34

Variation in chemical profiles and diversification of an alpine butterfly species complex

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Deciphering the factors driving reproductive isolation and speciation is central to our understanding of the build-up of divergence between lineages and the shaping of biodiversity patterns. Reproductive isolation may be based on intrinsic factors (e.g. genetic incompatibilities) or extrinsic factors (e.g. strong ecological divergence). Here we study the interplay of ecological divergence and hybridization in diversification in the *Coenonympha arcania/gardetta* species complex (*Coenonympha*, Nymphalidae: Satyrinae). In the French Alps, this complex is composed of three species, a widespread low elevation, wood edge species, (*C. arcania*), a high elevation grassland specialist species, (*C. gardetta*), and a species of hybrid origin found in intermediate habitats and elevations (*C. cephalidarwiniana*). In this complex, species-specific chemical traits are of high interest as they could be linked with adaptation to altitude (e.g. involvement of cuticular hydrocarbons against desiccation) and/or with reproductive isolation and speciation (e.g. pheromone-based mate choice) and therefore play an important role on its diversification. In the present study we will first describe the three species' chemical profiles (volatile and cuticular components) using GC-TOF-MS analysis, pointing out the components or their proportions discriminating species and sex. Second, we will compare the chemical profile of individuals of three possible contact zone modalities (*gardetta–arcania; gardetta–cephalidarwiniana; arcania–cephalidarwiniana*) in regard to known gene flow and therefore strength of reproductive isolation between each species pair.

Keywords: *Butterfly; chemical profiles; diversification*

Abstract Number: 95

Towards chemical ecology of *Tenebrio molitor* larvae and adults

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In a human's diet, entomophagy offers a nutritious, sustainable, and culturally significant alternative to traditional meat sources, and has the potential to play an important role in addressing global food security and environmental challenges. Mealworms (*Tenebrio molitor*) are easy to cultivate, high in protein, low in fat, rich in nutrients and suitable food source for humans. For insect breeders, it is important to know the biology and ecology of the insect, including chemical ecology. In the latter area, sex pheromones produced both by females and males, defensive substances and some repellent compounds have been studied. However, data on chemical ecology at larval stage, as well as many aspects of the issue at adult stage still remain uncovered.

To look for some natural chemicals which might regulate behaviour of mealworm was the aim of the present research. Essential oils (EO) as well as their most abundant components were the targets for repellent search suitable for mealworm larvae behaviour control. Six EOs were tested, those of: thymus (*Thymus vulgaris*), eucalyptus (*Eucalyptus globulus*), spearmint (*Mentha spicata*), lavandin (*Lavandula* x *hybrida*), lemongrass (*Cymbopogon flexousus*) and clove (*Eugenia caryophyllus*). Six most abundant components were also tested: 4-terpineol, carvone, limonene, myrcene, γ-terpinene and cis-sabinene hydrate.

The results revealed that the most effective repellent for mealworm larvae was EO of spearmint, moderate activity was shown by EO of clove and the least repellent were EOs of lemongrass, thymus, lavandin and especially of eucalyptus. The most repellent compounds were 4-terpineol and carvone.

Different substrates for female oviposition were tested looking for potential chemicals involved in oviposition regulation. The highest number of eggs was recorded on wheat and oat flour. Based on this finding, oat extract was used on sand to test an oviposition regulation response. The results indicated that females laid significantly more eggs in sand than in sand plus oat extract. Data on chemistry involved in egg laying regulation will be presented.

Keywords: *Mealworms; essential oils; oviposition*

Abstract Number: 157

Annotation and phylogenetic analysis of olfactory receptor genes in the two giant honey bees

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Chemoreception plays an important role in the life of honey bees. The remarkable abilities of honey bees to perceive diverse chemical signals and cues established them as excellent model systems to study olfactory communication and learning and memory. Six out of ten honey bee species occur in India among them the two giant honey bee species, *Apis dorsata* and *Apis laboriosa*. These two species are important pollinators in Asian ecosystems inhabiting different ecological niches. *A. dorsata* is widely distributed over the plains and plateaus of India whereas *A. laboriosa* is restricted to the mountainous regions of the Himalayas. Given the differences in the distribution pattern of the two closely-related species we were interested to know whether they show differences in the sets of olfactory receptor genes. As a manually curated list of ORs was not available for *A. laboriosa*, we annotated ORs by comparing RNA-seq based antennal transcriptome data with the published genome assembly from the NCBI database. Thus, we identified 162 functional and 19 pseudogenous OR genes in the genome of *A. laboriosa*. The number of functional olfactory receptor genes is similar to previously reported numbers in other *Apis* species, e.g., *A. mellifera* and *A. florea*. The majority of OR genes showed a 1:1 orthologous gene relation between *A. dorsata* and *A. laboriosa*. To validate potential functionality of the newly described ORs, we visualized the gene models along the RNA-seq tracks in the Apollo browser. Most of the ORs, including the 9-exon subfamily of putative cuticular hydrocarbons (CHC) receptors, were transcribed and supported by RNA-seq reads spanning predicted exon-intron boundaries This was followed by phylogenetic reconstruction of ORs from the two giant honey bees (*A. dorsata* and *A. laboriosa*), two cavity nesting honey bees (*A. mellifera* and *A. cerana*) and a dwarf honey bee (*A. florea*) where the ORs were organised into 21 clades/subfamilies. Our analysis revealed species-specific expansions of the CHC receptors and variation in specific OR subfamilies that might be related to local adaptations in the olfactory system of giant honey bees.

Keywords: *Asian honey bees; cuticular hydrocarbons; olfaction*

SYMPOSIUM 17: CHEMICAL ECOLOGY AND PEST CONTROL

Abstract Number: 92

Reverse chemical ecology in a moth: identification of new behaviorally-active semiochemicals

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Odorant receptors (ORs) are transmembrane proteins expressed in animal olfactory sensory neurons. They are at the core of odorant detection since they recognize odorants and trigger a neuronal response that will be transmitted to the central nervous system. However, most of these ORs are still orphans, which means the odorants that activate them are unknown. The so-called "reverse chemical ecology" or "molecular chemical ecology" approaches propose to use OR-ligand and/or OR-sequence characteristics to identify potential new ligands via a combination of modelling and experimentation, which have the potential to accelerate the discovery of new ligands. Using the crop pest moth *Spodoptera littoralis* (Lepidoptera; Noctuidae), we used such approaches for the study of insect ORs. Ligand-based virtual screening coupled to experimental validation led us to extend the range of semiochemicals active at the receptor and the behavioural levels.

Our work opens new routes for i) odorant receptor function analysis, ii) a better understanding of this species odor space, and iii) the development of novel insect pest control strategies targeting chemosensory receptors.

Keywords: *Odorant receptor; machine learning;* Spodoptera littoralis

Abstract Number: 110

Identification of salivary effector proteins from the generalist herbivore, *Spodoptera litura*

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Effector proteins make herbivores more virulent by suppressing plant defenses at multiple steps. However, no comprehensive study has been undertaken to identify effector proteins secreted from salivary glands of the chewing insect pest, *Spodoptera litura*. We have selected *S. litura* and *Arabidopsis thaliana* as model insect–plant interaction for our study. We hypothesized that generalist insect like *Spodoptera litura* are able to successfully attack their host plants by evading the plants' immune system and that effectors proteins, secreted by the insects into plant cells, are responsible for this ability. We further hypothesized that these effectors are responsible for attenuating plant defense by modifying or hijacking defense responsive genes at transcriptional level signaling complexes. We used a combination of next generation sequencing (NGS), functional genomics, proteomics and reverse genetics to identify and analyse effectors from *Spodoptera* and their targets in plants. Based on *de novo* transcriptome data in *S. litura*, 808 and 267 proteins from the head and salivary gland respectively were predicted as potential secreted protein with possible effector functions. We functionally characterized the role of two potential effector protein, Inducible metalloproteinase inhibitor protein-like (*Sl*-TIL) and cysteine proteinase like (*Sl*-CPL) from *S. litura*. *Sl-TIL* contains TIL domains (trypsin inhibitor like cysteine rich domains), which forms several disulphide bonds and keep the protein stable under adverse conditions. The *Sl-CPL* contains cathepsin propeptide inhibitor domain (I29) domains and cysteine-type peptidase activity. *Sl*-CPL functions to elicit the plant defense and does not suppress them as we predicted. *Sl*-TIL shows effector like activity in *Arabidopsis* as it increases *S. litura* feeding, when overexpressed in plants. Both the secreted proteins show diverse roles in host defense modulation and act on diverse targets in host plants.

Keywords: *Effector proteins; insect–plant interaction; plant immunity*

Cabbage aphids' response to chemical cues from conspecific and heterospecific infested plants

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Behavioural and electrophysiological studies on aphid responses to hostplant odours, although important, are still not exhaustive for some species. Moreover, most studies have not focused on whether these responses are mediated by odours from plants infested by conspecific and heterospecific individuals. Here, our goal was to identify the chemical cues involved in the attraction of the cabbage aphid (*Brevicoryne brassicae* L.) to cabbage plants. To achieve this, we collected volatiles emitted from uninfested cabbage plants, cabbage plants infested by either *B. brassicae* (conspecifics) or by diamondback moth (*Plutella xylostella*) larvae (heterospecifics), and from aphids alone. Choice tests revealed that *B. brassicae* were mostly attracted to odours from conspecific-infested cabbage plants; however, they also showed attraction to odours from uninfested plants and plants infested by *P. xylostella* larvae and were least attracted to odours from aphids alone. Using coupled Gas-chromatography-Electroantennographic detection studies (GC-EAD) and Gas chromatography-Mass spectrometry (GC-MS), we revealed some chemically active compounds that elicited *B. brassicae* olfactory response, with more of these compounds in the cabbage plant infested by conspecific than in the rest of the treatments. It is possible that the knowledge gained from this work could pave the way for synthetic lures as an integrated pest management approach for *B. brassicae*. Therefore, more studies would need to be conducted to validate and isolate those exact compounds that positively excited the cabbage aphids' olfactory response.

Keywords: *Olfactory response;* Brevicoryne brassicae*; volatile organic compounds*

Abstract Number: 214

Effect of sub-lethal dosage of mycotoxin, destruxin-A on *Helicoverpa armigera*: A transcriptome study

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Helicoverpa armigera is a voracious lepidopteran pest on a large number of crop plants, causing enormous losses to agricultural yield. In nature, the entomopathogenic fungus, *Metarhizium anisopliae*, infects *H. armigera* and is known to produce a wide array of insecticidal mycotoxins. Destruxin A is one such mycotoxin which has been well studied for its lethal effect on insect pests. In the present study, the effect of sub-lethal dosage of Destruxin A on this insect pest was studied at the transcriptome level. Although insecticidal, when *H. armigera* larvae are exposed to sub-lethal concentrations of this toxin, the larvae are able to detoxify the toxin in vivo and are able to recover from a tetanic paralysis within 4 hours of exposure. The transformed products, Destruxin A-diol, Destruxin E and Destruxin E-diol could be detected in the larvae treated with sub-lethal dosage of Destruxin A. The transcriptomic analysis showed the differential behavior of the gut and the rest of the body tissue to Destruxin A treatment.

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Keywords: *Destruxin;* Helicoverpa armigera*; sub-lethal dosage*

Abstract Number: 8

Studies on combination of para-pheromones and best lure type for fruit fly control

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The cucurbits belonging to the family Cucurbitaceae include more than 800 described species worldwide. Abiotic and biotic factors are responsible for yield reduction in cucurbits. Among the biotic factors, fruit flies belonging to the family of Tephritidae cause yield losses in the range of 30–100% in case of severe infestation. Several species of fruit flies have been reported to be associated with cucurbits in India. It is difficult to control them with insecticides as they are frugivores and symptoms are invisible until the fruits are rotten and fall off. Cue-Lure (4-(p-acetoxyphenyl)-2-butanone) and methyl eugenol (4-allyl-1,2-dimethoxybenzene) are para-pheromones used for monitoring and mass trapping of responsive fruit flies in India as well as in other countries. The lure mixture usually consists of the para-pheromone, ethyl alcohol and insecticide in the ratio of 6:4:1 respectively in which the para-pheromone concentration is around 55%. In order to ascertain the fruit flies infesting cucurbits, and the feasibility of combining para-pheromones and their effective concentration and lure types for monitoring and mass trapping of responsive fruit flies, experiments were conducted during 2020–2022. The results revealed that although several species of *Zeugodacus* and *Bactrocera* were reported in cucurbits only *Zeugodacus cucurbitae* and *Z. tau* actually infest cucurbits. The combining of Cue-Lure and methyl eugenol reduced the trapping efficiency of responsive fruit flies in comparison to individual lures of Cue-Lure and methyl eugenol. 25% concentration of Cue-Lure was found to be more efficient in trapping the maximum number of *Z. cucurbitae* than higher concentrations. The lure impregnated in wood or tablet formulation had almost similar trapping efficiency.

Keywords: *Fruit flies; para-pheromone; pest*

Exploration on mugwort-derived essential oil: A sustainable approach to mosquito vector management

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Dengue fever is the most common mosquito-borne disease, affecting a wide spectrum of the global population. *Aedes aegypti* (Diptera: Culicidae) is the vector of dengue fever, dengue hemorrhagic fever, chikungunya, and, most recently, Zika. Alternative approaches for better mosquito vector management are failing to control through chemical and synthetic insecticides. The use of plant-derived essential oil could be one of the alternative environmentally friendly forms of management. There is indigenous knowledge that the smoke from burning dried mugwort (*Artemisia*) leaves has been commonly used to repel and kill mosquito adults. In the present study, the aerial part of *Artemisia* sp. was collected from Ukhrul, Manipur and extracted essential oil (EO) by Clevenger apparatus was obtained. The oil is prepared in different dose concentrations of 1000,100, 50 and 10 ppm in acetone and tested on the mosquitoes (*Aedes aegypti*) by Y-tube olfactometry. The mosquitoes show repellency behavior with a response index of -0.6 on 100 ppm upon EO treatment. The response index is calculated using the number of insect responses to the test, control and the total number of insects used in the experiment. The chemical composition of the oil was analysed by GC-MS and the volatile organic compounds constituted in the oil were identified. Thus, the oil of mugwort can be a good source of repelling mosquitoes and might be a potentially sustainable approach to mosquito vector management. A detailed study of the oil and the insect chemical ecology will allow us to identify the chemical cues that can be used as bait to manipulate vector behaviours and would be a great help in the development of vector mosquito management.

Keywords: *Essential oil; mosquitoes; response index*

SYMPOSIUM 18: INTRA- AND INTERSPECIFIC CHEMICAL COMMUNICATION OF BARK BEETLES AS FOREST PESTS IN GLOBAL CHANGE

Abstract Number: 57

Early attack detection of infested trees on plant VOC emission

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The Eurasian Spruce Bark Beetle (*Ips typographus*, Coleoptera, Scolytinae) is a devastating pest of spruces in Eurasian forests. Various reasons, such as long-term drought and reduced defence mechanisms in stressed trees, have led to the development of bark beetle outbreaks. Functional prevention of the bark beetle calamities on spruce is the early detection of freshly infested trees based on personal observation by foresters. Then trees must be removed from the forest before the next bark beetle generation will emerge. Here, we present the development of an alternative early attack detection method based on changes in VOC emission by the freshly infested trees. We optimized the detection of VOC tree emission in the surrounding model logs in the laboratory by using either the headspace technique to sorb VOC to cartridges, or SPME fibre and analysing them by GC-FID. The proof-of-concept was conducted in the field by detecting the VOC emission of naturally-infested Norway spruces. Besides the measurement by analytical techniques, the VOC emissions were scanned by the non-specific sensor containing variable electronic chips – the electronic nose. From the quantity of chosen characteristic compounds (α-pinene, 2-methyl-3-buten-2-ol) the 3D VOC cloud was constructed. Optimization techniques on the model system will allow the measurement of a freshly infested tree in a real forest, with the perspective of being used for large-scale detection along with remote sensing methods.

Keywords: *Early attack detection; bark beetle management; VOCs*

Abstract Number: 100

Biochemical and physiologic changes in drought-affected *Picea abies* correlate with *Ips typographus* attacks

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Drought caused by climate change weakens the defence ability of European Norway spruce monocultures (*Picea abies* L.), making them vulnerable to bark beetle outbreaks, such as *Ips typographus* L. Therefore, this study aimed to determine the biochemical and physiological responses to drought stress in *P. abies* and the resultant vulnerability to *I. typographus*. In order to induce drought stress in *P. abies*, roofs were built to cover tree roots from rain, and then methyl jasmonate (MeJA) was applied to simulate the biotic attack. To identify tree responses, we measured the contents of non-structural carbohydrates (NSC) as key resources for secondary metabolites and nutrition, as well as phenolics and terpenes, which are essential defence metabolites. In addition, a host acceptance bioassay with individual male beetles was conducted in Eppendorf tubes attached to the trees. The results showed that trees in acute drought stress (5 months without water) were upregulated in the NSC content and downregulated in phenolics. The increase in the acceptance of males of *I. typographus* was also observed. After MeJA treatment, NSC concentrations decreased in trees, and the concentration of phenolics increased. We discuss the role of changes in NCS and defence compound production in acute drought-stressed trees to acceptance by *I. typographus*.

Keywords: Picea abies*; drought;* Ips typographus

Abstract Number: 149

Functional characterisation of an *Ips typographus* aggregation pheromone receptor and its paralogue

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The odorant receptors (ORs) present in the neurons of the antenna detect odour cues and initiate signal transduction in insects. Focusing on the ORs creates an opportunity to understand the communication system and evolution by understanding the function of ORs and ligand interactions, which might also lead to improved odour-based management. The spruce bark beetle *Ips typographus* is one of the most destructive pests of spruce forest in the Palearctic region. These beetles use odour cues to communicate and invade host trees. After choosing a suitable host, males release an aggregation pheromone consisting of 2-methyl-3-buten-2-ol and (4*S*)-*cis*-verbenol which is received by both the sexes to induce a mass attack. Hence, in this study we used 37 ecologically relevant compounds to reveal the *Ips typographus* OR response to its aggregation pheromone component (4*S*)-*cis*-verbenol and functionally characterise the paralogous OR of the aggregation pheromone receptor. It is the first time an OR responding to an aggregation pheromone component of its own species has been reported in any bark beetle. Using computational methods we predicted the OR–ligand interaction of the functionally characterised (4*S*)-*cis*-verbenol OR and its paralogous OR. Finally using site-directed mutagenesis variation in sensitivity to (4*S*)-*cis*-verbenol OR was observed by changing single predicted binding residues at different positions in the ligand binding pocket.

Keywords: Ips typographus*; odorant receptor (OR); OR–ligand interaction*

Functional evolution of odorant receptors in bark beetles

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Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a highly diverse subfamily of weevils, considered a natural part of forest ecosystems. However, the recurrent outbreaks reported in European forests classify them as serious forest pests. Pheromone communication is vital for bark beetle survival as they employ species-specific aggregation pheromones to attract conspecifics to their host trees for mating and resource exploitation. Within the Scolytinae, a clear phylogenetic pattern has been observed in pheromone compounds, as some components are more conserved than others. The differences in pheromone composition were found as great between closely related species compared to distantly related species indicating a rapid selection of certain pheromone components. We hypothesize rapid and heritable switches in the peripheral olfactory reception at the molecular level, *i.e.* in the structure, expression, and function of odorant receptors in closely related *Ips* species. To test this, we generated antennal transcriptomes from closely related species from Ips genus from the Czech Republic and identified the odorant receptors. Based on the expression and phylogeny, candidate receptors were selected and functionally characterized using *Drosophila melanogaster* transgenic expression system. We identified a new pheromone receptor and its orthologs that could better explain bark beetle survival. Our results contribute to the current knowledge on the functional evolution of olfaction in bark beetles, with future implications in developing olfaction-based pest control strategies.

Keywords: *Bark beetles; odorant receptors; evolution*

Abstract Number: 185

Bark beetle pheromones and anti-attractants produced by their associated symbiotic fungi

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Tree-killing bark beetles in conifer forests vector symbiotic fungi that are thought to help the beetles kill trees. Fungal symbionts emit diverse volatile blends that include bark beetle semiochemicals involved in mating and host localization. In one study over time, all 12 tested fungal isolates emitted beetle semiochemicals when growing in medium amended with linoleic acid. These semiochemicals included the spiroacetals chalcogran, *trans*-conophthorin and *exo*-brevicomin but also 2-methyl-3-buten-1-ol, the main aggregation pheromone component of the spruce bark beetle *Ips typographus.* The emission of these compounds was affected by the type and concentration of fatty acid present. Linoleic but not oleic acid promoted the synthesis of the spiroacetals. The authors of this study hypothesize that beetles probe the fatty acid composition of potential host trees to test their suitability for beetle development and release of semiochemicals by symbiotic fungi.

In another study the volatile emissions from isolates of the blue stain fungus *Endoconidiophora rufipennis* (ER) were collected by solid-phase microextraction (SPME) and analysed by gas chromatography- ass spectroscopy (GC-MS) over a period of 30 days.

Nine volatiles were emitted by ER in substantial amounts: isoamyl acetate, sulcatone, 2-phenethyl acetate, geranyl acetone, geranyl acetate, citronellyl acetate, (*R*)- and (*S*)-sulcatol, and (*R*)-sulcatol acetate. A late-peaking compound was geranyl acetone. In the field trapping experiment, three of the fungal volatiles (geranyl acetone, 2-phenethyl acetate and sulcatone) were tested in combination with a synthetic aggregation pheromone for *I. typographus*. Traps with geranyl acetone attracted lower numbers of *I. typographus* compared to traps with 2-phenethyl acetate, sulcatone or the pheromone alone as a control. The results showed that geranyl acetone acts as an anti-attractant and may act on *I. typographus* as a cue from an associated fungus to signal an overexploited host.

Keywords: *Tree-killing bark beetles; symbiont fungi; semiochemicals*

Abstract Number: 186

Linoleic acid promotes emission of bark beetle semiochemicals by fungal symbionts

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Tree-killing bark beetles in conifer forests vector symbiotic fungi that are thought to help the beetles kill trees. Fungal symbionts emit diverse volatile blends that include bark beetle semiochemicals involved in mating and host localization. In this study, all 12 tested fungal isolates emitted beetle semiochemicals when growing in medium amended with linoleic acid. These semiochemicals included the spiroacetals chalcogran, *trans*-conophthorin and *exo*-brevicomin but also 2-methyl-3-buten-1-ol, the main aggregation pheromone component of the spruce bark beetle *Ips typographus*. The emission of these compounds was affected by the type and concentration of fatty acid present. Accumulating evidence show that the fatty acid composition in conifer bark can facilitate colonization by bark beetles and symbiotic fungi, whereas the fatty acid composition of non-host trees can be detrimental for beetle larvae or fungi. We hypothesize that beetles probe the fatty acid composition of potential host trees to test their suitability for beetle development and release of semiochemicals by symbiotic fungi.

Keywords: *Tree-killing bark beetles; symbiont fungi; fatty acid composition*