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PLENARY LECTURES

ISCE Silver Medal

The other half of the story: How insects circumvent plant chemical defenses

Jonathan Gershenzon
Max Planck Institute for Chemical Ecology, Jena, Germany

Plants contain thousands of chemical compounds purported to be anti-herbivore defenses. Yet, in natural communities nearly every plant is attacked by some species of insect that feeds on it without apparent ill effects. In recent years, we have learned that the ability of insect herbivores to metabolize plant defense compounds is widespread. In fact, the chemical diversity of insect metabolites of plant defenses rivals the chemical diversity of the defenses themselves. Nevertheless, it is still unclear which metabolic processes are true detoxification reactions that benefit insects.

I will discuss some highlights of our recent work on insect detoxification of glucosinolates, the plant defenses responsible for the flavor of mustards and cabbages. These sulfur-rich defenses are activated on plant damage by glucose cleavage and rearrangement to poisonous products. Herbivorous insects deal with glucosinolate defenses in several different ways with varying consequences for their growth and survival. Glucosinolates can be detoxified by converting the parent protoxin to a non-hydrolyzable derivative, by diverting the hydrolysis reaction, or by waiting for the toxic hydrolysis products to form and then metabolizing them.

Under selection pressure, insect herbivores have developed many ways to circumvent the toxic arsenal of their host plants. More knowledge of these adaptations will increase our understanding of what plant defenses are most effective, what the limits are to an insect’s host range, and which insects are likely to become pests on a plant, as well as leading to new approaches to controlling pest insects in agriculture and forestry by targeting their detoxification systems.

Wittko Francke’s Daaks-Chemicals Memorial Lecture

Jerry Meinwald: Food, family, flute, and pheromones

Walter S. Leal
Department of Molecular and Cellular Biology, University of California, Davis, USA

I am absolutely delighted to have the opportunity to memorialize one of the Founding Fathers of chemical ecology – Jerrold (Jerry) Meinwald (January 16, 1927 – April 23, 2018). I will start with a brief introduction to Wittko Francke’s Daaks-Chemicals Memorial Lecture. Hopefully, everyone attending this lecture, including Jerry’s closest friends and family, will learn something new about Jerry. More importantly, I hope Jerry’s story will inspire the latest generation of chemical ecologists as much as he inspired many of us. Jerry was a “Master of Understatement” who “Touched so many lives.” What did he say when he received the National Medal of Science from then-President Barack Obama? Through Tom Eisner (June 25, 1929 – March 25, 2011), Jerry – a skilled natural product chemist – found the chemical defense mechanisms of arthropods a fascinating field, entirely ignored by his contemporary chemists. They presented their first chemical ecology paper at the 11th International Congress of Entomology in Vienna (1960) and published 132 papers in chemical ecology. In one particular year (1966), they published as many as five papers in Science. As a member of the Editorial Board of the Proceedings of the National Academy of Science, Jerry handled 651 manuscripts, many of them in chemical ecology. As his long-term collaborator Athula Attygalle said, Jerry was “an inspiring scientist and a true gentleman.” By the end of the presentation, those who did not know Jerry will fully agree with Athula, like us, who had the privilege of knowing Jerry, do. Lastly, we shall answer Cam Oehlschlager’s question to Jerry.

ISCE Silverstein-Simeone Award

Discovering the sex life of moths as driving force for inspiring collaborations

Astrid T. Groot
Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, the Netherlands

Sexual attraction plays a central role in speciation and biodiversity, as this determines the level of gene flow between populations. Moths are classical models for studying the evolution of sexual attraction through sex pheromones. Through a combination of genetic analyses and behavioral lab and field experiments, recently combined with theoretical modelling as well, we have investigated the causes and consequences of variation in sexual attraction that may lead to population divergence. In this presentation, I will highlight the most unexpected discoveries that we found in our analyses between species, within species, between populations and within populations. Some of these discoveries took > 20 years and all results were joint efforts of not only several people but also several labs. For me, these international, interdisciplinary and collaborative efforts are the driving force that makes research so inspiring. Therefore, I dedicate the Silverstein-Simeone award to all collaborators.

Invited Plenary Lecture

Scent-sitive families: Unraveling the complexity of interactions among family members

Sandra Steiger
Evolutionary Animal Ecology, University of Bayreuth, Germany

Social life is a highly diverse phenomenon and comes in many forms and facets. A fundamental social core unit of immense importance is the family, the association of one or two parents with their offspring. Temporary family living is widespread across the animal kingdom, occurring not only in vertebrates but also in a wide range of invertebrates. Family life is a cooperative event but is also full of conflict, and the evolution of communication processes has been considered essential for the integrity of the family unit and the coordination of interactions among family members. Despite chemical communication being prevalent among animals, our understanding of the evolution of social pheromones within a family context remains very limited. The contrast between the extensive research available in eusocial insects and the limited knowledge about chemical communication in "subsocial" species is remarkable. In my talk, I will journey into family interactions in subsocial species and show the fascinating complexity of these interactions. I will demonstrate how chemical communication can stabilize family life from the perspectives of (1) the entire family, (2) the father, (3) the mother, and (4) the offspring. I will show that socially induced reproductive "control" is not only a core feature of eusocial societies but plays a fundamental role in simple families. Moreover, our data suggest that offspring chemical signals are of high importance in the regulation of family life.

Early Career Award

Sterility-inducing mechanisms in social insects

Etya Amsalem
Department of Entomology, Center for Chemical Ecology, Center for Pollinator Research, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA 16802, USA

A key characteristic of sociality is the reproductive division of labor between fertile and sterile females. Reproductive conflicts within social insect colonies are often mediated by pheromones, which convey information about reproductive status or manipulate the reproductive capabilities of others. However, our understanding of the structure, function, diversity, and evolution of these reproductive signals remains limited. Pheromones regulating reproduction have been identified in only a few species, and the roles of non-queen players in regulating worker reproduction are frequently overlooked. Additionally, the scarcity of mechanistic and comparative studies of reproductive signals across insect species has hindered progress in this field.

Investigating ultimate and proximate questions related to reproductive signals in bumble bees reveals the structural diversity of these signals and their producers, as well as the critical role of social context in their function. Mechanistic studies of pheromone biosynthesis, worker brain responses to reproductive signals, and the role of learning in responding to queen pheromones further elucidate the modes of action of these signals and offer insights into their evolutionary pathways. Overall, my talk will provide an overview of reproductive signals in social insects, highlight the barriers faced in this research area, and discuss the insights gained from recent studies.

Applied Chemical Ecology Award

A voyage in search of King Solomon's ring

Miklós Tóth
Plant Protection Institute, Hungarian Academy of Science, Hungary

As long as I can remember I have always loved insects. As a teenage boy I was a keen collector of beetles, and at that time I frequently thought about how nice it would be to obtain King Solomon's Magic Ring, to understand what they talk about, and perhaps to deceive rare specimens by attracting them and putting them into my collection. A chance to start my journey in search of the ring opened up when in the middle of the seventies I joined the Plant Protection Institute, and as a young scientist I was put on the study of pheromones. In this presentation I will show you the most interesting cases I came across in investigating pheromones, other semiochemicals, and developing traps and other devices for agriculture, in moths, beetles and lacewings. As my journey approaches to its end, I must say that I enjoyed every minute of it. I wish the same to the youngsters just embarking upon a similar journey.

S1: Insect host-choice: Placing chemosensory genes in an ecological and evolutionary context

Chairs: *Sonja Bisch-Knaden, Alexander Haverkamp*

ORAL PRESENTATIONS

Attraction and aversion of noctuid moths to fermented food sources mediated by distinct olfactory receptors

Xiao-Qing Hou[1], Dan-Dan Zhang[3], Hanbo Zhao[1], Yang Liu[2], Christer Löfstedt[3], Guirong Wang[1,2*]
[1]Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Synthetic Biology Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China
[2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
[3]Department of Biology, Lund University, Lund, Sweden

Moths intake nutrients before and during mating, and hence gain survival and reproduction benefits. As a major food source for moths in times of nectar scarcity, fermented sugary substrate is more attractive to noctuid moths than moths from other families in field traps, although the molecular and genetic mechanisms underlying the difference remain unexplored. The release of volatiles is dynamic during fermentation and it is still unclear how this would affect the feeding preference of moths. Here, we identified eight compounds abundant in the dynamic volatile profiles of several sugary substrates during yeast fermentation. We showed that the attraction and aversion of cotton bollworm moths (*Helicoverpa armigera*) to the fermentation cultures at early and later stages were respectively mediated by isoamyl alcohol and octanoic acid. We deorphanized the olfactory receptors detecting these two compounds and found that they belonged to two distinct gene families and were functionally conserved across four noctuid subfamilies; HarmOR52 orthologues responding to the attractive isoamyl alcohol produced at early fermentation stage and HarmIR75q.1 orthologues responding to the aversive octanoic acid produced at later stage. We suggest that this functional conservation is an olfactory adaptation that has allowed noctuid moths to extend their diet to fermented food sources.

Identification of novel toxin resistance genes in *Drosophila* via Experimental Evolution, GWAS, and CRISPR screening

Michele Marconcini[1], Steeve Cruchet[1], Raghuvir Viswanatha[2], Srishti Goswami[2], Stephanie E. Mohr[2], Richard Benton[1]
[1]*Center for Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland*
[2]*Department of Genetics, Harvard Medical School, Boston, USA*

Insecticide resistance is a widespread challenge for the management of vectors transmitting pathogens and agricultural pests, requiring a better understanding of the genetic mechanisms underlying evolution of resistance. *Drosophila sechellia* is a compelling model for such studies as it naturally evolved resistance to octanoic acid (OA), an abundant chemical of its noni fruit host that is toxic for other insects, including close relatives *D. simulans* and *D. melanogaster.* We have used a multi-pronged strategy to identify genes contributing to OA resistance. We began by experimentally-evolving *D. simulans* strains with higher tolerance to OA, and determined the resulting genetic architecture. To identify specific candidate genes, we integrated this analysis with a genome wide association study of OA resistance in *D. simulans* as well as a genome-wide CRISPR selection screen upon OA exposure in *D. melanogaster* S2R+ cultured cells. We identified four candidates, with diverse predicted molecular and expression properties, and validated their relevance using loss-of-function analysis in *D. melanogaster*. Two of these genes displayed an increased expression in the experimentally-evolved strains, paralleling their higher levels of expression in *D. sechellia* compared to other drosophilids; transgene-mediate over-expression of one of these in *D. melanogaster* can promote *D. sechellia*-like levels of OA resistance. Our results hint at an adaptive role of these genes in shaping OA resistance both under laboratory conditions and during *D. sechellia*'s evolutionary history. This study emphasizes the power of integration of multiple genetic approaches to identify genes and cellular mechanisms underlying toxin resistance in insects.

Life-stage dependent behavior mimics chemosensory repertoire diversity in a belowground, specialist herbivore

Kyle J. Paddock, Jacob A. Corcoran
Biological Control of Insects, United States Department of Agriculture – Agricultural Research Service, Columbia, Missouri, USA

Insects rely on their chemosensory system to detect and translate environmental stimuli into relevant behaviors. The interface between the insect chemosensory system and the environment is populated by odorant (OR) and gustatory (GR) receptors. Over the course of development, life strategies change, and an insect may rely more heavily on specific chemosensory receptors. How receptor expression profiles change in combination with changing life strategies is not well understood. Using genomic and transcriptomic resources we annotated the OR and GR expression profiles across all life stages of the western corn rootworm (WCR), a major subterranean pest of corn in the US and Europe. Genomic analyses identified 193 ORs and 189 GRs, of which 125 and 116 were found to be expressed, respectively, in one or more WCR life stages. Expression profile analyses revealed first instar larvae possess a unique OR and GR repertoire distinct from other instars and adults, suggesting a role in host plant recognition. Odorant receptors of interest were then functionally characterized in mammalian cell lines, with various ORs responding to volatiles produced by corn roots, flowers, and the WCR sex pheromone. We then used RNAi to alter the expression levels of ORs tuned to important volatiles to disrupt WCR attraction or repellency in behavioral studies. These data highlight ORs with critical roles in WCR biology and serve as a proof of principle for modulating WCR behavior through interference of chemosensory gene expression levels.

The ecological significance of odorant receptors in *Pieris brassicae*

Qi Wang[1,2], Marcel Dicke[1], Dan-Dan Zhang[2], Alexander Haverkamp[1]
[1]Laboratory of Entomology, Wageningen University & Research, Wageningen, the Netherlands
[2]Department of Biology, Lund University, Lund, Sweden

Pieris brassicae is a widespread pest species and causes much economic loss. The olfactory-mediated tritrophic interaction among *P. brassicae*, cabbage plants and parasitoids has been extensively investigated, although the molecular mechanisms underlying this interaction have remained largely underexplored. Here we employed CRISPR/Cas9 to knock out the olfactory co-receptor *Orco*. Our findings revealed that the *Orco* knockout (KO) butterflies exhibited a complete loss of olfactory responses to most tested volatiles. Consequently, the majority of these KO butterflies failed to locate their mates and lay fertilized eggs, although a small subset of them can still manage to mate and reproduce. Furthermore, compared to wildtype (WT) caterpillars, *Orco* KO caterpillars displayed increased susceptibility to natural enemies *Cotesia glomerata* and decreased sensitivity to differently treated plants. To further explore the role of odorant receptors in tritrophic interactions, we knocked out another odorant receptor *OR45b* which is highly expressed in female antennae. We found that female KO butterflies laid more eggs on plants than wildtypes. Additionally, KO butterflies spent much less time than WT to make a choice between egg-infested and uninfested plants, independent of the plant they selected. Remarkably, gravid KO females were more attractive to egg parasitoids than WT females. These findings highlight the critical roles of olfactory receptors in host-plant selection, mate finding, and subsequent impacts on oviposition and interactions with natural enemies.

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Searching for honey bee odorant receptors tuned to floral scents: Opening the way to an understanding of how bees' olfactory system adapted to find floral resources

Arthur de Fouchier[1], Benjamin Andreu[2], Laura Pellicier[1], Marie-Faivre[1], Ludvine Brajon[1], Julie Carcaud[2], Emmanuelle Jacquin-Joly[1], Jean-Christophe Sandoz[2], Nicolas Montagné[1]
[1]Institute of Ecology and Environmental Sciences of Paris, Sorbonne Université, INRAE, Université Paris Cité, CNRS, IRD, UPEC, Versailles, France
[2]Evolution, Genomes, Behaviour and Ecology, CNRS, IRD, Université Paris-Saclay, Gif-sur-Yvette, France

Bees are major pollinators. They provide this essential ecosystem service to plants in exchange for nectar and pollen resources. Helping bees locate floral resources could help mitigate the current decline of pollinators. Bees locate suitable flowers in their environment using floral signals, including floral scents. To recognize profitable food sources among the diversity of available flowers, they need to detect efficiently both the presence and the relative abundances of relevant odorants in the complex floral bouquet. However, current knowledge of how olfactory cues drive the behavior of bees in the wild remains scarce. To detect odorants, insects use a range of odorant receptors (ORs) expressed in the olfactory sensory neurons of their antennae. The study of these receptors in multiple insect species has shown that phylogenetically closely related ORs seem to detect odorants of the same types or from similar sources. However, current knowledge on the function of bees OR is limited to only a handful of receptors. In this work, we explored the detection of floral odors by honeybee ORs with varying degrees of relatedness. We tested the response spectra of this selection of candidate ORs expressed in *Drosophila* to a panel of flowering plant odors from natural sources. We identified receptors detecting floral volatiles and showed that deorphanization of plant volatile-tuned ORs in honey bees is possible using complex odors. Further evolutionary analyses gave us insight into how the OR repertoires of bees adapted to detect floral scents.

Functional evolution of pheromone receptors in bark beetles

Twinkle Biswas[1,2], Cassie Sims[1,2], Jothi Kumar Yuvaraj[1], Rebecca E. Roberts[1], Christer Löfstedt[1,2], Martin N. Andersson[1,2]
[1] *Department of Biology, Lund University, Lund, Sweden*
[2] *Max Planck center next Generation Insect Chemical Ecology (nGICE), Department of Biology, Lund University, Lund, Sweden*

Aggressive conifer-feeding bark beetles (Coleoptera, Curculionidae, Scolytinae) can quickly destroy large areas of healthy forest. Successful host tree colonization is mediated by aggregation pheromones that rapidly attract large numbers of conspecifics to the tree, overwhelming its defenses. The Eurasian spruce bark beetle *Ips typographus* L. is the most devastating pest of Norway spruce, *Picea abies* (L.) Karst., in Europe. Its male-produced aggregation pheromone is comprised of (4*S*)-*cis*-verbenol and 2-methyl-3-buten-2-ol. Insect pheromone compounds are detected by specialized members of the insect odorant receptor (OR) family; however, only few pheromone receptors have been identified in bark beetles (and in other coleopterans). Here, we functionally characterized an OR in *I. typographus* that responds strongly to (4*S*)-*cis*-verbenol, and much weaker to three structurally similar compounds. This receptor is phylogenetically well separated from all previously characterized pheromone receptors in *I. typographus*, responding to ipsdienol, ipsenol, *E*-myrcenol, or lanierone, respectively, which are aggregation pheromone components in other *Ips* species. The scattered phylogenetic positions of these receptors suggests that pheromone-detecting functions have originated on several independent occasions during the evolution of the bark beetle OR family. Finally, we used computational predictions and experimental validation to reveal two amino acid residues in the characterized receptor that most likely are directly involved in pheromone binding. Altogether, the results of this study shed new light on the response specificity, evolutionary origins, and binding mechanisms of bark beetle pheromone receptors important for host colonization.

Evolution of receptors for plant odours and pheromones in phytophagous insects

Rémi Capoduro[1], Arthur de Fouchier[1], Ludvine Brajon[1], Arthur Comte[1], Gabriela Caballero-Vidal[1], Binu Antony[2], Emmanuelle Jacquin-Joly[1], Nicolas Montagné[1]
[1]Institute of Ecology and Environmental Sciences, INRAE & Sorbonne University, Paris, France
[2]Department of Plant Protection, King Saoud University, Riyad, Saudi Arabia

The evolution of odorant receptors is crucial for the adaptation of insects to their environment. In our laboratory, we are particularly interested in understanding how receptors for plant odours and pheromones have evolved in phytophagous insects. Using a combination of genomics, molecular biology and heterologous expression methods, we have characterised the response spectra of a large number of receptors in the moth *Spodoptera littoralis* and identified the role of some of these receptors in adult and caterpillar behaviour. Using these results and those of other research groups, we aim to reconstruct the evolutionary history of odorant receptors within the order Lepidoptera, and in particular to understand when and how receptors specific for sex pheromones appeared. More recently, work on palm weevils has led us to identify a close link between receptors that detect the odours of host plants and receptors for aggregation pheromones.

A gustatory receptor gene involved in host selection of Swallowtail butterflies

Katsuhisa Ozaki[1], Masasuke Ryuda[2], Juri Nishioka[3], Frédéric Marion-Poll[4], Teiichi Tanimura[5], Hiroshi Yoshikawa[1]
[1]JT Biohistory Research Hall, Osaka, Japan
[2]Analytical Research Center for Experimental Sciences, Saga University, Saga, Japan
[3]Graduate school of science, Osaka University, Osaka, Japan
[4]Physiologie de l'Insecte: Signalisation et Communication, INRA/UPMC, Versailles, France
[5]Graduate school of science, Kyushu University, Fukuoka, Japan

Most herbivorous insects use specific plants as host. Larvae immediately after hatching from eggs have small bodies and poor mobility. It is difficult for larvae to search for food on their own from a wide area. Unlike larvae, adults can fly and have a high ability to moving around. Female adult swallowtail butterflies do not eat plant leaves, but they accurately identify hosts for larvae and lay eggs on larval food. When female adults identify plants, the most important clue is the sense of taste they feel with their fore legs.

Phytochemical substances called "oviposition stimulants" have been identified from host plant for *Papilio xuthus* by previous work. By appropriately blending those, the solution is possible to induce egg laying behavior of female adults at the almost same frequency as their hosts. We have identified a gustatory receptor gene (*GR*) that specifically recognizes synephrine, which is one of stimulants.

We expressed the *GR* ectopically in cultured insect cells derived from tissues unrelated to taste function and stimulated them with oviposition stimulants. As a result, an increase in intracellular calcium concentration was observed only with synephrine.

We conducted oviposition experiments by free flight using female adults whose expression of the *GR* was inhibited by RNAi. As a result, when exposed to a chemical substance solution containing synephrine, the egg laying frequency of female adults decreased significantly.

We believe that in swallowtail butterflies, *GRs* expressed in the forelegs of female adults are involved in the identification and selection of host plants.

Identification and characterization of *Diatraea saccharalis* odorant receptor involved in the plant-insect-fungus interaction

Flávia P. Franco[1,2], Pingxi Xu[2], Felipe Ten Caten[3], Felipe G. Gonçalves[1], Emile Goutorbe[1], Marcio C. Silva-Filho[4], José Maurício S. Bento[1], Walter S. Leal[2]
[1]Departamento de Entomologia e Acarologia, ESALQ-USP, Piracicaba, SP, Brazil
[2]Department of Molecular and Cellular Biology, UC-Davis, CA, USA
[3]Centro de Inovação – INOVA USP, São Paulo, SP – Brazil
[4]Departamento de Genética, ESALQ-USP, Piracicaba, SP, Brazil

Plant volatile organic compounds (VOCs) play a crucial role in plant defense mechanisms against herbivores and in their communication with other organisms, including microorganisms and predators. Changes in plant VOCs emission due to microbial interactions can have significant consequences for plant-insect interactions, affecting the olfactory preferences of insects. Studies have shown that *Fusarium verticilliodes* can manipulate the sugarcane host plant VOCs emission and the sugarcane borer behavior, attracting caterpillars to feed on a contaminated diet and guiding females to oviposit in *F. verticillioides*-infected plants. Many studies have shown the expression and activity of odorant receptors (ORs) in insects; however, there is no information about *Diatraea saccharalis* ORs. Thus, the goal of this study was to identify and characterize *D. saccharalis* ORs that might mediate the plant-insect-pathogen interactions. We used next-generation sequencing to identify *D. saccharalis* ORs and the two-electrode voltage clamp technique to identify OR ligands. In this work, 14 gustatory receptors, 11 odorant receptors, 32 odor-binding proteins, 23 odor-degrading enzymes, 37 ionotropic receptors, and 3 sensory neuron membrane protein sequences were identified. The OR DsacOR26 in association with its co-receptor DsacOrco, showed to be highly sensitive to geranyl acetone and sulcatone. Geranyl acetone is released by *F. verticillioides* and sugarcane; however, its levels are increased in *F. verticillioides*-infected plants, while sulcatone is released by *D. saccharalis*-attacked plants alone or in association with the fungus. Both odorants affected insect behavior at different concentrations, indicating a role in plant-insect-fungus interaction and potential use in pest management.

Exploring the *Spodoptera exigua* caterpillar olfactory system

Ana Carrión-Moreno[1], **Daniel Pinos**[1], **Antonio Torres-Méndez**[2], **Lucía Prieto-Godino**[2], **María Cristina Crava**[1]
[1]*Biotechnological Pest Control Laboratory, Institute of Biotechnology and Biomedicine (BIOTECMED), Universitat de València, Burjassot (Valencia), Spain*
[2]*The Francis Crick Institute, London, UK*

Spodoptera exigua is a lepidopteran pest found worldwide, feeding on various crops and weeds during its caterpillar stage. Larvae rely on their olfaction for essential behaviours like detecting feeding substrates, identifying microorganism contamination, and avoiding predators. However, compared to adults, the larval olfactory system remains less characterized. Here, we used scanning electron microscopy to identify sensilla in the antennae and maxillary palps of *S. exigua* larvae potentially involved in olfaction. We found nine sensilla within the second and third antennal segments, four of which display the porous surface typical of olfactory sensilla. Maxillary palps also harbour eight sensilla, classified into five gustatory-types and three olfactory-types based on external shape. We further mapped the olfactory sensory neurons housed in each sensillum by Fluorescence in situ hybridization (FISH), using probes specific to the odorant receptor (OR) co-receptor (Orco) and the ionotropic receptor (IR) co-receptors IR8a and IR25a. Results indicate *Orco* and *IR8a* expression in antennal sensilla, supporting both IR- and OR-based olfactory modalities in this appendage. In contrast, only *Orco* was expressed in maxillary palp sensilla. Lastly, we have used the CRISPR-Cas9 system to generate an *Orco* gene knockout and address the importance of the OR-based olfaction in *S. exigua* larval olfactory-driven behaviour. Overall, our study offers valuable insights into *S. exigua*'s olfactory system, aiding further research into its ecology and the design of new olfactory-based control strategies.

Exploring gustatory genes involved in the herbivory in zoophytophagous stinkbug, *Nesidiocoris tenuis*

Takuya Uehara[1], Kaoru Saeki[1], Tomofumi Shibata[1,2], Ritsuko Murakami[3], Tomokazu Seko[3], Tomoya Katsuno[3], Aki Sagisaka[3], Masami Shimoda[2]
[1]Institute of Agrobiological Sciences, NARO, Japan
[2]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan
[3]Institute for Plant Protection, NARO, Japan

Nesidiocoris tenuis plays a critical role in managing micro-pests in tomato cultivation. This bug exhibits zoophytophagous nature, blending carnivorous and herbivorous food habits. This enables it to maintain a stable population even during periods of low pest density by utilizing companion plants until a pest outbreak occurs. However, excessive populations can cause these bugs to consume the crops themselves, inflicting damage. This behavior likely stems from their food preferences and chemosensory abilities.

To understand the genetic foundation of their food habits, we constructed a chromosomal-scale genome for *N. tenuis* and conducted a comparative analysis of chemosensory receptor genes among different stinkbug species. This suggested that gustatory receptor (GR) genes significantly influence their herbivorous behavior. We compared GR expression levels in phytophagous and zoophytophagous stinkbugs, identifying 13 candidates associated with plant-eating behavior.

We further investigated the functions of these genes using RNA interference. After administering double-stranded RNA for two days, we assessed changes in plant and egg-eating behaviors. Herbivory was measured by the number of stylet piercings on agar-based tomato media, and carnivory by the number of consumed eggs. Our experiments showed that specific GR gene knockdown led to reduced herbivory, but carnivorous behavior remained unchanged. Testing the impact on crop damage, we released GR-knockdown individuals into a greenhouse, observing a reduction in crop damage with no adverse effects on their survival rates.

These insights underline the potential of these genes for innovative breeding strategies for *N. tenuis* and shed light on the mechanisms behind sensory adaptation to food habits.

Deciphering the function of the most highly expressed odorant receptor in *Ips typhographus*

Jothi Kumar Yuvaraj[1], **Dineshkumar Kandasamy**[1], **Rebecca E. Roberts**[1], **Bill S. Hansson**[2], **Jonathan Gershenzon**[3], **Martin N. Andersson**[1]
[1]*Department of Biology, Lund University, SE-223 62 Lund, Sweden*
[2]*Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Jena, Germany*
[3]*Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany*

Insects rely on a diverse family of odorant receptors (ORs) to detect odors, enabling them to adapt to various ecological niches. Unravelling the function of ORs is important for understanding the sensory adaptations and chemical ecologies of insects. Such studies have in the past mainly targeted the ORs of species within Diptera and Lepidoptera, while Coleoptera, for instance, has received very little attention. We have used reverse chemical ecology approach and functionally characterized most highly expressed OR from the bark beetle *Ips typographus* using HEK293 cells. Then, we investigated the antennal distribution of this OR using single sensillum recordings (SSR) and *in situ* hybridization, followed by field- and laboratory experiments to evaluate the behavioural effect of the identified ligand. The most highly expressed ORs of *I. typographus* tuned exclusively to the monoterpene-derived ketone lanierone with an unknown ecological origin. In addition, the lanierone responding OSNs were co-localized with seven different previously described OSN classes, suggesting a promiscuous OSN co-localization principle in bark beetles.

POSTERS

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The degree of host specialization shapes the morphological and molecular organization of the sensory organs of Tephritidae fruit flies

Vincent Jacob[1], **Emma Persyn**[1,2,3], **Emmanuelle Jacquin-Joly**[2]
[1]UMR PVBMT, CIRAD, Saint-Pierre, La Réunion, France
[2]Institute of Ecology & Environmental Sciences of Paris, INRAE, Versailles, France
[3]UMR PVBMT, Université de la Réunion, Saint-Pierre, La Réunion, France

Finding a suitable oviposition site is a fundamental requirement for phytophagous insects, which calls on the insect's multiple sensory modalities, including vision and olfaction. To what extent host adaptation constrained the diversification of sensory organ morphology and of chemosensory proteins? The question is particularly tricky for polyphagous species, whose choice of host relies on multiple sensory cues. Fruit flies of the family Tephritidae (Diptera) not only encompass several significant agricultural pests but also exhibit a wide range of host preferences, stemming from multiple host shifts back and forth throughout their evolutionary history. We studied the morphology of head sensory organs on seventeen Tephritidae species from three tribes, and the odorant receptors (ORs) of thirteen species from two tribes, using transcriptomic analysis of antennae and maxillary palps. The dataset included species with similar and/or contrasted host range at various phylogenetic distance, including generalist fruit consumers and species specialized on a plant family. Independently of phylogeny, we found that generalist species have relatively bigger eyes ($F(8,37)= 4,8$, $p<0,001$), and a better visual sensitivity, compared with specialist species. We also found that the OR repertoire is significantly more conserved among generalist than among specialist species, in term of number of orthologous ORs ($F(1,9)=9$, $p=0.015$), OR expression level ($F(1,9)=5$, $p=0.05$), or for 10 ORs in term of proportion of amino-acid substitution (linear model, $p<0.05$). Overall, our observations reveal sensory characteristics linked to polyphagous diets, offering insights into the ecological drivers of sensory organ evolution across a broader phylogenetic spectrum than typically studied.

P-31

Natural polymorphisms altering ligand selectivity in a bark beetle pheromone receptor

Jibin Johny[1]*, Souleymane Diallo[1], Krystyna Nadachowska-Brzyska[2], Amit Roy[1], Blanka Kalinová[1], Ewald Große-Wilde[1], Fredrik Schlyter[1,3]
[1]Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Suchdol 16500, Czech Republic
[2]Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, 30-387, Kraków, Poland
[3]Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, 234 22 LOMMA, Sweden

Variations are critical to the survival of a species in ecological contests. Natural variations in the odorant receptors are well known, but such variations within an insect species and their effect on olfactory communication are understudied, especially in non-model organisms. The current study identified natural polymorphisms within an *Ips typographus* odorant receptor gene (ItypOR-X) distributed with similar frequency across European populations. We explored the functional significance of such variations by deorphanizing ItypOR-X and its variant ItypORXa using *Drosophila* empty-neuron system (DNS). We found that ItypORX was tuned to amitinol, a heterospecific pheromone component. In contrast, its variant was tuned to (*S*)-(–)-ipsenol, a conspecific pheromone component of *I. typographus,* indicating natural polymorphisms altering the ligand selectivity. The putative ligand binding sites identified by *in silico* approaches also support our finding. Our results provide additional evidence for the known functional divergence of odorant receptors in *Ips typographus.* As the Eurasian spruce bark beetle *Ips typographus*, is a significant threat to Norway spruce forests in Europe, and olfaction-based pest control strategies are being considered as a practical approach, our results highlight long-term complexities associated with managing these pests in forests.

P-32

Putative role of antenna-enriched sensory neuron membrane protein RferSNMPu1 in Asian Palm Weevil pheromone detection

Jibin Johny[1,2]*, **Mohammed Ali AlSaleh**[1], **Binu Antony**[1]
[1]*King Saud University, Center for Chemical Ecology and Functional Genomics, Chair of Date Palm Research, College of Food and Agricultural Sciences, Riyadh, Saudi Arabia*
[2]*Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic*

The red palm weevil (RPW) or Asian palm weevil, *Rhynchophorus ferrugineus* (Olivier), is an invasive pest of palm trees around the globe. As pheromone communication is crucial for their mass attack and survival, olfaction-based pest control strategies have been widely explored. To study the molecular basis of olfaction in RPW, we recently uncovered the functional role of olfactory family proteins, *viz.*, odorant binding protein (OBP) and odorant receptor (OR)-coreceptor (Orco) in pheromone detection. This study explored the putative role of sensory neuron membrane proteins (SNMPs) in pheromone detection. SNMPs are CD36 family proteins with two distinct olfactory roles in insects, as two classes: SNMP1 for transferring pheromone to receptor and SNMP2 for the pheromone clearing process. We identified four distinct SNMPs based on genome mapping and quantitative expression analysis. We further classified RferSNMPs using phylogeny and studied their tissue-specific expression and relative abundance in field-collected and lab-reared adult weevils, finally leading us to select *RferSNMPu1* as a candidate for functional analysis. We used RNA interference (RNAi) based knockdown of *RferSNMPu1*, resulting in a significant reduction in electroantennogram (EAG) responses to RPW aggregation pheromone components, 4-methyl-5-nonanol (ferrugineol) and 4-methyl-5-nonanone (ferrugineone). The structural modelling revealed the *RferSNMPu1's* alignment with *RferOR1* (pheromone receptor) and *RferOrco* and an ectodomain with tunnels likely involved in pheromone detection. *RferSNMPu1*'s putative role in pheromone detection provides valuable insight into understanding the olfaction in *R. ferrugineus* as well as in other Curculionids, as SNMPs are under-explored in terms of its functional role in insect olfaction.

P-36

Identification of the trail-following pheromone receptor in termites

Souleymane Diallo[1,2], Kateřina Kašparová[1,3], Jibin Johny[2], Jan Křivánek[1], Jana Nebesářová[3], Josef Šulc[1], David Sillam-Dussès[4], Pavlína Kyjaková[1], Jiří Vondrášek[1], Ondřej Lukšan[1], Ewald Grosse-Wilde[2], Robert Hanus[1]
[1]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
[2]*Czech University of Life Sciences, Prague, Czech Republic*
[3]*Faculty of Science, Charles University, Prague, Czech Republic*
[4]*University Sorbonne Paris Nord, 93430 Villetaneuse, France*

The chemical diversity of social insect pheromones received considerable attention in both major groups of social insects, the eusocial Hymenoptera and termites. By contrast, the knowledge on termite odorant receptors (ORs) remains strikingly poor compared to that in bees and ants, and has previously been restricted to modest attempts of *in silico* identifications. Here, we will present the first OR deorphanization in termites. Using the data from antennal transcriptome and genome of the termite *Prorhinotermes simplex* (Rhinotermitidae), we selected 4 candidate OR sequences, expressed them in Empty Neuron *Drosophila*, and functionally characterized using single sensillum recording (SSR) and a panel of termite semiochemicals. In one of the selected ORs (PsimOR14), we succeeded at obtaining strong and reliable responses for the main component of *P. simplex* trail-following pheromone, the diterpene neocembrene. PsimOR14 showed a narrow tuning to neocembrene; only one additional compound out of 72 tested (geranylgeraniol) generated non-negligible responses. Subsequently, we succeeded using SSR and *P. simplex* workers, in identifying the olfactory sensillum specifically responding to neocembrene, thus likely expressing PsimOR14. We further demonstrated the caste-specificity of PsimOR14 expression and performed homology modelling and molecular dynamics calculations to address the functional properties of PsimOR14.

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P-37

The walnut husk fly that chose quince! – The strange case of *Rhagoletis completa* oviposition on *Cydonia oblonga*

S. Kecskemeti[1], M.O. Szelényi[1], B. Kiss[2], O. Molnár[3], B.P. Molnár[1]
[1]Department of Chemical Ecology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Brunszvik utca 2., Martonvásár, H-2462 Hungary
[2]Department of Zoology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Brunszvik utca 2., Martonvásár, H-2462 Hungary
[3]Department of Plant Pathology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Brunszvik utca 2., Martonvásár, H-2462 Hungary

Walnut Husk Fly (WHF, *Rhagoletis completa*) is a highly specialized fruit fly, targeting walnut species of the Juglandaceae family. While females typically lay egg clusters exclusively in unripe walnut fruits, under no-choice experimental conditions they were observed ovipositing in other fruits like pear, nectarine, apple, bell pepper, potato, and tomato. Although rare, WHFs have been found to use alternative hosts in nature, like hawthorn.

In the year 2021 and 2022 we observed ovipositional activity of WHF females on European quince in a natural habitat. Ethological observations revealed courtship behavior, male-male aggression, and male guarding of oviposition sites, further indicating the acceptance of quince as a host. Genetic sequencing confirmed that observed flies on quince were indeed *R. completa*. We compared the similarities/differences of the volatile profile of European walnut and quince through GC-MS analyses and concluded that very few volatile compounds overlap between the plant species. To see which compound elicits a strong antennal and maxillary palpi response, we conducted electrophysiological experiments (GC-EAD/GC-EPD). These experiments revealed that apart from walnut volatiles, unique volatiles exclusive to quince also elicited responses from the antennae and palpi, which means that a receptor array of WHF is much larger than we have anticipated. The behavioral experiments showed that unmated females would choose quince fruit as opposed to walnut, while males do not discriminate between them. To our knowledge, this is the first report of quince utilized as an ovipositional host by *R. completa*.

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P-49

Evolution of chemosensory genes in the Chagas disease vectors of the genus *Rhodnius*

Marie Merle[1], **Gabin Rignaut**[1], **Florence Mougel**[1], **Jonathan Filée**[1], **Louise Maille**[1], **Elaine Folly-Ramos**[2], **Carlos Eduardo Almeida**[3], **Myriam Harry**[1]
[1]*UMR EGCE, Evolution, Génomes, Comportement et Ecologie, Université Paris-Saclay, CNRS, IRD, IDEEV, Gif-sur-Yvette, France*
[2]*Departamento de Engenharia e Meio Ambiente – DEMA, Universidade Federal da Paraíba, João Pessoa, Brazil*
[3]*Universidade Federal do Rio de Janeiro (UFRJ), Centro de Ciências da Saúde, Instituto de Biologia, Departamento de Zoologia, Rio de Janeiro, Brazil*

The evolution of chemosensory genes has been studied among *Rhodnius* species, hematophagous bugs that are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease endemic in Latin America. Among *Rhodnius* species, some are domiciliary, meaning they invade anthropized environments in which they can be subjected to differentiated sensory stimuli. Chemosensory genes, namely Chemosensory Binding Proteins (CSPs), Odorant Binding Proteins (OBPs), Odorant Receptors (ORs), Gustatory Receptors (GRs), and Ionotropic Receptors (IRs), were annotated in 13 *Rhodnius* genomes, including those of four domiciliary species. Compared to genes already annotated in *R. prolixus*, 1 CSP, 1 OBP, 7 ORs, 1 GR, and 2 IRs were newly extracted from our data. For the well-assembled genomes (BUSCO > 80%), from 113 to 186 chemosensory genes were annotated according to the species.

Gene phylogenies confirm various expansion events for all multigene families. Tests for positive selection performed on well-defined orthologous groups within each family revealed 2 CSPs, 3 OBPs, 9 ORs, 2 GRs, and 2 IRs with sites under positive selection. Furthermore, positive selection was observed between the domiciliary species and others for an additional 4 ORs and 2 IRs. Purifying selection was also detected for GR1, a receptor homologous to the fructose receptor in *Drosophila*, suggesting the essential role of sugar for hematophagous *Rhodnius*.

These results suggest a rapid rate of evolution for these chemosensory multigene families and open the way to future development in vector control using chemosensory manipulation.

S2: Deciphering olfactory communication in humans

Chair: *Ilona Croy*

ORAL PRESENTATIONS

Olfactory reference syndrome – initial investigations into a new diagnosis

Ilona Croy, Julia Überschär, Antonie Bierling
Clinical Psychology, Friedrich Schiller University of Jena, Germany

Olfactory reference syndrome is listed in the new, 11th edition of the World Health Organization’s classification of mental illnesses. The disorder is defined as the non-objectifiable conviction of emitting a repulsive body odor, combined with the conviction that others are repelled by the odor and corresponding avoidance behavior of social contacts. To investigate this phenomenon, we developed a criterion-based questionnaire and tested it on an initial sample of 277 people. This showed that beliefs about having an unpleasant body odor are widespread and not stable over time. Checking behavior is also widespread, but only a few people (<3%) report significant restrictions in social activities due to concerns about body odor. Body odor concerns relate to other mental symptoms, like anxiety and depression, but not to olfactory abilities.

How does the human sense of smell compare to other mammals? And what can we learn about human olfactory function from people with smell loss?

Thomas Hummel
Smell & Taste Clinic, Department of Otorhinolaryngology, Technische Universität Dresden, Germany

Without doubt, olfactory function is of high significance in insects, while most people would agree that it is the most dispensable sense in humans. However, in terms of sensitivity it has been shown for a number of odorants that humans outperform dogs and rats and bats. The sense of smell is important in humans! For example, we react on the body odors of our fellow human beings – the nose determines who we "can smell". The perception of scents and the perception of aromas when eating mean quality of life! And smell also influences our working life: For example, perfumers, bakers and chefs absolutely rely on the sense of smell! And the sense of smell is important for detecting fire/smoke or spoiled food. The significance of the sense of smell is illustrated by the lively complaints of many people with anosmia. Life is much poorer without smelling!

State-dependent variation of human body odors: From molecular changes to perception

Antonie L. Bierling[1,2,3], **Alexander Croy**[4], **Robert Hanus**[5], **Natan Horacek**[5], **Pavlina Kyjakova**[5], **Annkathrin Wunder**[6], **Helene Loos**[7,8], **Ilona Croy**[1,3]
[1]Department of Clinical Psychology, Friedrich-Schiller-Universität Jena, Germany
[2]Institute for Materials Science, Technische Universität Dresden, Dresden, Germany
[3]Clinic and Polyclinic for Psychotherapy and Psychosomatics, University Hospital C. G. Carus, Medical Faculty, Technische Universität Dresden, Dresden, Germany
[4]Institute of Physical Chemistry, Friedrich Schiller University Jena, 07737 Jena, Germany
[5]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[6]Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Department of Psychiatry and Psychotherapy, Schwabachanlage 6, 91054 Erlangen, Germany
[7]Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg
[8]Department of Sensory Analytics and Technologies, Fraunhofer Institute for Process Engineering and Packaging IVV

Body odors play an important role in social communication. For example, they influence sexual attraction, create a sense of belonging in the family, or allow us to infer emotions such as fear or happiness in other people. Still, most research on nonverbal communication is based on face perception, gesture, or voice. With this study we aim at characterizing the state-dependent variation of healthy human body odors and their perception.

To this end, axillary sweat was sampled from 40 young male donors in four different conditions (exercise, stress, sexual arousal, control) and the pooled samples were rated on six visual analogue scales and a newly developed multiple choice description matrix of qualitative descriptors by normosmic perceivers. Furthermore, the samples were analyzed for their chemical components using gas chromatography analysis, and electronic fingerprints were estimated using an electronic nose.

First results show that receivers can differentiate between some of the body odor condition and that this aligns with differences in the pattern of emitted volatile organic compounds. Further results will be presented.

Combined sensory and instrumental approaches to investigate the chemical basis of olfactory communication

Helene M. Loos
Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany

Volatiles emitted from organisms naturally depend on the organism's physiological state. Volatile profiles therefore contain information about this state, which is a prerequisite for functional chemical communication and its applications. It is however not trivial to determine which volatiles are relevant in conveying information to other organisms, and which volatiles are most suited for detection of a certain state by technical systems. In this overview presentation, gas chromatographic-mass spectrometric and gas chromatographic-olfactometric techniques for the characterization of volatile profiles of body odor samples are presented. Results obtained with the different techniques in the field of human chemical communication will be presented, focusing on emotion and age information in the volatile profiles of axillary sweat samples. An outlook will be given regarding the application of combined sensory and instrumental approaches in investigations on chemical communication in other species.

Chemistry of human body odor: Individuality vs. conserved signatures of psychophysiological states

Natan Horáček[1,2], **Antonie Bierling**[3], **Pavlína Kyjaková**[1], **Robert Hanus**[1], **Ilona Croy**[3]
[1]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic
[2]Faculty of Science, Charles University
[3]Department of Clinical Psychology, Friedrich-Schiller-Universität Jena, Germany

Human body odor conveys large body of information about our genetic background as well as physiological and psychophysiological states, including diseases and pathologies. This banal statement has a great support from our daily life experiences as well as from the research mainly conducted using olfactory perception studies with human raters or trained dogs. Yet, empirical knowledge on chemical underpinnings of differential body odor are rare, essentially due to past technical limitations in detection and separation of individual analytes from the complex blend of multiple hundreds of small molecules emitted by human body.

With the recent advent of gas phase metabolomics, many previously intangible questions related to human body odor became realistic targets of research. Here, we use a combination of olfactory perception rating and comprehensive two-dimensional gas chromatography coupled with MS detection (GC×GC-TOFMS) to search for axillary body odor patterns characteristic for different psychophysiological states of healthy male donors. More specifically, we analyzed the body odor changes upon sport exercise, upon exposure to psychosocial stress and upon sexual arousal stimulation. In my presentation, I will show that both, perception rating and chemical analyses allowed for efficient separation of control samples from the same donors and samples collected after sport and stress exposure. By contrast, we did not observe a systematic shift in body odor patterns upon sexual arousal. I will also disclose the candidate analytes responsible for the body odor shift among different psychophysiological states.

Funding: Pathfinder Open SMELLODI (101046369)

S3: Ecometabolomics: Unveiling the molecular landscape from discovery to ecosystem functioning

Chairs: *Emmanuel Defossez, Sergio Rasmann*

ORAL PRESENTATIONS

The Earth Metabolome Initiative, an introduction

Emmanuel Defossez[1,2], Pierre-Marie Allard[3], Meredith Schuman[4]
[1]*Institute of Biology, University of Neuchâtel, 2000 Neuchâtel, Switzerland*
[2]*Botanical garden of Neuchâtel, Switzerland*
[3]*Department of Biology, University of Fribourg, 1700 Fribourg, Switzerland*
[4]*University of Zurich Department of Geograhpy / Department of Chemistry 8057 Zürich, Switzerland*

Life is a complex and dynamic interplay of chemical structures, spanning multiple dimensions and scales from biogeochemical cycles to cellular metabolism. After 3.5 billion years of evolution, this ever-expanding network linking all forms of life on Earth is the structure that underpins the functioning of ecosystems and shapes biodiversity. Although, humans depend deeply on this chemodiversity for nutrition and medicines, we have yet identified only a minute fraction (less than 1%) of the millions of metabolites estimated to be produced across the Tree of Life. This fundamental aspect of biodiversity is a treasure chest yet to be unlocked, and with every species lost it is sinking out of reach.

By describing the ensemble of metabolites – the metabolome – of every organism across their habitat, the Earth Metabolome Initiative (EMI) aims to reveal the mechanisms that orchestrate and maintain living systems. This information is digitized and organized in an open knowledge base and accompanied by a metabolome biobank to reach 3 main objectives:

- Advance life sciences by systematically expanding knowledge on global chemodiversity, from less than 1% towards 100%; and placing it in the context of biosphere processes and functions.
- Benefit human society through the development of novel, sustainable solutions in agriculture, medicine and other fields.
- Protect biodiversity by providing critical information for monitoring, developing novel metrics, and informing predictive tools to prioritize protection and incentivizing conservation.

MS/MS molecular networking and molecular biology of citrus bacterial infection: Chemical interactions and future perspectives

M. Fátima G. F. da Silva[1], **Jessica C. Amaral**[1], **João B. Fernandes**[1], **Danielle F. Silva**[1], **Alain Hehn**[2]
[1]Chemistry Department, Federal University of São Carlos, CP 676, 13565-905 São Carlos, SP, Brazil
[2]University of Lorraine, Nancy, France

Huanglongbing (HLB) is the most destructive citrus disease and is caused by the bacterium *Candidatus* Liberibacter asiaticus (CLas), *C*. Liberibacter africanus and *C*. Liberibacter americanus. The development of resistant or tolerant varieties to the bacteria is still a long-term challenge. A study of the plant/bacteria interaction CLas was developed by LC-HRMS/qTOF in positive and negative ionization modes. Roots of healthy and HLB-infected plants of *Citrus sinensis* x *C. limonia* cultivated in a greenhouse, and the roots of healthy and infected *C. sinensis* seedlings cultivated in vitro were analyzed. Through the analysis of the molecular network, it was possible to observe an increase in the concentration of some coumarins in plants infected with HLB. The plant reaction was observed inhibiting the biosynthesis of linear derivatives in favor of others that are prenylated at C-8 (angular). The quantification of the expression of *DXR*, *DXS2*, *PT1* and *PT2* genes was carried out by RT-qPCR, in *C. sinensis* x *C. limonia* roots of healthy and symptomatic plants infected with CLas. There was a decrease in the expression of the *PT1* (involved in the linear coumarins formation) genes in plants infected with CLas compared to healthy ones. For the *PT2* gene (involved in the angular coumarins formation) there was an increase in the relative expression for infected plants compared to healthy ones. Thereby, this study will provide important information for genetic engineering in order to increase the levels of these compounds and, thus, improve resistance against phytopathogens.

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An innovative dynamic headspace collection technique (HSCD) for outdoor use and suitable for quantitative odor comparison

Jürgen Gross[1*], Ralf Kunath[2]
[1]Institute for Plant Protection in Fruit Crops and Viticulture, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Dossenheim, Germany
[2]FLUSYS GmbH, Offenbach, Germany

Volatile organic compounds (VOCs) emitted by insect pests or infected plants are usually present in low concentrations. Numerous collection techniques for VOCs have been developed in recent decades. All these different techniques have their limitations. The flow in dynamic headspace collection systems is influenced by internal and external factors. Especially in field experiments, it is important to measure comparable samples at the same time to avoid distortions due to temperature fluctuations during the day or changing intensity of solar radiation due to e.g. clouds between the VOC collections of different samples. The attempt to collect relevant VOCs in qualitatively and, in particular, quantitatively comparable ratios has led us to develop an innovative dynamic headspace collection device (HSCD). It is a derivative of two prototypes we described earlier. The technical features of the device are presented in detail. It was produced as a small series as part of the PurPest project (European Commission, 2023).

The HSCD possesses six parallel mounted odor collection systems, each consisting of a suction pump coupled to a digital mass flow controller (range: 0.1 to 1 ln/min). It has two sampling modes, the open loop sampling (OLS) and the closed loop sampling (CLS) mode. Ambient temperature and relative humidity are monitored during the sampling of VOCs and stored together with flow data on an USB-stick. All components are mounted in a trolley suitcase for mobility of the system.

This work was funded by the European Union (Horizon 2020 Farm2Fork) under the PurPest project through grant agreement 101060634.

Chemical exploration of the interactions within marine algal holobiont: A comparative metabolomic study

Gautier Demoulinger[1,2], **Cécile Le Guillard**[2], **Soizic Prado**[1]
[1]Unité Molécules de Communication et Adaptation des Micro-Organismes (UMR 7245), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, 75005 Paris, France
[2]Département R&D Nutrition Végétale, Centre Mondial de l'Innovation, Groupe Roullier, 35400 Saint-Malo, France

Seaweed harbour and interact with associated microbial communities, which contribute to their growth and defence mechanisms. These intricate interactions have led to the conceptualization of seaweed and their associated microbiota as a “supraorganism”, collectively referred to as the holobiont. Molecular investigations increasingly point to non-random associations between algae and their microbiota, strongly implying the existence of complex structured and dynamic interactions both within the microbiota itself and with the algal host.

Herein, we endeavour to elucidate the chemical interactions occurring between fungi and the algal host, through the determination of the fungal community's metabolome detected in the host, and the understanding of the role of fungal mediators in fungi-host interactions. The brown seaweed *Ascophyllum nodosum* has been selected regarding its economic interest in agronomy. Indeed, among the commercial products developed to stimulate plant growth and alleviate stresses, many contain brown seaweed extracts, among which *A. nodosum* is widely represented. However, the chemical nature and origin of the bioactive compounds present in *A. nodosum* extracts have not been fully characterized, even regarding secondary metabolites.

Thus, in order to decipher the entire metabolome of *A. nodosum* holobiont, we isolated and characterized the cultivable microbiota associated with the brown alga. To explore the chemical interactions within this entity, we developed an analytical workflow, based on untargeted metabolomics from LC-MS/MS data and molecular networking with various levels of restrictiveness.

Mining actinomycetes' metabolomes and genomes for anti-*Phytophthora infestans* compounds

Ola Abdelrahman[1,2], Quinn Coxon[1], Eliane Abou-Mansour[1], Floriane L'Haridon[1], Laurent Falquet[1,3], Pierre-Marie Allard[1], Laure Weisskopf[1,4]
[1]Department of Biology, University of Fribourg, Fribourg, Switzerland
[2]Department of Botany, University of Khartoum, Khartoum, Sudan
[3]Swiss Institute of Bioinformatics, Lausanne, Switzerland
[4]Food Research and Innovation Centre, University of Fribourg, Fribourg, Switzerland

Actinomycetes are well-known for their capacity to produce a wide array of specialized metabolites exhibiting diverse bioactivities. In a previous work, we examined a collection of 175 actinomycetes strains isolated from various Sudanese soils for their inhibitory activity against *P. infestans*, the causal agent of late blight disease in potato and tomato. A significant proportion of these strains demonstrated considerable inhibition or alteration of *P. infestans* mycelial growth. Considering the diverse inhibitory effects and morphological alterations induced by the strains, we aimed to identify the active metabolites responsible for these effects through a comparative metabolomic approach. To achieve this, we selected 63 strains displaying varying degrees of antagonistic activity against *P. infestans* and extracted their metabolites using ethyl acetate. Subsequent analysis via liquid chromatography coupled with mass spectrometry and spectral network analysis of our extracts revealed several compounds potentially active against *P. infestans*. These compounds, which were detected in active extracts but absent in inactive ones, included borrelidin, albonoursin, oxopropaline G, lansai A/C, actinomycin D, annimycin, antibiotic A 83586C, louisianin D, antimycin, collinomycin, among others. Furthermore, our analysis suggested that actinomycete surfactins, angucyclines, phenazines, and other compounds may be responsible for altering *P. infestans* morphology. The annotation of some of these compounds was validated by comparing them to purchased standards or by detecting the relevant biosynthetic gene clusters in the strains' genomes via antiSMASH analysis. This study provides a list of candidate compounds with potential as inhibitory agents against *P. infestans* and hints at broader applications against other plant pathogens.

Exploring the chemical complexity of *Petunia hybrida* pollen

Abigail Moreno-Pedraza[1,2], **Paula C.P. Bueno**[2], **Nicole M. van Dam**[1,2,3]
[1]*Institute of Biodiversity, Friedrich Schiller University Jena, Jena, Germany*
[2]*Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany*
[3]*German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany*

Plants produce pollen to transfer their genome to the female reproductive organs. Here we present an untargeted metabolomics approach that investigates the secondary metabolites present in pollen of *Petunia hybrida*. The aim of the project was to identify whether pollen metabolomes can be used to select pollen for plant breeding by linking them to desirable traits of the pollen donor. In contrast to the floral traits of *Petunia*: color and volatiles, non-volatiles compounds in pollen have been less studied and may also drive ecological interactions between pollinators.

We used two contrasting lines of *Petunia hybrida*: V26 (violet flowers) and W115 (white flowers). Plants were grown under greenhouse conditions. Fully opened flowers were collected, stamen dissected and pollen isolated. A subsequent three-in-one extraction solution was employed for a comprehensive analysis of pollen metabolome. The three extracts were analyzed using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) in positive and negative modes.

The results show clear differences in the metabolic profile of *Petunia* V26 and W115. Partial Least squares-discriminant analyses (PLS-DA) using the three columns in the two ionization modes show differential metabolites that correctly classified pollen from white and purple lines in all datasets. Using molecular networking and classification tools highlighted the most important features differentiating the groups. Flavonoid aglycones and phenylalanine derivatives were predominant in the line W115. Understanding which secondary metabolites are unique or significantly different in pollen will help us to further explore the ecological functions that these compounds potentially have.

Multifactorial daily variations in true lavender inflorescence chemistry in relation to daily pollinator dynamics in lavender fields

Mathias Fontez[1], **Joris Huguenin**[2], **Sandrine Moja**[1], **Frédéric Jullien**[1], **Magali Proffit**[2], **Florence Nicolè**[1]
[1]Laboratoire de Biotechnologies Végétales appliquées aux Plantes Aromatiques et Médicinales (LBVPAM), Université Jean Monnet, Saint-Étienne, France
[2]Centre d'Écologie Fonctionnelle et Évolutive (CEFE), Université de Montpellier, CNRS, Montpellier, France

True lavender (*Lavandula angustifolia* Mill.), originates from mid-altitude environments in the south of France and is an emblematic species of the garrigue Mediterranean ecosystem. It flowers for 2-3 weeks during summer, and it is a valuable nutritive resource for many insect pollinators. True lavender communicates with pollinating insects through chemical signals, including the emission of many volatile organic compounds (VOCs) by its inflorescences and nectar production. These chemical signals and the abundances of different pollinator cohorts vary quantitatively and qualitatively throughout the day. Daily joint variations of chemical signals and pollinator communities remain poorly studied in generalist plant-pollinator systems, as well as VOC dynamic emission monitoring in flowering plants with glandular trichomes storing structures. To investigate the true lavender interactions with pollinators, the daily variations in floral bouquet were monitored in relation to abiotic factors: temperature and light intensity with PTR-TOF-MS in a dynamic chamber system, as well as the daily joint variations in nectar volume and composition, VOC emission, and pollinator guilds abundances in lavender fields. These measures aim to reveal correlations between the daily flower metabolite production and pollinators dynamics observed in the natural environment, therefore paving the way for future investigations of the functional roles played by these various chemical signals.

The roles of functional and chemical traits in shaping plant community assemblies across an aridity gradient

Hanna Nomoto[1], **Rachda Berrached**[2], **Sergio Rasmann**[1]
[1]University of Neuchâtel, Faculty of Science, Institute of Biology, Rue Emile-Argand 11, 2000, Neuchâtel, Switzerland
[2]University of Mouloud Maameri of Tizi-Ouzou, Department of Biology, Tizi-Ouzou, Algeria

Assessing shifts in community assemblies across environmental gradients is important to predict consequences for ecosystems facing novel environments under climate change. While increasingly arid climates are expected to threaten the diversity within plant communities, the role of aridity in shaping community assemblies has predominantly been explored via traditional functional traits, while responses reflected in the plant metabolome remain largely unknown. We explored patterns of convergence and divergence reflected in functional and chemical traits for plant communities distributed along an aridity gradient in northern Algeria. We estimated SLA (specific leaf area), leaf area, LDMC (leaf dry matter content), stalk height, as well as phytochemical diversity at the levels of features, classes and structural composition, for the most common plant species within 8 sites. We quantified functional divergence, dispersion, diversity and community-weighed means for both functional traits and phytochemical properties. Our study revealed that aridity drove convergence of functional, but mainly chemical traits, suggesting that aridity can act as an environmental filter shaping the functional and phytochemical composition of plant assemblages. Stronger patterns of convergence observed for chemical properties compared to functional traits suggest that the plant metabolome is necessary to consider to better understand the causes and consequences of variation in community assemblies along environmental gradients. Importantly, our findings indicate that increasingly arid climates could act to alter not only functional, but also chemical diversity in plant communities, potentially impacting future ecosystem-level dynamics.

The Curious Case of Piper Pink Belly: Reactive Intermediates as Drivers of Chemodiversity Shaping Insect Interactions Across Ecological Gradients

Christopher S. Jeffrey
Department of Chemistry, Hitchcock Center for Chemical Ecology, University of Nevada, Reno, USA

Piper “pink belly” (*P. kelleyi – Piperaceae*) is a tropical shrub found in the cloud forests of Ecuador and Peru. Our studies reveal that the genetic structure of specialist herbivores feeding on this phytochemically diverse shrub is shaped by the unique variation in natural products that differ across elevations and light exposures. Field experiments have uncovered the chemical underpinnings of this remarkable variation, highlighting the role of reactive *ortho*-quinone methide intermediates as key drivers of chemical diversity. This presentation will discuss studies utilizing ecometabolomics and chemical synthesis to understand the mechanisms and origins of the foliar chemodiversity that shapes plant-insect interactions within the phytochemical landscape of this tropical forest system.

Chemically mediated interactions along elevation gradients and climate change

Sergio Rasmann
Institute of biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

Plants and insects have coexisted for millions of years, with a range of intricate and dynamic interactions occurring between them. One of the most important ways that plants interact with insects is through chemical compounds, which can attract, deter or even manipulate the behaviour of the insects. In some cases, these interactions can be adversarial, such as when plants produce toxins to defend against herbivore, while in other cases, they can be mutually beneficial, such as when plants send signals for attracting predators or parasitoids. These interactions vary across ecological gradients, including differences in climate, elevation and habitat, as well as differences in plant and insect species diversity. In this talk, I will expand on our current understanding of how plant chemistry changes along large-scale ecological gradients, and in turn, how such chemistry impacts multitrophic interactions between plants, insect herbivores and predators. Understanding the complex relationships between plants and insects across large-scale ecological gradients is essential for predicting the impacts of environmental change on ecosystems and for developing sustainable agricultural and conservation practices.

Keywords: plant-herbivore interaction, chemical ecology, tritrophic interactions, phytochemical diversity, ecological gradients of biodiversity

POSTERS

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GC coupled with infrared spectroscopy as key technology in the structure elucidation of trace compounds

Anton Möllerke, Johanna Kuhn, Matthew Stell, Stefan Schulz
Institute of Organic Chemistry, TU Braunschweig, 38106 Braunschweig, Germany

The amount of material of a natural compound and its purity frequently restricts the structure elucidation. While NMR-spectroscopy or X-ray crystallography often permit the unquestionable elucidation of structures, they require comparatively high amounts of isolated substance. This is often challenging, as the accessibility of biological material is constrained by the difficult collection and slow reproduction of organisms. Given this, natural extracts are often only available in small amounts of complex mixtures.

Gas chromatography/mass spectrometry (GC/MS) provides high sensitivity and separation of complex mixtures, enabling the analysis of extracts from small amounts of sample without further purification. For certain compounds such as alkanes, there are well-established rules for the interpretation of EI mass spectra. However, limitations exist for other compound classes such as substituted arenes.

We developed a stepwise protocol based on GC coupled with direct deposition infrared spectroscopy (DD-IR) to complement the GC/MS-based structure elucidation. GC/DD-IR is a highly effective analytical tool, capable of resolving the same complex mixtures and providing a similar sensitivity as GC/MS while providing additional information on functional groups. In addition, in contrast to mass spectra IR spectra can be calculated using density functional theory (DFT). A comparison of the GC/DD-IR data with DFT-generated spectra permitted the correct prediction of the constitution. The sequential application of GC/(HR)-MS, GC/DD-IR, DFT-calculations, and synthesis allows to time-efficiently decipher the structure of target compounds present in low amounts in complex mixtures. We describe this approach with our recent elucidation of highly hetero-substituted arenes from Collembola.

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Identification of bioactive essential compound extracted from Lemna using supercritical fluid extraction

Lilach Lily Mondaca[1-3], **Nurit Sigler**[2], **Ido Korman**[2]
[1]Science Department, Sapir Academic College, 7919, D.N. Hoff Ashkelon, Israel
[2]Eshkol Innovation Ltd. Ein HaBesor, Israel
[3]Gaza Envelope Research and Development Center, Netivot, Mailbox 100, 8771002, Israel

Lemna, commonly known as duckweed, is a small aquatic plant that belongs to the Lemnaceae family. It is characterized by its rapid growth and ability to reproduce quickly. Duckweeds, the smallest flowering plant that adapted to aquatic environment has a great potential as an alternative source of proteins and other metabolites due to its high growth rate when grown under optimal conditions. Lemna family contain hundreds of varieties with enormous number of different molecules include essential amino acids, fatty acids, antioxidants, antibacterial, and other important metabolites like zinc, manganese and others. Lemna has been gaining attention in recent years due to its potential as a sustainable and cost-effective source of various bioactive compounds, including sterols. Duckweed Species were shown to have high potential as source for various nutrients and other essential compounds. Phytosterols as a lipophilic compound, can be extracted from Duckweed using supercritical fluid extraction (SFE), which is a promising area of research. SFE is a technique used to extract lipophilic compound from Lemna using supercritical fluids, typically CO_2, as the solvent to extract the desired compounds from the plant material. SFE offers several advantages over traditional extraction methods, including higher selectivity, faster extraction times, and the ability to extract heat-sensitive compounds. It is an environmentally friendly and efficient technique that is widely used in the pharmaceutical, food, and nutraceutical industries. At the conference we will present SFE as the extraction method and the founder of phytosterols, carotenoids and tocopherols.

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MACE – Mass spectra for chemical ecology

Stefan Schulz, **Anton Möllerke**
Institute of Organic Chemistry, TU Braunschweig, Braunschweig, Germany

EI mass spectral libraries are an important tool for the identification and structure elucidation of natural products by GC/MS, a typical task in Chemical Ecology. Unfortunately, spectra of new compounds are usually only available as figures, even in recent publications. This makes it tedious to keep track of and difficult to integrate into user libraries. We have therefore implemented an open access data repository for EI mass spectra, called MACE. In this database, high quality spectra not found in common databases can be downloaded as a collection in a simple format that can be easily integrated into local spectral databases. The spectra are taken from original publications of synthesized or isolated compounds. Compounds found in widely used commercial databases such as NIST 17 or Wiley 7 are not included. MACE is therefore a complementary database of high quality EI mass spectra. MACE is designed to be an open-access collaborative community, requiring input from research groups worldwide. The list of compounds will be continuously expanded, hopefully with spectra submitted by other groups. Additions are very welcome. Submitted spectra will be checked for quality and added to MACE. The complete text file of the MACE library can be downloaded from Leopard, the research data repository of the TU Braunschweig, thus ensuring long-term data storage. The data can be freely distributed for non-commercial use under the Creative Commons license CC-BY-SA. There is a X account for news about MACE: @MACE4GCMS and a mailing list. To join, send a request to: mace@tu-bs.de

S4: Chemical ecology of forest insects

Chair: *Quentin Guignard*

ORAL PRESENTATIONS

(Why) do spruce bark beetles prefer drought stressed trees?

Sigrid Netherer
Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU University, Vienna, Austria

The recent outbreaks of the European spruce bark beetle *Ips typographus* have been triggered by disturbance agents such as storm throw, heat and drought. While weather extremes clearly correlate with volumes of timber loss on landscape and stand scale, the effects of drought stress on the susceptibility of individual trees to bark beetle attack are complex and still insufficiently understood. Not only the interactions between trees and pioneer bark beetles decide about early attack success but also associated microorganisms such as ophiostomatoid fungi challenge the chemical defense system of trees. Modelling studies, field and laboratory experiments deliver important results to advance bark beetle risk assessment, early warning systems and recommendations for damage prevention and mitigation.

Towards push-pull control of an invasive bark beetle, *Ips grandicollis*, using non-host volatiles and semiochemicals from sympatric species

Somia Afzal[1,2], **Helen F. Nahrung**[2], **Simon A. Lawson**[2], **Brian T. Sullivan**[3], **Kamal J.K. Gandhi**[4], **R. Andrew Hayes**[2]
[1]*School of Science, Technology and Engineering, University of the Sunshine Coast*
[2]*Forest Industries Research Centre, University of the Sunshine Coast, Ecosciences Precinct, Dutton Park, Qld, Australia*
[3]*USDA Forest Service, Southern Research Station, Pineville, LA*
[4]*Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA*

The eastern five-spined ips, *Ips grandicollis* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is native to the eastern USA and an invasive pest of exotic pines (*Pinus* spp.) in Australia. It has close associations with several species of ophiostomoid fungi that cause blue staining of the wood and reduce the commercial value of timber. This bark beetle uses semiochemicals produced by conspecifics and host trees to locate mates and trees for colonization and reproduction, while semiochemicals from competitors, non-hosts and unsuitable host volatiles may induce a repellent response. To exploit these behaviours in a management strategy, we are developing a push-pull technique for *I. grandicollis* employing aggregation pheromones to draw beetles away from pines (pull) and releasers semiochemicals to reduce landings and attacks (push). We tested the influence of 12 putative repellent compounds on trapping success with *I. grandicollis* aggregation pheromone lures (ipsenol and *cis*-verbenol) in the native (Georgia and Lousiana) and invasive (southeast Queensland) ranges of *I. grandicollis*. Of these, three compounds (methyl eugenol, 2-phenylethanol, and camphor) significantly reduced attraction to the attractive lure in both native (19-39%) and invasive (41-56%) ranges. Methyl eugenol and 2-phenylethanol additionally displayed additive repellent effects when applied as a binary combination in Louisiana, and using these two compounds with camphor as a tertiary combination inhibited beetle attraction in Queensland. Further investigations using these three effective repellents together will assist in the development of a sustainable push-pull management strategy for protecting pine trees, logs, and log stacks from *I. grandicollis* attack.

The potential of verbenone to complement the integrated management of *Ips typographus* in Central Europe

Tobias Frühbrodt[1,5], **Helge Löcken**[2], **Baoguo Du**[3], **Christopher J. Fettig**[4], **Peter H.W. Biedermann**[2], **Jürgen Kreuzwieser**[3], **Tim Burzlaff**[2], **Horst Delb**[1]
[1]Forest Research Institute Baden-Württemberg, Dept. Forest Protection, Wonnhaldestrasse 4, 79100 Freiburg, Germany
[2]Chair of Forest Entomology and Protection, University of Freiburg, Fohrenbühl 27, 79252 Stegen-Wittental, Germany
[3]Chair of Ecosystem Physiology, University of Freiburg, Georges-Köhler-Allee 53, 79110 Freiburg, Germany
[4]USDA Forest Service, Pacific Southwest Research Station, Davis, California, U.S. 95618
[5]Bavarian State Institute of Forestry, Dept. Forest Protection, Hans-Carl-von-Carlowitz-Platz 1, 85354 Freising, Germany

Ips typographus is the most damaging insect in European forests, threatening the existence of its main host, *Picea abies*, in some regions. Outbreaks of *I. typographus* are triggered by sudden changes to favorable abiotic conditions, such as drought or windthrow, which can provide large numbers of weakly defended host trees. Management approaches are mostly limited to salvage and sanitation logging, sometimes challenging to achieve due to logistic capacities. Verbenone is a volatile compound inhibiting the attraction of several tree-killing bark beetle species. Here, we summarize the outcome of four years of studies on the potential of (–)-verbenone (SPLAT® Verb, 10% w/w verbenone) to prevent infestation of *P. abies* by *I. typographus*. Attraction to pheromone-baited traps was inhibited by 34 to 93%, the applied SPLAT® Verb was effective for >80 days, and verbenone was detectable up to a distance of 14 m with an application of 75 g. Furthermore, SPLAT® Verb reduced and delayed the infestation on small (5-6 trees) windthrows under low to moderate infestation pressure, while no effect was obtained under high infestation pressure (≥ 76% infestation rate in untreated control). No relevant effect on infestation dynamics was observed on stored logs, possibly explained by the large amount of *P. abies* timber in relation to the amount of verbenone. We outline potential management options and research questions that are relevant to optimize the use of verbenone to complement the integrated management of *I. typographus*. SPLAT® Verb is not yet registered as a plant protection product in the EU.

Does beetle size influence olfactory selection of suitable host trees? The effect of beetle size on trap catches and electroantennographic (EAG) response to 1,8-cineole and (+)-isopinocamphone in female *Ips typographus*

Antonioni Acacio Campos Moliterno[1*#], Mayuri Kashinath Shewale[1#], Sara Basile[1], Rajarajan Ramakrishnan[1], Anna Jirošová[1]
[1]Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Czech Republic 16500 – moliterno@fld.czu.cz – # equal contribution*

The bark beetle, *Ips typographus* L., is a major pest species with significant ecological and economic impacts in the central European spruce forest. Beetle aggregation is coordinated by the emission of pheromones, which are associated with the volatile compounds emitted by host trees. In experiments using pheromone traps have demonstrated the ecological relevance of oxygenated monoterpenes (OMTs) in repelling/attracting *Ips typographus*. A high dose of 1,8-cineole, a potential marker of susceptibility of spruces against the attack of *Ips typographus*, inhibited the capture of beetles at pheromones, whereas the addition of (+)-isopinocamphone (Ph +OMT) increased captures compared to pheromone alone (Ph). These findings raise additional scientific questions:

Are there sex-specific or size-specific differential preferences for a mixture of Ph + OMT when compared to Ph alone?

Are there differences in the antennal sensitivity to 1,8-cineole and (+)-isopinocamphone between small and larger beetles?

Field trapping experiments in 2019 and 2022 exposed *Ips typographus* to varied 1,8-cineole and (+)-isopinocamphone doses alongside pheromones, or pheromones alone. Beetles were sorted by sex, and females' body length (pronotum and elytra) measured. Results showed smaller female beetles were most captured with a high 1,8-cineole dose + pheromones, compared to Ph. smaller females were captured more frequently with a low dose of isopinocamphone in combination with pheromones compared to a high dose. Electroantennographic (EAG) analysis revealed differential responses to 1,8-cineole between smaller and larger beetles. Larger females' preference for lower 1,8-cineole content may explain their tendency to select more suitable host trees, potentially enhancing reproductive fitness.

Options in the use of synthetic baits against the Central European pine bark beetles

Petr Doležal[1], **Markéta Davídková**[1], **Miloš Knížek**[1]
[1]*Forest Protection Service, Forestry and Game Management Research Institute, Prague, Czech Republic*

Over the last decade, Scots pine stands in Central Europe have been negatively affected by climate change, which has increased the frequency of bark beetle outbreaks. The most common bark beetles on pine trees are *Ips sexdentatus* and *Ips acuminatus*. Pheromone traps are a simple method of monitoring the occurrence and abundance of these species. Only one pheromone dispenser for *I. sexdentatus* – SEXOWIT (Witasek-Pflanzenschutz GmbH, Feldkirchen, Austria) – is available in Central Europe. In contrast, three baits for *I. acuminatus* are in use and could be tested in the study. These are Acumiprotect (SEDQ s.l., Barcelona, Spain), Pheagr IAC (Scitech s.r.o., Praha, CZ) and Acuwit (Witasek-Pflanzenschutz GmbH, Feldkirchen, Austria). Acumiprotect and Pheagr IAC were the most effective for trapping *I. acuminatus*. Pheagr IAC in particular had a longer life span and still caught individuals towards the end of the growing season. Sexowit, a bait designed to attract *I. sexdentatus*, was very ineffective as it only caught a few individuals during the growing season, except in places with a very high population density of *Ips sexdentatus*. In contrast, Acumiprotect, apart from its effectiveness for *I. acuminatus*, proved to be the most efficient bait for monitoring *I. sexdentatus* as it consistently caught adults throughout the growing season. Therefore, Acumiprotect appears to be a sufficient product for monitoring both *Ips acuminatus* and *Ips sexdentatus*.

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Styrene mediates interactions among spruce bark beetle, fungus and host tree

Dineshkumar Kandasamy[1,2,3], **Akanksha Jain**[3], **Jonathan Gershenzon**[3], **Almuth Hammerbacher**[4], **Martin N Andersson**[1,2]
[1]*Max Planck Center for Next Generation Insect Chemical Ecology (nGICE), Lund University, Sweden*
[2]*Department of Biology, Lund University, Lund, Sweden*
[3]*Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany*
[4]*Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa*

The success of the Eurasian spruce bark beetle, *Ips typographus,* as a lethal pest for Norway spruce (*Picea abies*) throughout Europe is largely attributed to its association with a suite of ascomycete blue-stain fungi. These fungi, differing in virulence, can exhaust or overwhelm tree chemical defences leading to tree death. Recent research has shown that a highly virulent fungus changes the volatile profile of spruce trees, aiding beetles to identify trees colonized by beneficial fungi. While these symbiotic fungi are known to break down non-volatile defense chemicals, it remains unknown whether any resulting byproducts could function as insect semiochemicals.

We found that a symbiotic virulent fungus produces an uncommon natural product, styrene by metabolizing a host tree non-volatile *trans*-cinnamic acid, a key intermediate in the biosynthesis of spruce phenolics defenses. While *trans*-cinnamic acid was not toxic to beetles, it inhibited symbiotic fungal growth. Adult beetles detect styrene and other similar chemicals via a specific olfactory sensory neuron type located on the antennae. Behavioural assays using walking beetles indicated attraction to styrene-emitting fungus over short distances. However, long-range field trapping experiments revealed that styrene inhibited the attraction of beetles to pheromones. Altogether, this study demonstrated that bark beetles use specific volatile cues originating from the fungal metabolism of a key intermediate compound in spruce phenolics biosynthetic pathway to distinguish between virulent and less-virulent fungi. This association with virulent fungi may aid beetles in avoiding toxic tree chemicals, ultimately increasing their fitness.

Trees respond to insect eggs on their leaves by improved defense against larvae

Monika Hilker
Institute of Biology, Freie Universitaet Berlin, Berlin, Germany

Plants have evolved a plethora of strategies to cope with the attack by herbivorous insects. They do not only notice insect infestation when damaged by feeding, but they also respond to insect egg depositions on their leaves. Both trees and herbaceous plants can take insect eggs as “warning” of impending larval herbivory. Egg-laden plants defend themselves more effectively against hatching larvae than plants that never have experienced egg depositions. Responses of *Pinus sylvestris* and *Ulmus minor* to insect eggs on their leaves show similarities to those of *Arabidopsis thaliana,* indicating some phylogenetically conserved, egg-inducible plant traits. Interestingly, elm and several herbaceous plants respond to larval feeding damage by higher concentrations of certain leaf phenylpropanoids when having received egg depositions prior to the feeding damage. Furthermore, both in the studied tree species and in *Arabidopsis,* defense against feeding larvae is not only linked with enhanced levels of jasmonic acid (JA). Levels of salicylic acid (SA) are also higher in plants that perceived insect egg depositions prior to larval feeding, suggesting a lack of antagonism between these phytohormones. While the abovementioned studies of pine and elm have been conducted with juvenile trees, future studies need to elucidate how mature trees in the forest respond to insect egg depositions.

Hundacker et al. 2024. doi: 10.1093/treephys/tpae008; Schott et al. 2022. doi: 10.1007/s00425-021-03803-0; Hundacker et al. 2022. doi: 10.1111/pce.14211; Lortzing et al. 2020. doi: 10.1038/s41598-020-72955-y

First line of defense: Eucalyptus leaf waxes influence insect feeding and infection by fungal pathogens

Almuth Hammerbacher[1], Johannes Christoff Joubert[1], Privilege Makunde[1], Myriam Solis[2]
[1]Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa
[2]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

Eucalyptus leaves have a complex wax layer, containing alkanes, alcohols, aldehydes, ketones, sterols, esters and long-chain fatty acids, primarily deposited as crystalloids. We focused on the biological roles of palmitic acid, a fatty acid occurring at different concentrations on leaf surfaces of diverse *Eucalyptus* genotypes and species, with the aim of understanding its role in herbivory and fungal infection. Topical applications of palmitic acid on leaves reduced surface tension, potentially reducing host recognition as well as insect attachment. Feeding trials with the Eucalyptus snout weevil (*Gonipterus* sp. n. 2) supported this, highlighting the effectiveness of palmitic acid as a feeding repellent. Palmitic acid also influenced the host specificity of two Eucalyptus lerp psyllid species (*Spondyliaspis* cf. *plicatuloides* and *Glycaspis brimblecombei*). However, a surprising duality emerged. Palmitic acid stimulated spore germination of the *Eucalyptus* foliar pathogen *Teratosphaeria destructans*. On resistant *Eucalyptus* varieties, it paradoxically also facilitated pathogen colonisation. These findings reveal the intricate relationship between leaf wax components and *Eucalyptus* defence. While palmitic acid provides protection against herbivory by some insects, its interaction with fungal pathogens presents a nuanced challenge. Our study portrays a “defensive trade-off,” underscoring the need for further exploration of the effects of palmitic acid on other economically important insect herbivores and foliar fungal pathogens.

The effects of defoliation and egg deposition by the Eucalyptus snout beetle, *Gonipterus* sp. n. 2 on the chemistry and gene expression of Eucalyptus leaves

Johannes Joubert[1,2], **Benice Sivparsad**[3], **Michelle Schröder**[1], **Ilaria Germishuizen**[3], **Jingyuan Chen**[5], **Brett Hurley**[1,2], **Jeremy Allison**[2,4], **Almuth Hammerbacher**[1,2]
[1]*Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa*
[2]*African Centre of Chemical Ecology, Innovation Africa Campus, University of Pretoria, Pretoria, South Africa*
[3]*Institute for Commercial Forestry Research, PO Box 100281, Scottsville 3209, Pietermaritzburg, South Africa*
[4]*Natural Resources Canada-Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Canada*
[5]*Zhuhai Branch of State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University, Zhuhai 519087, China*

Gonipterus sp. n. 2 is an invasive, commercially important weevil that causes large-scale defoliation of *Eucalyptus* trees. *Gonipterus* sp. n. 2 displays a very strong preference for certain *Eucalyptus* genotypes, however, this behaviour and the chemistry underlying it is poorly understood. To elucidate the feeding preference of *Gonipterus* sp. n. 2, we assessed the relative levels of susceptibility of 62 *Eucalyptus* genotypes from 23 species using a laboratory choice assay. We then selected a subset of 13 genotypes from two species spanning the full range of resistance for a semi-quantitative detailed metabolite profile analysis. The behavioural effects of the identified compounds were assessed through an *in vitro* feeding preference assay using artificial diets as well as under field conditions, using 12 unique genotypes. Which revealed three phagostimulants (1,8-cineole, oxalic acid, and sucrose) and two feeding deterrent compounds (shikimic acid and palmitic acid) for *Gonipterus* sp. n. 2. After determining the effects of constitutive foliar compounds, we wanted to assess both the chemistry and gene expression of the induced defense response caused by both feeding and oviposition of *Gonipterus* sp. n. 2 on a susceptible host. Which revealed an association between the weevil's oviposition secretions and the upregulation and accumulation of plant sugars and fatty acids.

Behavioral modulation of host plant provides saprophagous feeding opportunities to herbivorous longhorn beetle larva

Min-Soo Choi[1,2], Juhee Lee[3], Jeong-Min Kim[1,3], Sang-Gyu Kim[2], Youngsung Joo[1,3]
[1]School of Biological Sciences, Seoul National University, Seoul 00826, Republic of Korea
[2]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, 34141, Republic of Korea
[3]Department of Biological Sciences and Biotechnology, Chungbuk National University, Cheongju 28644, Republic of Korea

Feeding on living plants, a key innovation that resulted in the divergent evolution of insects, requires the circumvention of plant defensive barriers. This is typically achieved by chemical detoxification and sequestration of toxic secondary metabolites produced by plants. Nevertheless, insects exhibit various adaptive behaviors and the function of the behavior in feeding on living plants has been overlooked. Here, we studied the behavioral modulation of a host plant by a girdling longhorn beetle (*Phytoecia rufiventris* Gautier, Cerambycidae; Lamiinae) that generates partially dead tissue in the living plant. The dead tissue exhibited impaired defenses and accumulated nutrients, which dramatically increased larval growth through resource allocation from detoxification to growth. While girdling behavior was not beneficial to a non-girdling longhorn beetle (*Agapanthia amurensis*), it decreased the preference of *A. amurensis* on girdled plant which enabled *P. rufiventris* larva to avoid harmful competition. Collectively, our study suggests that the behavioral adaptation enables herbivorous larva to feed on dead tissue in living plants while excluding competitors.

Aggregation pheromone of *Cyrtogenius luteus*: An invasive bark beetle species damaging pine plantations in South America

Gianna Zinola[1], Jessica Pereyra[1], María Eugenia Amorós[1], Helena Pardo[2], Gustavo Seoane[3], Gabriela Piñeyro[4], Gonzalo Martínez[4], Andrés González[1]
[1] *Laboratory of Chemical Ecology, Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay*
[2] *Centro NanoMat, Facultad de Química, Instituto Polo Tecnológico de Pando, DETEMA, Universidad de la República, Montevideo 11800, Uruguay*
[3] *Laboratory of Organic Synthesis, Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay*
[4] *Laboratory of Entomology, National Forestry Research System, Instituto Nacional de Investigación Agropecuaria, Tacuarembó, Uruguay*

The asian bark beetle *Cyrtogenius luteus* (Curculionidae: Scolytinae) was first reported in pine forests in South America in 2012. In recent years, population outbreaks and damage in pine plantations have been reported in Uruguay. We here present our advances in the study of aggregation pheromones in *C. luteus*, and their potential use in its management. We conducted behavioral and chemical studies and identified a putative male aggregation pheromone. The candidate compound was synthezised and tested in the field in the fall and summer of 2023/24. Cross-vane traps were deployed in pine plantations with recent history of bark beetle damage. Generic host attractants (turpentine and ethanol) were used in combination with the synthetic pheromone. The pheromone was tested in different combinations and presentations. In low population levels (fall), the pheromone resulted in a remarkable 25-fold increase in adult captures in comparison with control traps baited with generic host volatiles. During the peak flight season (summer) the same comparison yielded a 5-fold increase in captures. The pheromone combined with ethanol (no turpentine) also showed high captures, whereas the pheromone by itself was not attractive. Finally, the pheromone inmersed in monolithic dispensers showed consistently higher captures than the control traps, but lower than traps with rubber septa, indicating that further development is needed for monolithic dispensers. The pheromone appears as a promissory tool for the management of *C. luteus*, both for early detection and surveillance under low population levels, as well as for mass trapping under high population conditions.

Chemical ecology of Capricorn beetles (Cerambycidae: *Cerambyx*)

Matic Gabor[1,2], **Alenka Žunič Kosi**[1], **Damjan Makuc**[3]
[1]Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia
[2]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [3]Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia

Despite the rich knowledge of pheromone use in longhorn beetles, surprisingly little is known about the chemical communication of Capricorn beetles (Cerambycidae: *Cerambyx*). As their saproxylic larvae bore tunnels into wood, Capricorn beetles are known for their role as ecosystem engineers. This study aims to identify their aggregation-sex pheromones and determine their role in reproductive isolation of six sympatric Capricorn beetle species in the Western Balkans. Additionally, effective management strategies are critically needed for *Cerambyx cerdo*, which is both protected and considered a pest. Developing a targeted monitoring tool based on pheromone lure could significantly improve monitoring efforts, which are essential for the species effective management. So far, we have studied three of the six species. Male aeration samples of *C. cerdo*, *Cerambyx scopolii*, and *Cerambyx miles*, contained compounds that elicited a response on the respective antennae of two species studied by gas-chromatography, coupled with electroantennography (GC-EAD). Using NMR spectroscopy, we determined the chemical structures of the sole compound emitted by *C. cerdo*, both compounds emitted by *C. scopolii*, and one of several emitted by *C. miles*. We will test the role of these compounds as aggregation-sex pheromones of the three species by laboratory and field bioassays and to extend our investigation to three additional species: *Cerambyx welensii*, *Cerambyx nodulosus*, and *Cerambyx carinatus*. The bioassays also aim to explore the potential involvement of their volatile compounds in mediating reproductive isolation and provide new insights into the evolution and ecology of Capricorn beetles.

The chemical communication of *Plagionotus* longhorn beetles (Cerambycidae) and possible practical applications

Zoltán Imrei[1], Zsófia Lohonyai[1,3], Mikael A. Molander[2], Eszter Matula[1,3], Éva Bálintné Csonka[1], Edit Orgován[1,3], Yunfan Zou[4], Zsófia Kováts[5], Gergely Janik[6], Krisztina Harmincz[5], Inis B. Winde[2], Michael J. Domingue[7], József Muskovits[8], Lawrence M. Hanks[9], József Fail[3], György Csóka[6], Mattias C. Larsson[2], Jocelyn G. Millar[4], Miklós Tóth[1]
[1]HUN-REN Centre for Agricultural Research, Plant Protection Institute, 1022 Budapest, Herman O. u. 15., Hungary, e-mail: imrei.zoltan@atk.hu
[2]Department of Plant Protection Biology, Swedish Univ. Agr. Sci., Alnarp, Sweden
[3]Department of Entomology, Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences, 1118 Budapest, Ménesi út 44., Hungary
[4]Department of Entomology, University of California, Riverside, CA 92521, USA
[5]Institute of Plant Protection, University of Debrecen, 4032 Debrecen, Böszörményi út 138., Hungary
[6]Department of Forest Protection, Forest Research Institute, Sopron University, 3232 Mátrafüred, Hegyalja út 18., Hungary
[7]Penn State University Department of Entomology, USA
[8]Independent, 1113 Budapest, Tardoskedd u. 9., Hungary
[9]University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

In Hungary, three species occur of longhorn beetles belonging to the genus *Plagionotus*, all of which are widespread in Europe. *P. arcuatus* and *P. detritus* are common species of oak forests, while *P. floralis* occurs in significant numbers in old, neglected alfalfa fields. All three species have a significant impact on their environment, which may also have economic implications, but due to the lack of suitable sampling methods, these are little known. A light green funnel trap with light-reflecting ability in the 500-550 nm wavelength range was combined with 1-phenethyl alcohol and 3-methyleugenol was developed suitable for monitoring purposes of *P. floralis*. From the pheromone emitted by the males of *P. arcuatus*, we identified hydroxyketone-type compounds forming a homologous sequence of (*R*)-3-hydroxyhexan-2-one, (*R*)-3-hydroxyoctan-2-one and (*R*)-3-hydroxydecan-2-one. According to our behavioral field studies, the combination of C6 and C10 compounds attracted both males and females demonstrating the identification of an aggregation pheromone. In the case of the males of *P. detritus*, we identified two hydroxyketone-type volatile compounds, (*R*)-3-hydroxyhexan-2-one and (*S*)-2-hydroxyoctan-3-one, the combination of which was demonstrated to be an aggregation pheromone, and attracted the predatory clerid *Clerus mutillarius* (Coleoptera: Cleridae). The attractant effect on *C. mutillarius* was also confirmed in the case of other longhorn beetle pheromone combinations, namely a generic attractant of multiple longhorn beetle species tested in a Central European oak forest, which suggests eavesdropping on the pheromones of cerambycids. Such attractant baits might be used to manipulate the local population density of predators.

Enhancing conservation strategies for endangered saproxylic beetles using pheromone-based tools

Alenka Žunič Kosi, Špela Ambrožič Ergaver, Andrej Kapla, Matjaž Bedjanič, Matic Gabor, Al Vrezec
Department of Organisms and Ecosystems Research, National Institute of Biology, Večna pot 121, SI-1000 Ljubljana, Slovenia

Saproxylic beetles are crucial for biodiversity conservation and are often used as indicators of forest ecosystem health. Given the threats of habitat loss and environmental change, there is an urgent need for effective conservation strategies for these species. This study aims to enhance conservation efforts for endangered saproxylic beetles through the use of pheromone-based monitoring and management strategies. Specifically, we investigated the effectiveness of a sex-specific aggregation pheromone of *Rosalia alpina* (Coloptera: Cerambycidae) in population assessments and conservation practices. Extensive field experiments were conducted, deploying pheromone-baited traps at various sites to test different pheromone concentrations for optimal trapping conditions. The study also examined the correlation between beetle flight activity and environmental variables, such as light intensity and temperature. Additionally, preliminary experiments were conducted to test the potential use of pheromones for manipulating oviposition site selection. Results indicated that pheromone-baited traps significantly increased the detection and capture rates of *R. alpina* compared to conventional methods. The traps were effective even at reduced pheromone doses, suggesting a cost-effective approach that minimizes the ecological impact. Environmental conditions, notably light intensity and temperature significantly influenced the flight activity of the beetles, with distinct patterns observed between males and females. Pheromone monitoring represents a significant advancement in the conservation of endangered saproxylic beetles. This method, particularly when combined with photo traps, offers a non-invasive, efficient, and scalable solution that can play a crucial role in biodiversity conservation initiatives.

Chemical ecology of acute oak decline

Jozsef Vuts[1], Gareth Thomas[1], Marine Cambon[2], James McDonald[2], John Caulfield[1], Sandra Denman[3]
[1]Protecting Crops and the Environment, Rothamsted Research, Harpenden, UK
[2]School of Biosciences, IMI, BIFoR, University of Birmingham, Birmingham, UK
[3]Forest Research, Centre for Forestry and Climate Change, Surrey, UK

Acute Oak Decline (AOD) poses a threat to native oak trees in the United Kingdom. Three bacterial pathogens are frequently isolated from necrotic stem lesions characterising AOD. Larval galleries of *Agrilus biguttatus* (Coleoptera: Buprestidae) commonly co-occur with these lesions, indicating beetles may be involved in vectoring AOD bacteria. Also, the presence of larvae increases lesion size, potentially triggered by larval chemical cues. Beetles fly to the tree crown, feed on leaves and mate upon maturation, gravid females then oviposit in bark crevices. Volatile organic compounds (VOCs) from healthy trees show a role for host location, although impacts on beetle behaviour of VOCs following AOD infection are unknown. Our hypotheses: 1) VOCs from symptomatic foliage attract beetles more so than healthy tree VOCs; 2) AOD bacterial VOCs attract gravid females to the stem of AOD trees; 3) chemical cues from *A. biguttatus* larvae trigger the growth and virulence of *Brenneria goodwinii,* the most abundant AOD-associated bacterium. VOC collections from AOD-symptomatic tree foliage contained four unique compounds that induced a behavioural preference in four-arm olfactometry assays in virgin females when added to healthy foliage. Olfactometry revealed greater preference of gravid female beetles to a combination of a synthetic bark blend plus headspace extract from AOD bacteria, compared to the bark blend alone, suggesting infected bark is more attractive than non-infected. Larval extracts from *A. biguttatus* elicited *B. goodwinii* growth and final cell density, as well as upregulated key virulence genes. This knowledge may help develop environmentally benign management strategies for AOD.

POSTERS

P-05

Response of *Picea abies* to simulate acute drought stress and its correlations with susceptibility to the bark beetles *Ips typographus* (L.) and *I. duplicatus*

Sara Basile[1], Barbora Stříbrská[1], Alina Kalyniukova[1], Jaromír Hradecký[1], Jiri Synek[1], Jonathan Gershersonn[2], Anna Jirošová[1]
[1]Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 16500 Prague, Czech Republic
[2]Max Planck Institute of Chemical Ecology, Jena, Germany

Central European Norway spruce monocultures (*Picea abies* L.) are threatened by violent outbreaks of bark beetles, such as *Ips typographus* (L.) and *I. duplicatus*. The overpopulation of bark beetles results from drought periods caused by climate change, weakening spruces' defense ability. Therefore, this study aimed to investigate Norway spruce's physiological and defense responses to acute drought stress and their susceptibility to *I. typographus* and *I. duplicatus*. Drought stress was simulated by covering the roots of trees with an artificial roof in April 2021 for five months, shielding them from rainwater while controlling trees that were naturally watered by rainfall. We continuously monitored the soil water potential, bark temperature, stem growth, and sap flow.

Furthermore, non-structural carbohydrates (NSCs), as resources for secondary metabolites and nutrition, and phenolics and terpenes as defensive metabolites were measured in July and August 2021 to identify tree responses. In addition, two distinct bioassays using single male *I. typographus* and *I. duplicatus* were conducted to evaluate host selection. The results showed that roofed trees exhibited drought stress in July, decreased tree trunk growth, and increased NSC content. Phenolics and Terpenes were unaffected except for an increase in diterpenes. In bioassay, *I. typographus* preferred boring into the bark of roofed trees in August in tubes and in September in boxes. This beetle acceptance correlated with increased levels of NSC in the phloem. Both species showed higher mobility in the boxes experiment in August and September on roofed trees but not in July.

P-07

Beech bark beetle *Taphrorychus bicolor* and the host volatiles of *Fagus sylvatica*

Jaromír Bláha, Michal Neugebauer, Jaromír Hradecký
Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, Czech Republic

In recent decades, there has been significant stress in forest stands, with more frequent disturbances and bark beetle calamities. Both coniferous and deciduous trees are stressed. Most attention has been aimed to the main commercial tree species (spruce, pine) and their bark beetles, while the deciduous tree bark beetles has been poorly studied. We focused on the economically most important deciduous tree species in the Czech Republic, the European beech *Fagus sylvatica* L., and its bark beetle *Taphrorychus bicolor* (Herbst, 1793). We collected volatiles from branches and leaves of European beech using the dynamic headspace method, and the collected compounds were dissolved in hexane. The obtained sample was analyzed using GC-EAD (gas chromatography coupled with electroantennographic detection). The separated compounds were released onto the antenna of *Taphrorychus bicolor* connected to glass capillary electrodes filled with Ringer's solution. We found that the bark beetle *Taphrorychus bicolor* responded to the compounds *trans*-2-hexenal, eucalyptol, γ-terpinene, and terpinolene. However, the behavioral answer of these compounds still needs to be investigated. Surprisingly, the bark beetle *Taphrorychus bicolor* did not respond to important monoterpenes found in the mixture of volatiles of conifers and deciduous trees, such as pinenes, camphene, limonene, sabinene, phellandrene, and others. These monoterpenes are significant for the orientation of spruce bark beetles such as *Ips typographus* and *Ips duplicatus*.

P-12

Chirality of α-pinene: Switch from plant kairomone to aggregation pheromone in the cypress bark beetle, *Phloeosinus aubei*

Gábor Bozsik[1], Armin Tröger[2], Stefan Schulz[3], Béla Péter Molnár[1], Kristóf Hegedüs[4], Tibor Soós[4], Anna Teski[1], Gábor Szőcs[1]
[1]Department of Chemical Ecology, Plant Protection Institute, Centre for Agricultural Research, HUN-REN, Budapest, Hungary
[2]Institute for Organic Chemistry, University of Hamburg, Hamburg, Germany
[3]Institute of Organic Chemistry, TU Braunschweig, Braunschweig, Germany
[4]Institute of Organic Chemistry, Research Centre for Natural Sciences, Budapest, Hungary

The cypress bark beetle (CBB), (*Phloeosinus aubei*), (Coleoptera: Curculionidae: Scolytinae) is a native pest of Cupressaceae in the Mediterranean region. Its recent expansion northwards in Europe is associated with severe damage to scale-leafed conifers in ornamental tree nurseries and in urban green areas.

In search for host plant kairomones, we collected volatiles from drought-stressed stems of the American arborvitae, *Thuja occidentalis* cv. 'Smaragd' trunks, preferred by females for building breeding galleries. GC-EAD studies revealed that CBB females perceive highly volatile components of plant odor. The most abundant compound was α-pinene, emitted by *Thuja* as a racemic mixture of (–)- and (+)-enantiomers, in a ratio of 2:1 (chiral GC-MS).

In search of the aggregation pheromone, unmated females were removed from nuptial chambers (entrance hall of breeding galleries), mid- and hindguts were excised and extracted with hexane. GC-EAD analysis showed that α-pinene and myrtenol elicited strong antennal responses. GC-MS analysis using a chiral column showed that CBB produces enantiomerically pure (–)-α-pinene and (–)-myrtenol. Behavioral tests in a four-choice arena olfactometer and in field trapping tests demonstrated that both compounds act together in the aggregation pheromone system.

Our results indicate that CBB uses pure (–)-α-pinene, as an aggregation pheromone component in a background plant odor, dominated by racemic α-pinene.

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The impact of flight on subsequent semiochemical-mediated communication in the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytinae)

Maya Evenden[1], Kelsey Jones[1], Leanne Petro[1], Antonia Musso[1], Rahmatollah Rajabzadeh[2], Guncha Ishangulyyeva[2], Nadir Erbilgin[2]
[1]Department of Biological Sciences, University of Alberta, Edmonton, Canada
[2]Department of Renewable Resources, University of Alberta, Edmonton, Canada

Flight polyphenisms occur as discrete or continuous traits in insects. The mountain pine beetle (*Dendroctonus ponderosae*) exhibits polyphenic variation in flight distance but consequences of flight variation on subsequent semiochemical-mediated communication is unknown. This study assessed the effect of flight on pheromone production in male and female beetles, and orientation response to host volatiles and pheromones. A 23-h flight treatment was applied to beetles using computer-linked flight mills. In experiment 1, both flown and unflown (control) beetles entered lodgepole pine bolts, which were aerated to collect pheromone. In experiments 2 and 3, flown and unflown female beetles were assayed in a 4-way olfactometer to measure their response to lodgepole pine and the aggregation pheromone *trans*-verbenol, respectively. Production of *trans*-verbenol by female beetles was influenced by both percent weight lost during flight and flight distance. Male production of *exo*-brevicomin was affected by beetle condition following flight but not by the energy used during flight. Flight treatment did not directly affect orientation to host volatiles, but the energy use during flight and the body condition of beetles after flight did. Beetles with higher lipid content that were in better condition following flight treatment spent less time near the phloem sources than beetles with low lipid content in poor condition. In contrast, orientation to aggregation pheromone was not influenced by flight treatment. These novel results give new insight into the effect of energy use through flight on semiochemical-mediated behaviours and the polyphenic flight of mountain pine beetles.

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Functional analysis of odorant-binding proteins XaffOBP9 and XaffOBP10 in *Xyleborus affinis*

Qian Wang, Kai Zhang, Qi Wu, Zheyuan Xu, Linan Deng, Xiang Zhou, Jixing Guo
School of Tropical Agriculture and Forestry, Hainan University, Danzhou, China

Xyleborus affinis is a major pest of rubber trees in the Hainan rubber planting area of China. This insect relies on its sense of smell to carry out important activities, including locating hosts and finding mates. To understand the molecular basis of odor recognition, we identified and analyzed two odorant binding proteins (OBPs), XaffOBP9 and XaffOBP10, which are highly expressed in *X. affinis*. Sequence analysis revealed that XaffOBP9 has a 411 bp open reading frame (ORF) encoding 136 amino acids, while XaffOBP10 has a 402 bp ORF encoding 133 amino acids. Both XaffOBP9 and XaffOBP10 belong to the Minus-C OBPs. Fluorescence binding assays showed XaffOBP9's strong affinity for 14 ligands, particularly camphene, □-pinene, and Myrcene, while XaffOBP10 showed no binding. Molecular docking revealed that XaffOBP9 mainly bound to the ligands through hydrophobic and van der Waals force interactions. Three amino acids, Leu71, Tyr106, and Leu114, played a role in the binding of XaffOBP9 to various ligands and in the formation of the binding pocket. Site-directed mutagenesis confirmed Leu114 as the key binding site for XaffOBP9, with Tyr106 and Leu71 playing supporting roles. In conclusion, this study analyzed the sequence and binding characteristics of XaffOBP9 and XaffOBP10 with semiochemicals, which may be helpful for the integrated management of *X. affinis*.

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What is in the belly of the beast: The gut microbial community of the *Eucalyptus* snout beetle

Rosa Sophie Knoppersen[1,3], **Teresa Anne Coutinho**[1,3], **Almuth Hammerbacher**[2,3]
[1]*Department of Biochemistry, Genetics and Microbiology*
[2]*Department of Zoology and Entomology*
[3]*Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*

The Eucalyptus snout beetle is an invasive insect pest that causes extensive damage to *Eucalyptus* plantations in South Africa, among other countries. The beetle has a preference for young *Eucalyptus* leaves, which contain bioactive essential oils. Despite the toxicity of these compounds, the Eucalyptus snout beetle can tolerate and overcome high concentrations of essential oils across different *Eucalyptu*s hosts. While the beetle's inherent detoxification mechanisms remain unknown, there is an indication that the gut microbial community of *Gonipterus* might be involved in these processes. To investigate this hypothesis, we catalogued the microbial communities of leaves from two biochemically different *Eucalyptus* genotypes, as well as the gut and frass of beetles fed on the leaves of these genotypes. We additionally conducted a comparative study of terpene profiles in *Eucalyptus* leaves and frass to determine the detoxification products following digestion. Metabolic analysis between leaves and frass indicated significant decreases in terpenoids with subsequent increases in microbial-based biotransformed products within the frass. Metabarcoding results revealed that, despite host differences, the gut microbiota of the beetles was consistently dominated by Saccharomycetaceae and Enterobacteriaceae. Many of these members are known to include genes associated with plant secondary metabolite detoxification, suggesting a crucial role of the beetle's gut microbiome in overcoming *Eucalyptus* defences.

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3-Carene metabolism: A potential target for RNAi-based *Ips typographus* pest management

Aisha Naseer[1], Vivek Vikram Singh[1], Gothandapani Sellamuthu[1], Kanakachari Mogilicherla[1,2], Amit Roy[1]
[1]*Faculty of Forestry and Wood Sciences, Czech University of Life Sciences in Prague, Prague, Czech Republic*
[2]*ICAR-Indian Institute of Rice Research (IIRR), Rajendra Nagar, Hyderabad, Telangana, India*

Ips typographus (ESBB) is an important forest pest of *Picea abies* (Norway spruce) that has been causing catastrophic ecological and economical loss in central European forests, despite a well-elaborated chemical defense system of its host trees. 3-carene is an important monoterpene produced abundantly as an induced defense in *P. abies*. ESBB successfully infest the host in a coordinated mass attack owing to its complex detoxification machinery. We assessed individual spruce monoterpenes for their toxicity on ESBB and identified that 3-carene has the lowest LC70 value. Further, the transcriptomics and proteomics study of 3-carene fumigated ESBB revealed common genes between proteo-transcriptomic analysis involving up-regulation of detoxification and defense genes, and downregulation of developmental genes. We applied various molecular techniques to validate and co-relate our results. In addition, we aim to apply RNAi-based gene silencing method to functionally validate some of the genes involved in detoxification.

Keywords: *Ips typographus*, 3-carene, RNA-seq, proteomics, RNAi

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The roles of volatile organic compounds in tri-trophic interactions along altitudinal gradient in *Salix* species

Shristee Panthee[1], **Sofian A. Renoult** [2], **Monica Barman**[1], **Martin Volf** [2], **Nicole M. van Dam**[1]
[1]Plant Biotic interactions, Leibniz Institute for Vegetables and Ornamental Crops (IGZ) e.V., Großbeeren, Germany
[2]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

Biotic and abiotic factors vary along altitudinal gradients influencing volatile organic compound (VOCs)-mediated multi-trophic interactions. Similarly, the large changes in community composition that plants and insects exhibit along an altitudinal gradient influence the dynamics of tri-trophic interactions. At lower elevations, VOC-mediated interactions between plants, insect herbivores, and their predators are typically more intense due to higher temperatures. In contrast, the intensity of such interactions gradually diminishes at higher elevations. This suggests that VOCs have distinct ecological roles at different elevations. Altitudinal gradients are unique natural settings to investigate the tri-trophic interaction patterns mediated by VOCs under varying biotic and abiotic environments. In our study, we predicted that the VOC emission and predation rates would vary with altitude, exhibiting lower VOC emission and predation rates at high altitudes and higher VOC emission and predation rates at low altitudes. We used Methyl Jasmonate (MeJA) to induce VOC emissions from 111 *Salix* trees. Furthermore, to observe tri-trophic interaction in field conditions, we placed 1332 dummy caterpillars in three distinct colors (green, brown, and yellow) and measured predation rates along an altitudinal gradient. VOCs were sampled using PDMS tubes and processed in TDU-GC-MS TD-GC-MS consisting of a DB-Wax capillary column. By combining predation rates with MeJA-induced VOC profiles we analysed how changes in VOC emissions over an altitudinal gradient relate to the type and intensity of predation. Understanding the tri-trophic interaction dynamics will help in comprehending the ecological functions of VOCs with altitude. S.P. and N.M.v.D.

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Characterization of olfactory sensory neurons in non-aggressive *Ips cembrae* and *Ips acuminatus* and its comparison to aggressive *Ips typographus*

Mayuri Kashinath Shewale[1], **Dineshkumar Kandasamy**[2], **Jiří Synek**[1], **Martin N. Andersson**[2], **Anna Jirošová**[1]
[1]*Czech university of life sciences Prague, Faculty of forestry and wood sciences, Prague, Czech Republic*
[2]*Department of Biology, Lund University, Lund, Sweden*

Coniferophagous bark beetles spend most of their life inside host tissues, with many species being economically important pests, causing widespread tree mortality. Their behaviour is mainly driven by complex olfactory signals, with the aggregation pheromone being the most prominent along with host, non-host (NHVs) and microbial volatiles. These signals are detected by olfactory sensory neurons (OSNs) housed in hair-like sensilla on the antenna. The peripheral olfactory physiology is unknown in many *Ips* species, but well-studied in the aggressive spruce bark beetle, *I. typographus* (IT). Here, we studied *I. cembrae* (IC), a primary pest of European larch trees, and *I. acuminatus* (IAc), which infests Scots pine. We screened responses of OSNs present in antennal olfactory sensilla using single sensillum recordings (SSR) in these two species and compared with existing data from *I. typographus*. Odor stimuli included 57 ecologically relevant compounds, including interspecific and intraspecific pheromones, host, and non-host compounds. Our aim was to find out the most abundant OSNs in the two species and whether the arrangement of OSNs on the antenna was conserved across species. Most of the identified OSN classes in IC and IAc were primarily tuned to pheromone compounds: ipsdienol, ipsenol, amitinol, and *cis*-verbenol, respectively. One class primarily responded to isopinocamphone with other similar oxygenated monoterpenes, and a few OSN classes responded to NHVs. Interestingly, we found no primary OSN class responding to any of the host monoterpenes in either species. Overall, our results add knowledge to peripheral olfactory detection of chemical cues in IC and IAc.

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Selection of candidate carboxylesterase genes involved in the biosynthesis of pheromonal *cis*-verbenol in the bark beetle *Ips typographus*

Jaroslav Strádal[1], **Rajarajan Ramakrishnan**[1], **Ondřej Lukšan**[2], **Michal Tupec**[2], **Jiří Synek**[1], **Stanislav Macháček**[2], **Iva Pichová**[2], **Anna Jirošová**[1]
[1]*Department of Forest Protection and Entomology, Czech University of Life Sciences Prague (CZU), Prague, Czech Republic*
[2]*Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic*

The aggregation pheromone of the European spruce bark beetle *Ips typographus* (Linnaeus, 1758), a critical factor in the coordination of the beetle's mass attacks, comprises key components including 2-methyl-3-buten-2-ol, ipsdienol, and *cis*-verbenol. The latter is notably synthesized from the tree host precursor α-pinene via a hydroxylation as part of the detoxification process. Recent advancements, such as high-quality transcriptomic analysis of *Ips typographus*, have led to the proposition of a second, alternative *cis*-verbenol biosynthetic pathway. This pathway involves a novel mechanism for the storage and release of *cis*-verbenol from fatty acid ester conjugates, potentially mediated by as-yet-unidentified carboxylesterases (CarEs). This discovery elucidates how pioneer beetles can emit *cis*-verbenol independently of the host-derived α-pinene. Despite these advances, the specific genes responsible for CarEs secretion remain unknown.

To address this knowledge gap, we have sequenced various life stages of the bark beetle. In the obtained transcriptome, through local alignment and phylogenetic analysis, all homologous sequences to previously characterized esterizes have been selected. In the final step, differential gene expression analyses were conducted. This approach allowed us to select two genes possibly secreting enzymes that make verbenyl ester conjugates and two responsible for their hydrolysis in pheromone-producing males. The expression of these genes was validated in relevant beetle stages using qPCR. The aim of this work was to select possible candidate genes involved in this unique pheromone biosynthetic step and to functionally characterize them in the future.

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Defense chemistry and physiological responses of mature Norway spruce (*Picea abies*) to bark beetle (*Ips typographus*) infestation: Potential parameters for early attack detection method

B. Stříbrská, J. Čepl, J. Hradecký, A.A.C. Moliterno, A. Jirošová
Faculty of Forestry and Wood Sciences, Excellent Team for Mitigation, Czech University of Life Sciences Prague, Czech Republic

Outbreaks of the Eurasian Spruce Bark Beetle (*Ips typographus*) severely impact Norway spruce forests in Central Europe. Forest damage is exacerbated by abiotic disturbances and fragmentation, worsening spruce forest vulnerability. This presentation investigates how Norway spruce trees respond to bark beetle infestations, examining defense chemistry parameters, physiology, and factors related to bark beetle attraction and tree acceptance. Our goal is to provide ecological insights into these processes and propose innovative methods for the early detection of *Ips typographus* attacks. We monitored and sampled freshly infested and non-infested Norway spruce trees in central Czechia. We conducted bioassays with living beetles. Following an attack, we observed a sudden surge in monoterpene emissions, reductions in sap flow, terminated tree stem increment, and increased bark temperature. The rapid increase in monoterpene emissions shows promise for developing a novel early detection methodology for bark beetle attacks. Therefore, we conducted a detailed study of the distribution of these compounds. The major monoterpene α-pinene was selected as representative, and its content in the air surrounding infested trees was collected using two analytical techniques: dynamic absorption to cartridges and solid-phase microextraction (SPME) and quantified by GCMS. Lab trials were conducted to optimize the method, and further detection of α-pinene distribution around infested spruce trees in real forest conditions was carried out. This resulted in the construction of a three-dimensional map of α-pinene distribution around infested trees, providing foundational knowledge for potential large-scale bark beetle detection. These findings pave the way for alternative pest management methods using scanning technologies.

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Everything you always wanted to know about bark beetles rearing, but were afraid to ask

Jiří Synek, **Jaromír Bláha, Roman Modlinger**
Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Czech Republic

Bark beetles (Curculionidae: Scolytinae) cause large-scale forest destruction and considerable ecological and economic damage. They are a widely studied group that have a high degree of sociality and chemical communication, but there is still much unknown. Thus, there is a lot of pressure to have a constant year-round supply of fresh and living insects for laboratory and field experiments. Therefore, it is necessary to establish laboratory rearings capable of producing thousands of bark beetles per year. At FLD, CZU, breedings were created in which we focused mainly on the Ipini tribe, and the aim of this poster is to share the methodology so others can avoid the problems that we faced. The rearings consist of five rooms: Main breeding room, dirty lab, clean lab, and two cold rooms. In the main breeding room, it is necessary to control the temperature, humidity, lighting and, above all, air circulation. In the dirty laboratory, the initial acceptance of the material takes place, as well as the processing of logs and the development of the F0 generation. Due to the large production of wood shavings, it is not possible to work with microscopes here, and therefore a clean laboratory is important. Separate cooling rooms for infested and uninfested logs are important to keep the infested logs fresh for as long as possible and also to slow down the development. Since the establishment of the rearings five years ago, more than 15 species of bark beetles have been continuously bred on deciduous and coniferous trees.

P-82

Traps with synthetic, long lasting bisexual generic attractants as a new method supplementing light traps for assessing composition and sex ratios of Macroheterocera assemblages

Szabolcs Szanyi[1], Zoltán Varga[2], Júlia K. Jósvai[3], Miklós Tóth[3], Antal Nagy[1]
[1]University of Debrecen, Faculty of the Agricultural and Food Sciences and Environmental Management, Institute of Plant Protection, Debrecen, P. O. Box 400, H-4002, Hungary
[2]Department of Evolutionary Zoology and Human Biology, University of Debrecen, H-4032 Debrecen, Egyetem tér 1. Hungary
[3]Plant Protection Institute, CAR, ELKH, Budapest, P. O. Box. 102, H-1525, Hungary

In the IPM strategy many traps are used for pest monitoring. Lepidopterans are regularly sampled with light- and then sex pheromone traps. The weakness of these methods can be eliminated by using attractant-baited traps that can catch many species but are more selective than light traps. They are as simple as sex pheromone traps but can attract both sexes. In studies on the effectiveness and selectivity of lures made in the last 10 years high number of non-pest Noctuidae were caught, that pointed out the alternative use of the tested lures in faunistical and ecological studies.

In the forest of Velyka Dobron' (W. Ukraine) we compare the performance of light and lured traps to study whether the lured traps can give additional information on the faunal composition and sex ratios.

During the study more than 330 Macrolepidoptera species were caught. The selectivity and efficiency of traps shoved significant differences. The light trap had the widest range, but the two tested lures also could attract more than 120 species with different selectivity. Our result proved that the parallel use of light and lured traps provides detailed faunistical data and can unravel structural and functional traits (e.g. sex ratios) of the assemblages strengthening the ecological background of both biodiversity- and plant protection monitoring.

Szabolcs Szanyi's research was financed by the National Research Development and Innovation Office (NKFIH, grant PD 138329).

S5: Beyond eusocial animals: chemical ecology of group living and subsocial species

Chair: *Sandra Steiger*

ORAL PRESENTATIONS

Carrion volatiles – Ecological succession and temperature-dependent competition among burying beetles

Stephen T. Trumbo[1], **Sandra Steiger**[2]
[1]*Dept. Ecology and Evolutionary Biology, University of Connecticut, Waterbury, USA*
[2]*Lehrstuhl für Evolutionäre Tierökologie, Universität Bayreuth, Bayreuth, Germany*

Burying beetles (Silphinae) utilize sulfur-based volatiles produced by microbes to locate resources for feeding (well-rotted carrion) and for breeding (small, fresh vertebrate carcass). We used both single-species and community-wide studies to examine how beetles respond to and manipulate these volatiles, and to anticipate changes related to a warming climate. Methyl thiocyanate was an important attractant for breeding beetles, while dimethyl trisulfide attracted feeders but deterred breeders. When breeding *Nicrophorus orbicollis* locate and bury a carcass during a nesting attempt, they alter the microbial community on the carcass so that less attractant and more deterrent volatiles are produced. This is hypothesized to reduce competition from rivals that would take over the carcass and kill any young in the nest. In a community-level study, designed to simulate the release of higher amounts of volatiles associated with a warming climate, carcasses supplemented with methyl thiocyanate were more attractive to four species of burying beetles. The smallest species, however, was displaced more often and won fewer resources for breeding. The results suggest that behaviorally subordinate species may be impacted by more intense interspecific competition with climate change, and their geographic range is likely to shift in response.

Guardians of the grave: Microbial management in burying beetles and its implications for carrion preservation and rival avoidance

Eric Grubmüller, Sandra Steiger
Evolutionary Animal Ecology, University of Bayreuth, Bayreuth, Germany

Burying beetles (*Nicrophorus* sp.) belong to a wide range of arthropods engaging in parental care. During the breeding process, both parents exhibit various care behaviors, including pre-hatching care, such as preparing a small vertebrate carcass as a food source for their offspring, and post-hatching care, such as the regurgitation of carrion food to the larvae. While the larvae benefit in terms of growth and survival from post-hatching care, the exact function of the parents' investment in pre-hatching care remains unclear. Recent studies suggest that pre-hatching care and the associated microbial management via excretions have, on one hand, positive effects as they manipulate the cadaveric VOC emissions to conceal the carrion from rivals, but also negative effects as they impede larval self-feeding by forming an inaccessible ball and removing microbes that act pre-digestively. Here, we show in *Nicrophorus vespilloides* that when using natural forest soil as a substrate, larval survival was much higher on parentally prepared than non-prepared carcasses. Moreover, we found that parentally prepared cadavers emitted different amounts of sulfur-based volatiles, already known for their attracting or deterring effects on *Nicrophorus*, than aged carcasses. Finally, a field experiment revealed that the aged and prepared carcasses were discovered at different frequencies by rivals. In summary, our results reveal that the management of the microbial community in the pre-hatch phase serves a double function: it makes the carcass a less toxic environment and manipulates the VOC emissions to conceal the carcasses and offspring from rivals.

Scent-sational drama: Larval chemical signals and their effect on maternal behavior

Jacqueline Sahm[1], Beatrice Brobeil[1], Eric Grubmüller[1], Taina Conrad[1], Matthias Schott[2], Johannes Stökl[1], Sandra Steiger[1]
[1]Department of Evolutionary Animal Ecology, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany
[2]Department of Animal Ecology I, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany

Parents and offspring in family associations face conflicts over the optimal level of parental investments and resource allocation. Those conflicts can be mediated by offspring signals reflecting their nutritional need, manipulating the parents to adjust their care behavior, and resolving the parent-offspring conflict. Insects mainly use chemical signals to regulate their social life including living in family associations. However, previous studies have primarily focused on the chemistry of adults, examining the effects of biotic and abiotic factors on their chemical profiles, while largely neglecting chemical substances released by offspring. Using *Nicrophorus vespilloides* as a study system, a beetle species which provides elaborate biparental care, we studied the changes in volatile organic compounds (VOCs) and cuticular hydrocarbon (CHC) profiles during the larval development to determine if larval chemistry reflects their nutritional need. We found that larval cuticular and VOC profiles change with the developmental and nutritional state of offspring. Second instar larvae – which receive the highest feeding rates compared to the other larval instars – produce the highest levels of specific volatiles. Further, food deprived larvae express higher levels of specific volatiles compared to fed larvae. Our results suggests that larvae can communicate their nutritional state to their parents, potentially influencing parental care decisions. Using electroantennography and a novel bioassay, we could show that female react to larval odor and change their behavior. Therefore, our study revealed promising candidates for a begging pheromone which affects mother-offspring interactions and with that the resolution of parent-offspring conflict.

The chemistry of reciprocal protection in an animal-microbe symbiosis

Bernal Matarrita-Carranza[1], **Chantal Selina Ingham**[2], **Tobias Engl**[1,2], **Martin Kaltenpoth**[1,2]
[1]Department of Insect Symbiosis, Max Planck Institute for Chemical Ecology, Jena, Germany
[2]Department of Evolutionary Ecology, Institute of Organismic and Molecular Evolution, Johannes Gutenberg-University Mainz, Mainz, Germany

Symbiotic associations with microbes are important driving forces of evolutionary innovation. In particular, many insects harbor beneficial symbionts that are transmitted vertically across generations and contribute to their host's nutrition, digestion, detoxification, or defense. However, the chemical mediators controlling transmission, ensuring specificity, and protecting the symbionts from environmental stressors and host immune effectors remain poorly understood. In the protective alliance between beewolf wasps and antibiotic-producing *Streptomyces* bacteria, we observed a localized transcriptional profile in the symbiont-bearing antennal gland reservoirs that appears to ensure specificity during transmission from the reservoirs to the brood cell via specific immune effectors. During the subsequent period in the brood cell environment, the symbionts are exposed to high concentrations of nitric oxide that the beewolf egg produces to kill antagonistic fungi. When investigating how the symbiont manages to survive these hostile conditions, we discovered that the beewolf protects the bacteria from nitric oxide by embalming them with the hydrocarbon-rich antennal gland secretion that serves as a diffusion barrier against the toxic gas. Our results uncover host chemical mediators underlying the maintenance and specificity of a protective alliance and thereby ensuring its stability over long evolutionary timescales.

Impact of host species on cuticular hydrocarbon profiles in the parasitoid wasp *Leptopilina victoriae*: Implications for sexual communication

Johannes Stökl
Department of Evolutionary Animal Ecology, University of Bayreuth, Bayreuth, Germany

Insect CHCs exhibit significant phenotypic plasticity that is influenced by a variety of environmental and internal factors. One notable factor is diet, which can significantly alter CHC composition and, in turn, impact CHC-based chemical communication. This effect may be especially pronounced in parasitoid wasps, whose dietary options are limited to their host organisms. Despite its potential importance, only a few studies have investigated the role of the host on CHC profiles and chemical communication in parasitoid wasps. *Leptopilina victoriae* is a parasitoid wasp that parasitizes *Drosophila* larvae. In this species, CHCs also act as female sex pheromone that facilitates sex recognition and triggers male courtship. Our research demonstrates that male and female *L. victoriae* exhibit distinct CHC profiles when reared on *Drosophila melanogaster* and *Drosophila simulans*, respectively. This divergence emerges as early as one generation after a change in host species. Additionally, we found that the fatty acid composition varies between *D. melanogaster* and *D. simulans* larvae, potentially explaining the observed differences in CHCs. Despite the significant variations in CHC profiles among females reared on different hosts, our study found no difference in the duration of male courtship behavior when males were exposed to females from the same or a different host species.

Consequences of intraspecific mate recognition signal variability

Maximilian Steurer, Simon Franke, Joachim Ruther, Tamara Pokorny
Institute of Zoology, University of Regensburg, Regensburg, Germany

In many insects, mate recognition is conferred by chemical signals and cues. Reliable recognition of suitable mates can be accomplished through a tight coordination between signal and receiver. Thus, intraspecific variability of sex pheromones should be low. However, many species rely on cuticular hydrocarbons (CHCs) for close-range mate recognition, even though the CHC profile can be affected by several biotic and abiotic factors. The consequences of the resulting variability of CHCs used for mate recognition are hitherto largely unknown. To investigate the effect of CHC variability, we studied a parasitoid wasp in which males use the females' CHCs for close-range mate recognition. Additionally, this species shows a natural, within-population CHC polymorphism. Females exhibit one of three distinct CHC phenotypes (chemotypes), one of which is similar to male-typical CHC profiles. While male-derived CHCs were not bioactive, males reacted with copulation attempts to CHCs of females of all three chemotypes. Choice assays revealed a preference hierarchy towards female-derived CHC profiles. With the chemotype most similar to male CHC profiles, interestingly, being the most attractive. Bioactivity of artificial mixtures of female-derived CHCs was similar to that of chemotype-pure female CHC profiles. In contrast, the addition of male-derived CHCs to those of the most attractive female chemotype reduced male responses. While female quality was not directly linked to female chemotype, our results indicate that the entire, complex composition of the CHC profile is evaluated for mate recognition and assessment.

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Leaf-damaging behavior is widespread among queens and workers of different bumblebee species

Priska Flury[1], Sofie Stade[1], Consuelo M. De Moraes[1], Mark C. Mescher[1]
[1]Department of Environmental Systems Sciences, ETH Zürich, 8092 Zürich, Switzerland

Recently, it has been shown that bumblebee workers damage leaves of flowerless plants in a very characteristic way when facing pollen scarcity [1]. This leaf damage was found to accelerate flower production of two plant species, mustard (*Brassica nigra*) and tomato (*Solanum lycopersicum*) [1]. However, the ecological and adaptive importance of this plant-pollinator interaction remains uncertain. We explored the leaf-damaging behavior of queens of different life cycle stages, followed by behavioral assays with wild-caught queens to determine the taxonomic distribution of the behavior.

We show that leaf-damaging behavior is performed by young (unmated and mated) queens of *Bombus terrestris* and that the effect on flower production is comparable to worker inflicted damage. Furthermore, we report that wild-caught queens from 12 species of variable ecology, natural history and social organization damage leaves, suggesting a widespread distribution of the behavior.

Taken together, our results indicate that leaf-damaging is a typical component of the behavioral repertoire of many *Bombus* species. This widespread distribution and the observed effects on flower production suggest an adaptive significance of leaf-damaging behavior for bumblebees. It might be especially relevant in early spring when flowering plants are scarce and queens are solely responsible for establishing a new colony.

[1] Pashalidou FG, Lambert H, Peybernes T, Mescher MC, De Moraes CM. Bumble bees damage plant leaves and accelerate flower production when pollen is scarce. Science. (2020) May 22;368(6493):881-884. doi: 10.1126/science.aay0496. PMID: 32439792

S6: Chemical ecology of symbiotic interactions

Chair: *Martin Kaltenpoth*

ORAL PRESENTATIONS

Resource competition regulates the cnidarian-algal symbiosis

Nils Rädecker
École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

The evolutionary success of the cnidarian-algal symbiosis has given rise to the formation of coral reef ecosystems. Yet, climate change and other anthropogenic impacts are disrupting this symbiosis at increasing frequency and scale. Identifying the causes of this symbiotic collapse requires understanding the processes that maintained this endosymbiosis in the first place. Combining physiological, omics, and NanoSIMS imaging approaches, we investigated the metabolic regulation of symbiotic interactions in the stable and stressed cnidarian-algal symbiosis. We show that, in a stable state, mutualistic nutrient exchange in the symbiosis is passively maintained by competition for inorganic nutrients between the host and its symbionts. A breakdown of this resource competition during heat stress, in turn, destabilizes nutrient cycling and, thus, the symbiosis itself. Taken together, we conclude that the functioning of this symbiosis is rooted in nutritional feedback between heterotrophic and phototrophic metabolisms. While this passive regulation has underpinned the evolutionary success of corals for millions of years, it also renders these organisms highly vulnerable to the rapid environmental change of the Anthropocene.

Toxin-producing endosymbionts protect soil fungi from micropredators

Ingrid Richter[1], **Hannah Büttner**[1], **Sarah P. Niehs**[1], **Silvia Radosa**[2], **Koen Vandelannoote**[3], **Zoltán Cseresnyés**[4], **Iuliia Ferling**[2], **Benjamin Dose**[1], **Ruman Gerst**[4], **Marc Thilo Figge**[4, 5], **Timothy P. Stinear**[3], **Sacha J. Pidot**[3], **Falk Hillmann**[2], **Christian Hertweck**[1, 5]
[1]*Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany*
[2]*Junior Research Group Evolution of Microbial Interactions, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany*
[3]*Department of Microbiology and Immunology at the Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia*
[4]*Research Group Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany*
[5]*Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University Jena, Jena, Germany*

The phytopathogenic fungus *Rhizopus microsporus* harbors a bacterial endosymbiont (*Mycetohabitans rhizoxinica*) for the production of the toxin rhizoxin, the causative agent of rice seedling blight. This toxinogenic bacterial-fungal alliance is, however, not restricted to plant disease, but has been detected in numerous environmental isolates from geographically distinct sites covering all five continents. Yet, the ecological role of rhizoxin beyond rice seedling blight has been unknown.

Here we show that rhizoxin serves the fungal host in fending off protozoan and metazoan predators. Fluorescence microscopy and co-culture experiments with the fungivorous amoeba *Protostelium aurantium* revealed that ingestion of *R. microsporus* spores is toxic to *P. aurantium*. This amoebicidal effect is caused by the bacterial rhizoxin congener rhizoxin S2, which is also lethal towards the model nematode *Caenorhabditis elegans*. By combining stereomicroscopy, automated image analyses, and quantification of nematode movement we show that the fungivorous nematode *Aphelenchus avenae* actively feeds on *R. microsporus* that is lacking endosymbionts, whereas worms co-incubated with symbiotic *R. microsporus* are significantly less lively.

This work uncovers an unexpected ecological role of rhizoxin as a shield against micropredators. This finding suggests that predators may function as an evolutionary driving force to maintain toxin-producing endosymbionts in non-pathogenic fungi.

The potyviral protein 6K2 from turnip mosaic virus increases plant resilience to drought

Ved Prakash[1], **Chad T. Nihranz**[2], **Clare L. Casteel**[2]
[1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA
[2]Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Sciences, Cornell University, Ithaca, NY, USA

Virus infection can increase drought tolerance of infected plants compared with noninfected plants; however, the mechanisms mediating virus-induced drought tolerance remain unclear. In this study, we demonstrate turnip mosaic virus (TuMV) infection increases *Arabidopsis thaliana* survival under drought compared with uninfected plants. To determine if specific TuMV proteins mediate drought tolerance, we cloned the coding sequence for each of the major viral proteins and generated transgenic *A. thaliana* that constitutively express each protein. Three TuMV proteins, 6K1, 6K2, and NIa-Pro, enhanced drought tolerance of *A. thaliana* when expressed constitutively in plants compared with controls. While in the control plant, transcripts related to abscisic acid (ABA) biosynthesis and ABA levels were induced under drought, there were no changes in ABA or related transcripts in plants expressing 6K2 under drought compared with well-watered conditions. Expression of 6K2 also conveyed drought tolerance in another host plant, *Nicotiana benthamiana*, when expressed using a virus overexpression construct. In contrast to ABA, 6K2 expression enhanced salicylic acid (SA) accumulation in both *Arabidopsis* and *N. benthamiana*. These results suggest 6K2-induced drought tolerance is mediated through increased SA levels and SA-dependent induction of plant secondary metabolites, osmolytes, and antioxidants that convey drought tolerance.

Bacteria vs amoebae — the battle of the microbes

Harikumar R. Suma[1], **Pierre Stallforth**[1,2]
[1]*Department of Paleobiotechnology, Leibniz Institute for Natural Product Research and Infection Biology – Leibniz-HKI, Beutenbergstrasse 11a, 07745 Jena, Germany*
[2]*Faculty of Chemistry and Earth Sciences, Friedrich Schiller University (FSU), Jena, Germany*

In habitats such as soil, bacteria are constantly exposed to predation by nematodes or amoebae. To counteract these predation pressures, bacteria have evolved intricate defence mechanisms. The biosynthesis of toxic small molecules, represents a potent defence mechanism to kill adversaries.

The soil bacterium, *Pseudomonas fluorescens* HKI0770, produces pyreudione alkaloids that protect against amoebal predation. Although the mode of action of the pyreudiones has been elucidated, the spatiotemporal dynamics underlying this interaction remain unknown.

Using a combination of microscopy and analytical techniques, we elucidated the intricate relationship between *P. fluorescens* HKI0770 and the bacterivorous amoeba *Dictyostelium discoideum*. We used the chromatic bacteria toolbox for intraspecific differentiation of the amoebicide-producing wildtype and the non-producing mutant. Co-culture studies between the amoeba and the fluorescent bacterial strains indicated that – intriguingly – both strains were ingested by the amoeba. Further, we identified that a pleiotropic control on the production of toxic small molecules alters the feeding behaviour of amoeba, turning inedible bacteria into edible ones.

Overall, the study of amoeba–bacteria interactions provides a valuable framework for understanding the ecology and evolution of predator–prey interactions as well as the other aspects of symbiotic microbial interactions. Our findings also shed light on the roles of protists in driving the structure and function of microbial communities, with broader implications for microbiome engineering.

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Beneficial soil microbes enhance indirect plant defenses induced by insect egg deposition

Tuğcan Alınç[1], Ezio Peri[1], Livio Torta[1], Salvatore Guarino[2], Bart Lievens[3,4], Stefano Colazza[1], Antonino Cusumano[1]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, Building 5, 90128 Palermo, Italy
[2]Institute of Biosciences and Bioresources (IBBR), National Research Council of Italy (CNR), Via Ugo La Malfa 153, 90146 Palermo, Italy
[3]CMPG Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Department of Microbial and Molecular Systems (M2S), KU Leuven, Willem De Croylaan 46, B-3001 Leuven, Belgium
[4]Leuven Plant Institute (LPI), KU Leuven, B-3001 Leuven, Belgium

Plants can respond to insect egg deposition by emitting oviposition-induced plant volatiles (OIPVs) recruiting parasitoids. The recruitment of herbivore antagonists in response to egg deposition is considered an indirect defense strategy that is widespread in the plant kingdom. In recent years, there has been increasing evidence showing that microbial colonization can influence the strength of plant responses to insect herbivory, yet no information is available on how beneficial microbes modulate indirect defenses induced by insect egg deposition.

In this work, we evaluated the effects of inoculation with the beneficial soil fungus *Trichoderma harzianum* strain T22 on a tritrophic system consisting of tomato, the southern green stink bug *Nezara viridula* and its associated egg parasitoid *Trissolcus basalis*. In olfactometer assays, we found that root inoculation with *T. harzianum* T22 enhanced egg parasitoid attraction towards tomato plants induced by *N. viridula* feeding and oviposition activities. In particular, the egg parasitoid preferred OIPVs emitted by tomato plants previously inoculated with *T. harzianum* T22 over OIPVs emitted by non-inoculated plants. Furthermore, chemical analysis showed that root inoculation with *T. harzianum* T22 resulted in changes in the composition of OIPVs, which was consistent with the behavioral observations. Among the compounds that strongly contribute to the chemical differences between OIPVs from non-inoculated and inoculated plants, chemical analysis identified green leaf volatiles ((*Z*)-3-hepten-1-ol, (*E,E*)-2,4-hexadienal), along with terpenoids (terpinen 4-ol, α-tujene and δ-elemene).

Taken together our results indicate that beneficial soil microbes enhance indirect plant defenses induced by oviposition, broadening our understanding of plant responses to insect eggs.

Symbiont interactions and chemical mediators in a defensive symbiosis

Alina Nick[1], Ramya Ganesan[1], Jana Krabbe[2], Christian Hertweck[2], Martin Kaltenpoth[1]
[1]Department of Insect Symbiosis, Max-Planck Institute for Chemical Ecology, Jena Germany
[2]Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Jena

Beneficial associations between insects and microbes are widespread and often crucial for host survival. Research has focused on the mutualistic benefits that single microbial partners provide to their hosts. However, our understanding of complex microbial communities with intricate interactions between the members and their influence on composition and functionality of symbiosis remains limited. Here, we address symbiont-symbiont interactions in the context of a multipartite defensive symbiosis.

Lagria villosa beetles (Coleoptera: Tenebrionidae) engage in a symbiosis with multiple strains of *Burkholderia gladioli* that protect the beetle's offspring from fungal infestation. *B. gladioli* Lv-StB is most abundant and produces the antifungal compound lagriamide. *B. gladioli* Lv-StA is only occasionally present but it is culturable *in vitro* and has the ability to produce multiple secondary metabolites that also confer protection against fungi.

To investigate the strain dynamics during colonization of specialized cuticular organs, Lv-StA was applied to naturally Lv-StB infected eggs and larvae. The results indicate that both strains can colonize the beetle's organs individually, in combination, and in succession, indicating that the strains do not outcompete each other and the symbiosis remains open to environmental microbes.

To study the impact of individual secondary metabolites produced by Lv-StA, we generated single knockout mutants and started conducting *in vivo* bioassays against a natural fungal antagonist of *Lagria villosa*. We anticipate to reveal if one compound is essential to the defense or if a combination of multiple compounds is needed.

Overall, we hope to contribute to the understanding of symbiont-symbiont interactions in complex systems.

Impact of 8-HQA in the number and diversity of orally secreted bacteria in *Spodoptera exigua* and its interaction with plant defences

Daniel Pinos[1], Elena García Marin[1], Beatriz Ramírez Serrano[2], Jordi Gamir[3], Cristina M Crava[1]
[1]*Biotechnological Pest Control group, Universitary Institute BIOTECMED, University of Valencia, Burjassot, Spain*
[2]*Estación experimental del Zaidin, CSIC, Granada, Spain*
[3]*Plant Immunity and Biochemistry group, Univesitat Jaume I, Castellóin de la Plana, Spain*

The 8-hydroxyquinoline-2-carboxylic acid (8-HQA) is a siderophore specifically detected in the regurgitant of *Spodoptera* species. It affects the availability of metal ions in the gut and, in *S. littoralis*, it regulates the microbial communities present in this organ. During the larval stage, some *Spodoptera* species release bacteria in the oral secretion that inhibit induced plant defenses. Thus, we hypothesized that if 8-HQA alters the microbial communities in the gut, this may impact the bacteria present in the oral secretion and, eventually, affect the activation of plant defences. To answer this question, we used CRISPR/Cas9 to knock out the enzyme kynurenine 3-monooxygenase (KMO) in *Spodoptera exigua*, which is a polyphagous noctuid pest that overlaps in host plant use with *S. littoralis* in the greenhouses of the Mediterranean regions.

As expected, 8-HQA was undetectable in *S. exigua kmo* mutant oral secretions. The next step was the assessment of the bacterial community present in the three compartments of larval guts (foregut, midgut, and hindgut) using 16S rRNA gene metabarcoding from cDNA obtained from both wild-type and *kmo* mutants of *S. exigua* larvae. In parallel, bacterial load and diversity of microbes present in the oral secretions of both strains were obtained from DNA metabarcoding. Lastly the effect of the presence of 8-HQA was assessed by comparing the performance of both strains when feeding on tomato plants.

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Agro-ecological implications of fly-yeast associations

Guillermo Rehermann[1], Amrita Chakraborty[2], Christine Petig[3], Sammar Khalil[4], Paul G. Becher[1]
[1]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden
[2]Czech University of Life Sciences Prague, Prague, Czech Republic
[3]Gottfried Wilhelm Leibniz University Hannover, Hannover, Germany
[4]Department of Biosystem and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden

Dipteran flies live in close association with microbial communities. Chemicals emitted from symbiotic microbes are known to mediate ecological interactions between host plants and flies.

Drosophila suzukii (Diptera: Drosophilidae) is a polyphagous pest of berries and other soft fruits. We first demonstrate that *D. suzukii* and the yeast *Hanseniaspora uvarum* (Ascomycota: Saccharomycetes) engage in niche construction in a reciprocal manner in the context of their shared fruit habitat. The mutualistic interaction between *D. suzukii* and *H. uvarum* increased flies' performance and supported yeast growth, while supressing the development of the antagonistic fungus, *Botrytis cinerea* (Ascomycota: Sclerotiniaceae), on raspberry fruit.

Conversely to the fruit-infesting pest, flower flies (Diptera: Syrphidae) are ecosystem service providers. In particular, *Eupoedes corollae* adults are pollinators and the larvae are predators on pest insects such as aphids. Here, we show the capacity of *E. corollae* for vectoring the beneficial and naturally occurring yeast *Metschnikowia fructicola* (Ascomycota: Saccharomycetes) to strawberry flowers. Yeast vectored by flies to flowers reduced the development of plant pathogens such as *B. cinerea* grey mould on fruit. Our results suggest that flower flies can be used as 'flying agents' in strawberry production combining pollination and yeast entomo-vectoring when visiting flowers.

The collective findings advance the fundamental understanding of the establishment and maintenance of multi-trophic interactions in flies. Moreover, they illustrate the potential to exploit microbial diversity to develop sustainable management practices of pollinators and pests.

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Fungal alkaloids mediate defense against bruchid beetles in field populations of an arborescent *Ipomoea*

Alberto Prado[1], Susana Pineda-Solis[1], Roberto Garibay-Orijel[2], Donald Windsor[3,] Jean-Luc Boevé[4]
[1]Escuela Nacional de Estudios Superiores Unidad Juriquilla, Universidad Nacional Autónoma de México, Querétaro, México
[2]Instituto de Biología, Universidad Nacional Autónoma de México, CDMX, México
[3]Smithsonian Tropical Research Institute, Panamá, Rep. de Panamá
[4]OD Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

Many Convolvulaceae species harbor heritable fungal endophytes from which alkaloids are translocated to reproductive tissues of the plant host. Evidence for the distribution and ecological role of these fungal alkaloids, however, is lacking or incomplete for many host species and growth forms. Here we report on the quantities of alkaloids present in the leaves and seeds of the arborescent morning glory, *Ipomoea murucoides* (Convolvulaceae). Young leaf samples taken from wild plants harbored one of two fungal taxa. Seeds had higher concentrations of the indolizidine alkaloid swainsonine than leaves. Additionally, seeds from trees harboring *Ceramothyrium* (Chaetothyriales) fungi exhibited less bruchid damage and had higher concentrations of swainsonine than seeds from trees harboring *Truncatella* (Xylariales) fungi. Five sesquiterpenes were detected in the leaf trichomes of both types of trees. The seed content in nortropane alkaloids, tropine and tropinone, did not differ significantly among the two fungal symbionts. Overall, our field data support the defensive-symbiosis hypothesis for swainsonine as proposed by Clay (2014) where the fungal partner supplies chemical defenses to the host. It is likely that the host allocates the defensive chemicals from leaves to seeds, protecting them from seed predators such as bruchid beetles.

POSTERS

P-26

Metabolism of spruce defensive phenolic compounds by bark beetle's symbiotic fungus

Baoyu Hu[1], Jonathan Gershenzon[1], Ruo Sun[1]
[1]Department of Biochemistry, Max-Planck Institute for Chemical Ecology, Germany

In the past decade, outbreaks of the spruce bark beetles *Ips typographus* have led to devastating consequences for spruce trees across Europe and Asia. Successful attack by these bark beetles seems to require the presence of fungal symbionts. These free-living, ascomycete associates may help by supplying nutrients, detoxifying spruce defenses, or protecting against beetle pathogens, but there is still little evidence for these benefits. We are investigating how a major bark beetle fungal symbiont, *Endoconidiophora polonica*, metabolizes stilbenes, one of the principal groups of phenolic compounds produced by Norway spruce (*Picea abies*), and its impact on the bark beetle. We revealed that *E. polonica* metabolizes stilbene glycosides and converts them into corresponding aglycones and *O*-methylated derivatives. In addition, we determined some of the properties of the enzymes and genes involved. The resulting stilbene metabolites display inhibitory effects on the bark beetle entomopathogenic fungus *Beauvaria bassiana* and the ubiquitous fungal parasite *Trichoderma harzianum*. This inhibition may aid in the survival of both the beetle and its associated fungus, but further study is needed to assess its importance in promoting successful attack by spruce bark beetles.

P-30

Ingested soil bacteria breach gut epithelia and prime systemic immunity without pathogenic effects

Seonghan Jang[1], Kota Ishigami[2,3], Peter Margaert[4], Yoshitomo Kikuchi[2,3]
[1]*Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea*
[2]*Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology, Hokkaido Center, 062-8517 Sapporo, Japan*
[3]*Graduate School of Agriculture, Hokkaido University, 060-8589 Sapporo, Japan*
[4]*Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198 Gif-sur-Yvette, France*

Insects possess a well-developed innate immunity to protect themselves against environmental pathogens, lacking the acquired immunity and immune memory found in vertebrates. Recent studies have identified a phenomenon called immune priming, where sub-lethal doses of pathogens or non-pathogenic microbes stimulate immunity, preventing future infections. Immune priming can occur through the injection of microbes into the hemolymph or ingestion of a microorganism-containing diet. However, the mechanisms behind immune priming in insects remain unclear. The bean bug *Riptortus pedestris* acquires its gut microbiota from soil, forming a complex microbial community in the anterior midgut (M3) and a mono-species *Caballeronia* symbiont population in the posterior midgut (M4). Our research demonstrates that a *Burkholderia sensu stricto* strain in the M3 region stimulates systemic immunity by penetrating gut epithelia and migrating into the hemolymph. This activation, involving humoral and cellular responses, does not negatively affect host fitness but protects against subsequent pathogenic infections. Disrupting contact between commensal *Burkholderia* and gut epithelia weakens host immunity to pre-infection levels, increasing vulnerability to pathogenic infections, indicating the importance of persistent environmental bacteria acquisition for effective immunity. Although gut epithelia penetration is known for some enteropathogenic bacteria, our study shows a soil-derived gut microbiota member priming host immunity without harming the host. This type of gut symbiotic bacteria remains underexplored, necessitating further molecular, immunological, and histological studies. Our findings reveal how insects utilize soil bacteria for pathogen defense and offer a new perspective on bacterial intestinal barrier breaching, which has been generally discussed only in terms of pathology.

P-35

Chemical profile of different oregano accessions originated from the same region (Mount Vertiscos) and their effect on plant growth promoting rhizobacteria

Aggeliki Ainalidou, Aikaterini Triantafyllou, Ioannis Mourtzinos, Katerina Karamanoli
Faculty of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

The current study aims to evaluate botanical, chemical and antimicrobial characteristics of different oregano accessions (Individuals, I) cultivated in the same field and originated from a local natural population of the region of Vertisco mountain in Greece. Fourteen oregano individuals (coded: I1-I14) that differed in their morphological characteristics, taxonomically classified as *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart, also known as "Greek oregano", were selected for further investigation. Essential oil composition, volatile organic compounds (VOCs) emission, plant flavonoids and their antioxidant capacity were explored. According to our results, essential oil yield ranged from 1.63% to 5.98% d.w. in oregano I7 and I13, respectively. The main constituent of all essential oils, and of VOCs was carvacrol with the highest value in I5 (81.217%) and the lower in I8 (52.4%). Evaluation of flavonoid concentration and antioxidant capacity revealed I4 as the most active individual. Antimicrobial activity of the essential oils and of the VOCs derived from accessions with the higher and the lower concentration of carvacrol and flavonoids, against two plant growth promoting rhizobacteria (PGPR) collected from oregano rhizosphere was explored. Further, the effect of VOCs emitted of selected oregano accessions in the interaction of PGP strains with the fungal pathogen *Botrytis cinerea*, that can cause necrosis in oregano leaves, was assessed.

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P-38

Unravelling mycobiome of Scots pine beetles (Coleoptera: Scolytinae)

Arunabha Khara, Amrita Chakraborty, Jiri Synek, Amit Roy
Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

The escalating severity and frequency of pine bark beetle attacks due to climate change have underscored the pivotal role of the fungal community associated with these beetles in enhancing their infestation and survival on defence-rich pine trees. However, a significant gap exists in understanding the environmental acquisition and stage-specific associations with different pine-feeding bark beetles. Moreover, there is limited knowledge regarding acquiring fungal species from pine trees to beetle mycobiomes and alteration in plant microbiomes following beetle feeding. To address these gaps, we conducted the first comparative fungal metabarcoding study, elucidating the fungal communities in pine trees before and after beetle feeding and across various life stages of two dominant pine-feeding bark beetles, *Ips sexdentatus* and *Ips acuminatus*. We identified significant differences in fungal abundance among beetle life stages and between beetle species, emphasizing developmental stage-specific influence on fungal assemblages. Moreover, we observed distinct fungal communities in lab-reared and wild-collected adult beetles, indicating environmental influences on beetle mycobiome. Our results also highlighted a higher abundance of shared fungal communities between infested pine wood and adult beetles, suggesting the impact of beetle feeding on fungal composition. Functional predictions underscored the potential ecological roles of these fungal genera in supporting beetle infestation success. Our study provides novel insights into fungal associations influenced by environmental factors, host interactions, and beetle life stages, laying the foundation for future functional studies on pine beetles and their fungal associates.

Keywords: Bark beetles, core mycobiome, *Ips acuminatus*, *Ips sexdentatus*, fungi, ITS2.

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P-75

The endophytic fungus *Mortierella hyalina* mitigates nitrogen starvation in *Arabidopsis thaliana* plants

Nataliia Svietlova[1], Michael Reichelt[2], Liza Zhyr[1], Veit Grabe[3], Axel Mithöfer[1]
[1]Max Planck Institute for Chemical Ecology, Research Group Plant Defense Physiology, 07745 Jena, Germany
[2]Max Planck Institute for Chemical Ecology, Department of Biochemistry, 07745 Jena, Germany
[3]Max Planck Institute for Chemical Ecology, Microscopic Imaging Service Group, 07745 Jena, Germany

Beneficial microorganisms, in particular endophytic fungi, can positively affect the growth of many host plants under biotic and abiotic stress conditions and, in some cases, can mitigate the effect of stress on plants. Our study investigated the role of the non-mycorrhizal, endophytic growth-promoting soil fungus *Mortierella hyalina* on *Arabidopsis thaliana* plants under different levels of N-starvation. Our data suggest that *M. hyalina* can alleviate N-starvation stress in several different ways: (i) the fungus supplies nitrogen (^{15}N) to the N-starving plant; (ii) the presence of the fungus can restore the plants' amino acid homeostasis that was out of balance due to N-starvation. In our case, the stress-induced accumulation of branched-chain amino acids was reduced. In addition, there was no accumulation of defense-related phytohormones upon the plant-fungus interaction, indicating that the plant tolerates the presence of *M. hyalina* well. In summary, the plants do not need to invest in defense; thus resources are preserved for growth, which in turn benefits the fungus, suggesting that this interaction should be considered a mutualistic symbiosis.

S7: Influence of man-made stressors on the chemical communication of insects

Chairs: *Manfred Ayasse, Samuel Boff*

ORAL PRESENTATIONS

Impact of air pollution on plant-pollinator chemical communication: Main effects and future perspectives

Magali Proffit
CEFE, University of Montpellier, CNRS, EPHE, IRD, Montpellier, France

Since the beginning of the industrial era, ecosystems have been facing massive anthropogenic changes that may alter interspecific interactions, for instance the chemical communication between plants and pollinators. Among these changes, the increase in certain atmospheric pollutants can affect every step of such communication: a) the emission of volatile organic compounds (VOCs) by flowers (i.e., the emitter), b) VOCs lifetime in the atmosphere (i.e., the signal) and c) the sensory capabilities of pollinators (i.e., the receiver). We tested the potential effects of ozone (O_3), one of the major atmospheric pollutants, on two Mediterranean plant-pollinator interactions highly contrasted in terms of specificity and cognitive capacities of their pollinators. Under controlled conditions, we separately exposed plants, VOCs or insects to an acute O_3 concentration, mimicking an O_3 pollution peak characteristic in the Mediterranean basin. In addition, dose-response experiments were performed to determine the concentration thresholds at which O_3 altered pollinators' floral odor recognition. The results of our experiments confirm a generalized impact of O_3 exposure on each step of plant-pollinator chemical communication, yet the thresholds at which these effects are observed differ between the two systems studied. The consequences of these multiple effects of O_3 and the current gaps in our understanding of the effects of atmospheric pollutants on plant-pollinator interactions will be discussed.

Effect of cultural practices on companion plant VOC emissions and the impact on their antibiosis and antixenosis effect on aphid

Louna Rizzi, Gabin Mardoc, Arnaud Da Rocha, Hélène Gautier
UR1115 PSH Plantes et Systèmes de Culture Horticoles, INRAE, F84914 Avignon, France; Avignon Université, Avignon, France

Intercropping with companion plant (CP) is one of the potential new strategies for optimising crop protection and reducing pesticide use. Through the emission of volatile organic compounds (VOCs), CP can have antibiosis and/or antixenosis effects on aphids. However, these effects often prove too modest and variable to effectively replace aphicide treatments. In the PPR CPA Cap Zero Phyto project, we tested the hypothesis that combining different cultural practices could yield a synergistic effect, thereby enhancing the efficacy of CP against aphids. UVC flashes are currently studied for their defense-eliciting effects, including the activation of the salicylic acid pathway, facilitating the synthesis and storage of secondary metabolites. In addition, according to the "Growth-Differentiation Balance Hypothesis" (GDBH), lowering nitrogen supply would lead to an excess accumulation of carbohydrates, allocated to the formation of secondary compounds crucial for defense. Under controlled conditions, using basil as a model plant, we investigated the effects of a nitrogen diet and/or the application of an UVC flash on the VOC emission dynamics and their consequences on aphid fecundity and orientation behaviour (*Myzus persicae* and *Dysaphis plantaginea*). These effects will also be studied on other CP in order to identity which CP and cultural practices should be used to control aphids in orchards.

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The smell of drought: Water limitation alters plant volatile emission and parasitoid attraction to herbivore-infested plants

Ilka Vosteen[1, 2], **Zhijia Huang**[1], **Shahinoor Rahman**[1], **Elisa Venturini**[1], **Laura Zuidema**[2], **Michael Rostàs**[1]
[1]*Agricultural Entomology, Department for Crop Science, Georg August University Göttingen, Germany*
[2]*Laboratory of Entomology, Wageningen University & Research, the Netherlands*

Intensity and frequency of droughts increase across the globe. As drought stress severely alters phytohormonal signaling and plant metabolism, it is difficult to predict its effect on the emission of herbivore-induced plant volatiles (HIPVs) and attraction of parasitoids, which use HIPVs as host-finding cues. Moreover, it is challenging to disentangle species-specific effects from the effect of the mode of drought stress (e.g. intensity, duration). We will present data from two study systems, 'sugar beet – black bean aphids – *Aphidius colemani*' and 'cabbage – cabbage white caterpillars – *Cotesia glomerata*' to show how different systems respond to drought stress.

Long-term drought stress resulted in reduced emission of total HIPVs per sugar beet plant, as drought-stressed plants were much smaller than controls. Parasitoid attraction positively correlated with total HIPV emission per plant, resulting in highest attraction to well-watered plants. Short-term drought stress similarly reduced parasitoid attraction to sugar beet volatiles without reducing plant size and stomata were found to be closed in the drought stress treatments. If drought-stressed plants were watered prior to the behavioral assay to induce stomata opening, the previously drought-stressed plant became highly attractive. We are currently analyzing the resulting volatile profiles to understand the effect of stomata conductance on HIPV emission.

Short-term drought altered HIPV emission from cabbage; with some compounds increasing and others decreasing upon drought stress, but this did not change parasitoid attraction. These results suggest that *C. glomerate* mainly relies on those volatile compounds that are not affected by drought to find host infested-plants.

Impacts of drought stress on the chemical communication between Styrian oil pumpkin and bumblebee pollinators

Monica Barman[1, 2], **Raimund Tenhaken**[1], **Stefan Dötterl**[1]
[1]*Dept. of Environment and Biodiversity, Paris Lodron University of Salzburg, Hellbrunnerstr. 34, 5020 Salzburg, Austria*
[2]*Current address: Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Theodor-Echtermeyer-Weg 1, 14979, Großbeeren, Germany*

The globally changing climatic condition is alarmingly increasing the incidences of drought and/or reduced soil water content in several regions of the world. This increased water shortage is predicted to have a huge impact on the process of pollination in both naturally and agriculturally grown plants. One commercially cultivated plant with reduced yield during years with little rain is the renowned Styrian oil pumpkin (*Cucurbita pepo* subsp. *pepo* var. *styriaca* Greb.), a crop that relies on insect pollination for setting fruits. We studied effects of drought on flower production, floral morphological traits, the quantity and composition of nectar, and the emitted flower scent. We also assessed antennal responses of bumblebee pollinators (via GC/EAD, gas chromatography coupled to electroantennographic detection) to the floral scents. We found that drought stress resulted in negative effects on flower numbers and on floral display size and nectar. Floral scents were not affected on a per flower basis, however, we found that the emissions of several phenylpropanoid/benzenoid compounds per gram of fresh floral tissue weight were higher in flowers of drought-stressed than control plants. This correlated with higher expression levels of genes involved in the biosynthesis of these compounds in drought-stressed plants. Drought had negative effects on the visitation of flowers by bumblebee pollinators. The investigations of this study provide crucial clues on how drought affects the flower production, the biosynthesis of floral scents and the communication of an insect-pollinated plant with their pollinators, and how drought overall has potentially negative effects on crop yield.

Effect of land-use associated stressors on bumblebee health and cuticular hydrocarbons

Florian Straub, Markus Birkenbach, Samuel Boff, Lena Wilfert, Jonas Kuppler, Manfred Ayasse
Ulm University, Institute for Evolutionary Ecology and Conservation Genomics, Ulm, Germany

In agricultural landscapes, bees face a variety of stressors, including insecticides and poor-quality food that were found to negatively affect health of the bees, including learning behavior and orientation during foraging. Effects of pesticides and poor-quality food on chemical communication, however, have rarely been investigated. Furthermore, although pesticides and poor-quality food individually have been shown to affect bumblebee health negatively, few studies have focused on stressor interactions, a scenario expected in intensively used agricultural landscapes. In field studies and, in addition, in a fully-factorial laboratory experiment, we assessed the effects of land-use associated stressors such as food quality and pesticides on cuticular hydrocarbons (CHCs), behavior and several health traits in bumblebees. We found that both stressors, food quality and insecticide exposure, affected various health traits at the colony and individual level in *Bombus terrestris*. Both stressors and their interaction changed the workers´ CHC bouquets and reduced worker interactions. The decrease in interactions observed in our study might be the result of changes in chemical profiles and a disturbed communication system. This hypothesis is supported by the finding that changes were mainly in those CHCs that in former studies were found to be involved in intra-nest communication. The synergistic effects we found highlight the need of combining stressors in risk assessments. Moreover, this provides a more realistic understanding of the complex effects of man-made stressors on health outcomes and chemical communication of bees.

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They poisoned our relationship: Toxicants and nutritional stress disrupt queen-worker communication in the honey bee

Margarita Orlova, Oscary Dotel, Darnell Weaver
State University of New York, Polytechnic Institute, Utica, NY, USA

Honeybees heavily rely on communication, especially chemical signaling, for performance of virtually all activities in the colony. Modern agricultural practices expose honeybees to an unprecedented number of stressors, including toxicants, pathogens and poor nutrition, yet we know very little about the effects of these stressors on chemical communication in honeybee colonies. We investigated the effects of common stressors affecting honeybees – pesticides and nutritional deficiency – on communication between queens and workers, focusing on behavioral interactions and semiochemical production. Our results suggest that a combination of a commonly used pesticide – imidacloprid – and nutritional deficiency produces a far greater detrimental effect on queen-worker interactions than each of these stressors alone, reducing the participation of workers in the retinue response and causing a shift in the cuticular profile of the queen. Another cholinergic pesticide – pyrethrin – produces a similar detrimental effect on retinue behavior, but no discernible effects on queen chemical profile, in addition to slowing down the workers' behavioral development. Our findings highlight the need for further research into the detrimental effects of stressors on honeybee behavior beyond acute toxicity and into the mechanisms underlying these effects.

This work was supported by the Honeybee Health grant from the North American Pollinator Protection Campaign.

Impact of man-made stressors on sex communication of wild solitary bees

Samuel Boff[1,2], **Jessica Hieber**[1], **Manfred Ayasse**[2]
[1]Institute of Botany, Ulm University, Ulm, Germany; [2] Institute of Evolutionary Ecology and Conservation Genomics, Ulm University, Ulm, Germany

Pesticides have been found to harm the reproduction of wild bees. However, little is known about whether these impairments are due to issues in chemical communication. In our research, we aimed to fill this gap by studying the sub-lethal effects of pesticide exposure on the mating behavior of three European wild bee species. During our trials, we compared exposed bees to control bees. We recorded pre-copulatory behaviors and copulation attempts. Additionally, we used gas chromatography to study changes in the composition of cuticular hydrocarbons (CHCs). We found that pesticide exposure reduced mating attempts in all species and negatively affected copulation/male efficiency in mason bees. Female rejection after exposure was observed in all species. Chemical analyses of the CHCs revealed changes in the production and composition of chemical volatiles, some of which were identified as electrophysiologically active compounds that stimulate mating behavior in male bees. Our study demonstrates that man-made stressors impact the mating behavior of wild bees. Additionally, we monitored wild bees in conventional and organic farming areas. We found that conventional farming leads to changes in the cuticular hydrocarbons and pheromones of the bees, influencing the overall attractiveness of females in bioassays.

POSTERS

P-19

Different climatic conditions – different success? Temperature and photoperiod induced changes in mating behavior and cuticular hydrocarbon profile in fruit pest *Drosophila suzukii*

Ferenc Deutsch[1,2], Zsolt Kárpáti[1,3], Balázs Kiss[1], Thomas Schmitt[3]
[1]Zoology Department, Plant Protection Institute Centre for Agricultural Research, HUN-REN, Martonvásár, Hungary
[2]Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences, Department of Plant Protection, Gödöllő, Hungary
[3]Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Würzburg, Germany

Photoperiod and temperature changes often trigger seasonal plasticity in insects. When suboptimal climatic conditions arise, insects can enter reproductive diapause. This often leads to a delay in development and changes in behavior. Reproductive diapause might also affect the cuticular hydrocarbon (CHC) profile which is responsible to prevent the insect from desiccation. In addition, CHCs are often important cues and signals for mate recognition. We chemically and behaviorally investigated the CHC profiles in *Drosophila suzukii*, an important pest species of soft-skinned fruits. Males and females of one- and five-day-old winter and summer morphs, were examined. We conducted mating behavioral assays with same and mixed morph pairs. According to the gas chromatography/mass spectrometry analyses, CHC profiles differed with age and morph, but no significant differences was found between the sexes. The behavioral assays revealed that summer morph mated earlier in their adult life than winter morph pairs. They also mated for a shorter durations and produced more offspring than the winter morphs. We hypothesize that the different CHC profiles of winter morphs may be an adaptation to cold winter conditions, by synthesizing a CHC profile which protects from cold temperatures and desiccation, potentially at the cost of reduced mate recognition cues.

P-27

Parasitoid response to temporal HIPVs dynamics and HIPVs emitted by drought-stressed plants

Zhijia Huang, Michael Rostás, Ilka Vosteen
Department for Crop Sciences, Georg-August-Universität Göttingen, Göttingen, Germany

The larval parasitoid *Cotesia glomerata* attacks first and second instar caterpillars of *Pieris brassicae* as host. The parasitoid uses HIPVs as long-range cues for navigation to the host-infested plant. Acute drought stress alters the HIPV blends emitted by host-infested cabbage plants, but this does not affect parasitoid attraction. We speculate that *C. glomerata* relies on certain key compounds that are emitted in relatively stable ratios to identify host-infested plants.

We also conducted behavioral test to explore how *C. glomerata* responds to temporal HIPVs dynamics. Cabbage white butterflies were allowed to lay their eggs on cabbage and the hatching larvae were left to feed 1, 3 or 5 days before the plants were tested. We found that *C. glomerata* prefers to land on plants with the most severe damage even though the hosts on it are too old for successful development of parasitoid larvae. This suggests that the amounts of attractive key compounds increase with increasing feeding damage and duration of infestation. By collecting and analyzing volatiles we aim to identify the key compounds that are responsible for the attraction of *C. glomerata*.

Project supported by the China Scholarship Council.

S8: Biochemistry and evolution of specialized metabolites in chemical interactions

Chair: *Dorothea Tholl*

ORAL PRESENTATIONS

Pyrrolizidine alkaloids – a model for pathway evolution and insect counter adaptations

Dietrich Ober
Chemical Ecology and Molecular Evolution, Botanical Institute and Kiel Botanic Gardens, Kiel University, Kiel, Germany

Pyrrolizidine alkaloids (PAs) are a class of secondary compounds that are produced by plants as chemical defense against herbivores. They are part of fascinating interactions between the plant and specialized insects that have evolved counter-adaptations to these toxins. Such adaptations include not only the evolution of mechanisms for detoxification, but also for sequestration of these toxins in insects. Within the angiosperms, several plant lineages have been described that produce PAs. Studies on the first pathway-specific enzyme, homospermidine synthase, have shown that the pathway has evolved several times independently during angiosperm evolution. Therefore, this system is a promising tool to study the evolution of pathways in plant secondary metabolism by comparative approaches. Recent results on the formation of chemical backbone structures will be presented, including some unexpected observations resulting from protein characterization.

Biosynthesis of the allelopathic alkaloid gramine in barley by a cryptic oxidative rearrangement

Sara Leite Dias[1], Ling Chuang[2,3], Shenyu Liu[2,3], Benedikt Seligmann[2], Fabian L. Brendel[1], Benjamin G. Chavez[1], Robert E. Hoffie[4], Iris Hoffie[4], Jochen Kumlehn[4], Arne Bültemeier[2,3], Johanna Wolf[2], Marco Herde[5], Claus-Peter Witte[5], John C. D'Auria[1], Jakob Franke[2,3]
[1]*Department of Molecular Genetics, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Seeland OT Gatersleben, Germany*
[2]*Institute of Botany, Leibniz University Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany*
[3]*Centre of Biomolecular Drug Research, Leibniz University Hannover, Schneiderberg 38, 30167 Hannover, German*
[4]*Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Seeland OT Gatersleben, Germany*
[5]*Department of Molecular Nutrition and Biochemistry of Plants, Leibniz University Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany*

Gramine is an alkaloid found in barley that provides protection against insects and affects their palatability to ruminants. The biochemical basis for the formation of gramine from the amino acid tryptophan has remained unresolved. We identify a gene cluster in barley containing two genes, encoding a previously reported N-methyltransferase as well as a cytochrome P450 monooxygenase, that we name AMI synthase (AMIS). We show that these two genes enable the production of gramine in the heterologous species *Nicotiana benthamiana*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae*, and constitute the necessary genetic complementation to reactivate gramine biosynthesis in the barley variety Golden Promise that does not produce it. To further characterize CYP76M57 in its natural host, we mutated the gene by Cas endonuclease technology in the cultivar Tafeno, which prevented the production of gramine in the plant. Based on in vitro experiments with yeast microsomes, we demonstrate that CYP76M57 performs a cryptic oxidative rearrangement of tryptophan to an iminium intermediate. Taken together, our findings reveal how the gramine scaffold is generated from a simple amino acid. The discovery of the genetic basis of gramine formation enables access to breeding initiatives that aim to reduce pesticide use and harness the biological activity of gramine for barley cultivation.

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Epigenetic weapons in plant-herbivore interactions: Sulforaphane disrupts histone deacetylases, gene expression, and larval development in *Spodoptera exigua* (but not *Trichoplusia ni*)

Thomas Arnold, Dana Somers, David Kushner, Marisa Arreola, Ashley Groff, Whitney Finney
Department of Biology, Program in Biochemistry and Molecular Biology, Dickinson College, Carlisle, PA 17013, USA

Cruciferous plants produce sulforaphane (SFN), an inhibitor of nuclear histone deacetylases (HDACs). In humans, the consumption of SFN alters enzyme activities, DNA-histone binding, and gene expression within minutes. We recently demonstrated that SFN consumed by the generalist grazer *Spodoptera exigua* inhibits HDAC enzyme activity in fat body tissues and ovaries. HDAC enzyme activities and larval development were slowed by 50%. Similar results were obtained when SFN was applied topically to eggs. RNA-seq analyses confirm that exposure to SFN down-regulated genes associated with energy conversion pathways while up-regulating those encoding ribosomal proteins. In contrast, the co-evolved specialist feeder *Trichoplusia ni* was not negatively impacted by SFN. While SFN inhibits HDAC enzymes isolated from this spe*cies in vitro* neither the consumption nor topical application of SFN inhibited HDAC enzymes in developing larvae. Subsequently, *T. ni.* development was not slowed by SFN. Similarly, gene expression was altered to a lesser degree, compared to *S. exigua*. This resistance could be overwhelmed by unnaturally high levels of SFN or by pharmaceutical HDAC inhibitors. These results demonstrate that dietary SFN can interfere with the epigenetic machinery of insects, supporting the hypothesis that plant-derived HDAC inhibitors may serve as “epigenetic weapons” against herbivores. The effects of these epigenetic weapons occur in reproductive tissues and may be transferred to future generations of herbivores. Interestingly, the effects of SFN on insect gene expression is similar to those observed when HDAC inhibitors are used to treat human cancers and neurodegenerative disorders.

CluSeek: A bioinformatics tool for discovering novel gene clusters in GenBank

Ondrej Hrebicek, Stanislav Kadlcik, Jiri Janata, Jana Kamanova, Zdenek Kamenik
Institute of Microbiology, Czech Academy of Sciences, Czech Republic

We introduce CluSeek, a novel Python-based bioinformatics tool tailored for the identification, visualization, and analysis of gene clusters – groups of co-localized genes and their surrounding regions. Unlike existing tools reliant on pre-existing libraries of known gene clusters, CluSeek operates on GenBank data, enabling the retrieval of gene clusters containing homologs of user-defined genes regardless of the associated phenotype. This sets CluSeek apart from other genome mining tools such as antiSMASH[1]. We showcase CluSeek's capabilities through a case study involving the genome mining of specialized metabolites featuring a distinctive 4-alkyl-L-proline motif. Importantly, CluSeek's utility extends beyond biosynthetic gene clusters for bioactive metabolites. It offers versatility in exploring diverse areas including bacterial secretion systems, chemotaxis, nitrate assimilation, sulfur metabolism, and evolutionary aspects associated with these phenomena. CluSeek is open-source software accessible at https://cluseek.com, and it has a user-friendly graphical interface tailored for non-experts in IT, facilitating easy launch and operation.

Reference: 1. Blin, K. et al. 2023 *Nucleic Acids Res.* doi: 10.1093/nar/gkad344

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Very long chain alkyl ascarosides as biosynthetic intermediates in *C. elegans'* ascaroside biosynthesis

Rocío Rivera Sánchez, Antoine Etourneau, Marie-Désirée Schlemper-Scheidt, Hanna Laaroussi, Stephan H. von Reuss
Laboratory for Bioanalytical Chemistry, University of Neuchatel, Neuchatel, Switzerland

Chemical signaling in nematodes, such as the model organism *Caenorhabditis elegans*, involves ascarosides, glycolipids of the 3,6-dideoxysugar L-ascarylose linked to ($\omega - 1$)-hydroxy fatty acid-type aglycones. Ascaroside biogenesis depends on the co-option of the peroxisomal β-oxidation cycle, which shortens very long chain fatty acids as well as ascaroside aglycones and thereby creates homologous series of short chain acyl ascarosides with diverse biological functions [1]. Comparative analysis of peroxisomal β-oxidation mutants revealed very long chain acyl ascarosides with up to 31 carbons as precursors for the short chain signaling molecules, but their biosynthetic origin has remained enigmatic [1,2].

Using a combination of reverse genetics based on comparative HPLC-HR-MS analysis of *C. elegans* wildtype and mutant metabolomes, stable isotope labeling experiments, and total synthesis we demonstrate that ascaroside biosynthesis proceeds via very long chain alkyl ascarosides, such as asc-ωC21-H, asc-C25-H, asc-*iso*C26-H, or asc-C29/C31-H. Furthermore, we identify specific cytochrome P450s and a cytochrome P450 reductase that are involved in the hydroxylation of alkyl ascarosides, thus, characterizing the entry points into short chain ascaroside biogenesis upstream of the peroxisomal β-oxidation cycle, which are widely conserved among the nematodes.

[1] von Reuss et al. (2012) *J. Am. Chem. Soc. 134*, 1817.

[2] von Reuss et al. (2017) *Anal. Chem. 89*, 10570.

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Sex pheromone biosynthesis in the oriental fruit moth *Grapholita molesta* involves unique Δ8 desaturation

Marie Inger Dam[1], Bao-Jian Ding[1,5], Kristina Brauburger[1], Hong-Lei Wang[1,5], Daniel Powell[1,2], Astrid T. Groot[3], David G. Heckel[4], Christer Löfstedt[1,5]
[1]Department of Biology, Lund University, Lund, Sweden
[2]Centre for Bioinnovation, University of the Sunshine Coast, Australia
[3]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands
[4]Max Planck Institute for Chemical Ecology, Jena, Germany
[5]Institute of Biological Interaction, Xianghu lab, Hangzhou, China

The oriental fruit moth *Grapholita molesta* is distributed throughout temperate regions, and considered to be a pest in peach production and other high-value fruit crops in the rose family. Insecticide treatment has led to resistance development, but the use of sex pheromones in pest management has shown great promise. The major female sex pheromone component of *G. molesta* has been identified as (*Z*)-8-dodecenyl acetate, and the minor components as (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecenol, and dodecanol.

We investigated the pheromone biosynthesis pathway in *G. molesta* with the aim of harnessing pathway genes in the biological production of sex pheromone for use in pest management. *In vivo* labelling experiments supported an uncommon Δ8 desaturase interacting with lauric acid producing the fatty acid precursors of the unsaturated pheromone components. One desaturase gene candidate was highly expressed in the female pheromone gland transcriptome. CRISPR/Cas9 knock-out of this desaturase gene blocked the production of the pheromone completely. Expression in yeast- and Sf9 insect cells, however, failed to demonstrate the expected activity, instead suggesting an ancestral desaturase activity in the heterologous systems.

A desaturase with unique Δ8 functionality is involved in *G. molesta* pheromone biosynthesis. CRISPR/Cas9 knock-out is a good alternative for gene characterisation in Tortricid moths, while heterologous expression often does not work as expected.

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The predictive power of AlphaFold-generated protein structures of insect fatty acyl desaturases

Stanislav Machacek, **Michal Tupec, Natan Horacek, Pavlina Kyjakova, Ondrej Luksan, Iva Pichova, Robert Hanus**
Institute of Organic Chemistry and Biochemistry CAS. Flemingovo náměstí 2, 16000 Prague, Czech Republic

Fatty acyl desaturases (FADs) are membrane-bound enzymes that catalyze the introduction of a double bond into a fatty acyl (FA) molecule and are among the enzymes involved in the biosynthesis of insect pheromones.

Here, we characterized FADs involved in the fatty acid-derived pheromone production in termites (*P. simplex*, *S. trispinosus*). We have selected the ten most abundant FAD-gene candidates using bioinformatic analysis of data from available databases and from transcriptomes obtained from different tissues of termites. The selected candidate genes have been functionally characterized in *S. cerevisiae*. Generation of FAD structures using AlphaFold can help us to predict position of the substrate in the binding tunnel, position of the newly introduced double bond in FA, and the substrate specificity of FADs. In addition, the constructed structures enable to identify amino acid residues from the binding tunnel, which are critical for the enzyme specificity.

The functional characterization of FADs in combination with the AlphaFold modeling appears to be a tool that can accelerate the characterization of enzymes in various organisms.

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Novel type of Δ5 desaturases biosynthesizing eicosanoid precursors in termites

Michal Tupec, Stanislav Macháček, Ondřej Lukšan, Iva Pichová, Robert Hanus
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 2, 16000 Prague, Czech Republic

In animals, the eicosanoids modulate immune response, reproduction, development and homeostasis. They are biosynthesized from polyunsaturated fatty acids (PUFAs) by a set of specific oxygenating and cyclizing enzymes. In vertebrates and in some invertebrates, several front-end desaturases (FEDs) participate in the biosynthesis of arachidonic acid (AA) which is a major eicosanoid precursor. However, no such enzymes have been found and characterized in insects. There is only limited information on AA and eicosanoid biosynthesis in these ubiquitous animals based on isotope labelling. Additionally, some eicosanoids have been shown to regulate physiological processes in insects.

While searching for desaturases involved in trail-following pheromone biosynthesis in termites (*Prorhinotermes simplex*), we discovered several enzymes with rather basic metabolic functions. We have characterized Δ9 and Δ12 desaturases, which together transform saturated stearic acid into linoleic acid which is an essential PUFA for many animals including all vertebrates.

Here, we report another group of desaturases which presumably engage in the biosynthesis of eicosanoid precursors in termites. These newly characterized enzymes are Δ5 desaturases, accepting a wide range of substrates. Remarkably, they are structurally distinct from FEDs which perform a similar function in vertebrates. By combination of FA analysis in termite tissues, phylogenetic analysis, structure prediction, and functional characterization, we draw the possible basis of function of these novel insect Δ5 desaturases and their involvement in AA biosynthesis.

This project was financially supported by the Czech Science Foundation (20-17194S) and the Czech Academy of Sciences (RVO:61388963).

Highly accurate discovery of terpene synthases powered by machine learning reveals functional terpene cyclization in Archaea

Raman Samusevich[1,2], Téo Hebra[1], Roman Bushuiev[1,2], Anton Bushuiev[2], Tereza Čalounová[1], Helena Smrčková[1], Ratthachat Chatpatanasiri[2], Jonáš Kulhánek[2], Milana Perković[1], Martin Engst[1], Adéla Tajovská[1], Josef Sivic[2], Tomáš Pluskal[1]
[1]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
[2]*Czech Institute of Informatics, Robotics and Cybernetics (CIIRC), Czech Technical University in Prague, Czech Republic*

Terpene synthases (TPSs) generate the scaffolds of the largest class of natural products, including several first-line medicines. A wealth of knowledge on the complexity of TPS reactions has been accumulated through decades of scientific research. However, millions of uncharacterized TPS sequences can be found in genomic databases and accurate computational prediction of their function remains an unsolved challenge. We curated a dataset of 2,500 characterized TPS reactions and developed a method to devise highly accurate machine-learning models for functional annotation in a low-data regime. Our models significantly outperform existing methods for TPS detection and substrate prediction. By applying the models to large protein sequence databases, we discovered and experimentally validated a number of new TPS enzymes previously undetected by state-of-the-art bioinformatic tools, including the first reported TPSs in Archaea. Furthermore, we described a new TPS structural domain and distinct subtypes of previously known domains. Our results demonstrate the potential of machine learning to speed up the discovery and characterization of novel enzymes.

Unlocking animal specialized metabolite biosynthesis: The case of an octocoral briarane diterpene gene cluster family

Natalie E. Grayson[1,6], **Paul D. Scesa**[2,6], **Malia L. Moore**[1,3], **Jean-Baptiste Ledoux**[4], **Todd P. Michael**[1,3], **Immo Burkhardt**[1], **Eric W. Schmidt**[2], **Bradley S. Moore**[1,5]
[1] *Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA*
[2] *Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT, USA*
[3] *Salk Institute for Biological Studies, La Jolla, CA, USA*
[4] *CIIMAR*
[5] *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA*
[6] *These authors contributed equally*

Specialized metabolites, integral for defining organisms' niches, provide distinctive traits and facilitate communication. Bacteria, plants, and animals exhibit diverse strategies in specialized metabolite production. Bacteria organize most of the genes responsible for specialized metabolite biosynthesis into clusters, while plants often organize pathways toward common intermediates into clusters. However, there are no biosynthetic gene clusters verified from animals. The only examples are recently discovered putative biosynthetic gene clusters in octocorals. These sessile marine invertebrates lack tough exterior skeletons. Instead, they depend on the biosynthesis of specialized metabolites, specifically terpenes, as chemical defenses. Through high-quality genome assemblies and heterologous biosynthesis, we validate the first animal biosynthetic gene cluster. An octocoral gene cluster containing a terpene cyclase, cytochrome P450s, and a short-chain dehydrogenase is involved in the production of a complex terpene. The terpene product most likely represents an advanced common intermediate in the biosynthetic pathway to briarane terpenoids, a coral specific structural class comprising over 600 known compounds. Notably, microsyntenic analysis showed that briarane producing octocoral across genera have the conserved gene cluster, which is unprecedented amongst metazoans. These results raise the question about the extent of such organizational trends in animal metabolic evolution.

Evolutionary origin of terpenoid biosynthesis in termites

Jitka Šťáfková[1], **Natan Horáček**[1,2], **Anna Amirianová**[1,3], **Daniel Jungwirth**[1,2], **Pavlína Kyjaková**[1], **Robert Hanus**[1]
[1]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
[2]*Faculty of Science, Charles University, Prague, Czech Republic*
[3]*University of Chemistry and Technology Prague, Czech Republic*

Terpenoids are a rich and diverse class of natural compounds and their biosynthesis in plants and microbes has long been the subject of intensive research. In the animal kingdom, it is primarily the insects which employ terpenoids in chemical communication and defence. The responsible terpene synthases have only been functionally characterized in a handful of species. Unlike the plant enzymes, they arose by independent duplications and neofunctionalizations of isoprenyl diphosphate synthase genes within individual insect lineages. As termites are renowned for their diverse array of terpenoids, we set out to map the emergence of terpenoid biosynthesis in this lineage. We searched for homologs of isoprenyl diphosphate synthases across termite species and identified a duplication in one of these, geranylgeranyl pyrophosphate synthase, as an apomorphy of the modern termite lineage Neoisoptera. Terpenoids are known to be present only in Neoisoptera, making these duplicated genes logical candidates for terpene synthase function. Indeed, using heterologous protein expression and functional assays, we succeeded in identifying two terpene synthases in *Embiratermes neotenicus* responsible for generation of the sesquiterpene queen pheromone (3*R*,6*E*)-nerolidol and the diterpene neocembrene, a component of queen-specific volatile blend. Likewise, we found an *Nasutitermes takasagoensis* enzyme to generate neocembrene, presumed in this species to be the precursor for an array of polycyclic oxygenated diterpenoids. Altogether, we conclude that termite terpene synthases arose by a single duplication event roughly 100 million years ago in an ancestor of Neoisoptera, followed by further duplications and neofunctionalizations.

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A novel terpene synthase underlying anti-aphrodisiac pheromone production in *Heliconius* butterflies

Kathy Darragh[1,2,a], Anna Orteu[1], Daniella Black[1,3], Kelsey J. R. P. Byers[1,2,b], Daiane Szczerbowski[4], Ian A. Warren[1], Pasi Rastas[5], Ana Pinharanda[1,c], John W. Davey[1,d], Sylvia Fernanda Garza[2], Diana Abondano Almeida[2], Richard M. Merrill[2,6], W. Owen McMillan[2], Stefan Schulz[4], Chris D. Jiggins[1,2]
[1] *Department of Zoology, University of Cambridge, Cambridge, UK*
[2] *Smithsonian Tropical Research Institute, Panama, Panama*
[3] *School of Biology, University of St Andrews, St Andrews, UK*
[4] *Institute of Organic Chemistry, Department of Life Sciences, Technische Universitat Braunschweig, Braunschweig, Germany*
[5] *Institute of Biotechnology, University of Helsinki, Helsinki, Finland*
[6] *Division of Evolutionary Biology, Ludwig-Maximilians-Universitat Munchen, Munich, Germany*
Current addresses:
[a] *Department of Biology, Indiana University, Bloomington, USA*
[b] *Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK*
[c] *Department of Biological Sciences, Columbia University, New York, USA*
[d] *Bioscience Technology Facility, Department of Biology, University of York, York, UK*

A major outstanding question in biology is how metabolism has evolved and diversified to produce the chemical diversity found in nature. To unravel how biosynthetic pathways evolve, we need to identify the genetic basis of compound production. Terpenes are one of the most structurally diverse compound classes and have key functions in interactions between organisms such as predator defense and mate choice. While the biosynthesis of terpenes has been described in plants and microorganisms, only a few terpene synthases have been identified in insects. In fact, it was previously thought that insects were unable to synthesize terpenes and instead sequestered them from plants. To unravel the genetic basis of terpene synthesis in *Heliconius* butterflies, we combined linkage mapping, gene expression, and enzyme assays to identify and functionally validate the gene. The novel terpene synthase I discovered is unrelated to previously described plant enzymes, demonstrating that this case of chemical convergence has independent evolutionary origins. Even more excitingly, the butterfly terpene synthase is from a different gene family to the previously described insect enzymes and does not share the same amino acid changes. This provides an opportunity to study how the same enzymatic activity can evolve repeatedly from different starting points, to better understand the predictability and flexibility of evolution.

Terpene biosynthesis in the sandfly *Lutzomyia longipalpis*

Charles Ducker[1], Antônio Euzébio Goulart Santana[2], John A. Pickett[3], Neil J. Oldham[1]
[1]*School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, UK*
[2]*Center of Engineering and Agrarian Science, Federal University of Alagoas, Maceio 57100-000, Brazil*
[3]*School of Chemistry, Cardiff University, Cardiff CF10 3AT, UK*

Leishmaniasis is a debilitating and often fatal neglected tropical disease. Males from the *Leishmania*-harbouring sandfly, *Lutzomyia longipalpis*, produce sex and aggregation terpene pheromones from diphosphate-containing isoprenoid chains derived from the mevalonate pathway. One of these is the diterpene sobralene, for which geranylgeranyl diphosphate (GGPP) is the isoprenoid precursor. We have identified a novel terpene synthase (*ll*TPS) from *L. longipalpis*, which was recombinantly expressed in bacteria and purified for functional analysis. The molecular weight in solution of *ll*TPS was determined to be 86.4 kDa by native MS, corresponding to a dimeric assembly. *In vitro* enzymatic GC-MS assays showed that *ll*TPS is an active enzyme, capable of converting geranyl diphosphate into various monoterpenes and monoterpenoid alcohols including (*E/Z*)-β-ocimene and linalool. Incubation of *ll*TPS with (*E,E*)-farnesyl diphosphate (FPP) yielded the sesquiterpene (*E*)-β-farnesene while (*Z,E*)-FPP and (*Z,Z*)-FPP gave a mixture of bisabolene isomers. Most notably, *ll*TPS produced a mixture of diterpenes when incubated with (*E,E,E*)-GGPP, and the major product was found to be sobralene when compared against an authentic male pheromone extract from a sobralene-producing sandfly population from Ceará state, Brazil. The minor diterpene components from enzyme assays included verticillene isomers, which were also observed in the pheromone extract. To our knowledge, this is the first identification of a true insect diterpene synthase, and presents a potential biosynthetic route for sustainable pheromone production for use in mass trapping strategies.

POSTERS

P-01

Salt stress activated the Warburg Effect in *Brassica napus* L.

Tülay Alp Öztürk, Şerife Palabiyik, Melike Bor
Ege University, Faculty of Science, Department of Biology 35100 Bornova Izmir, Türkiye

Adaptation to the changing environment is among the biggest challenges of plants and due to their sessile nature, they have evolved a wide variety of defense and resilience mechanisms. The Warburg Effect, was first defined in cancer cells in which glucose is converted to lactate although oxygen is abundant. In plants how Warburg effect is activated, its mechanism and its interaction with different metabolic pathways is unknown. Therefore, we aimed to examine whether the Warburg effect is observed in plant cells under salt stress conditions, and if it is observed, whether it provides an advantage for plant cells. We treated young seedlings of *Brassica napus* plants with 100 mM NaCl and sampled the fast growing meristematic tissues from the shoot and root tips. We assayed lactate and proline contents, lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) enzyme activities in these samples. The lactate content and LDH activity showed different trends in leaves and roots. In general, both lactate content and LDH activities increased in the roots while, LDH activity increased and lactate content decreased in the leaves during the first days of the salt stress treatment. Under salinity, PDC activities were increased in the leaf and root samples while ADH showed a different trend in which the onset of incline was different in leaf and root samples. We propose that lactate might be converted to pyruvate in order to provide an additional energy source to support growth and development under salt stress conditions in *Brassica napus* plants.

P-16

The effect of modulator molecules on GABA-shunt in *Brassica napus* L.

İrem Çetinkaya, Tülay Alp Öztürk, Melike Bor
Ege University, Faculty of Science, Department of Biology 35100 Bornova-Izmir, Türkiye

Gamma amino butyric acid (GABA) is a component of the free amino acid pool in all organisms and its function as a neurotransmission inhibitor molecule in the central nervous system of the mammals is very well defined. In plants, GABA and GABA-shunt play an active role in the different physiological processes including the adjustment of cytosolic pH, maintenance of carbon and nitrogen balance, control of stomatal movements, regulation of growth and by-pass for the tricarboxylic acid (TCA) cycle, signal transduction and defense responses. Although some of these processes have been studied in detail at the molecular level, how they are regulated and controlled is not well known. The regulation of GABA receptors through positive and negative modulator molecules is an important concept for excitation and inhibition processes in the central nervous system in animals. In this context, modulator molecules might also affect GABA levels and activation of the GABA-shunt in plant cells. The sudden changes and alterations in the GABA content in plant cells especially under stress conditions might be related to a similar regulation process as it is in mammals. The aim of our project is to determine how the agonist modulator, gallic acid, and antagonist modulator, picrotoxin, affect GABA content and GABA-shunt in plant cells. In the light of this aim, we conducted experiments with different concentrations of gallic acid (0, 0.5 and 1 mM) and picrotoxin (0, 0.1 and 1 µmol) in *Brassica napus* plants and compared how GABA contents and GABA-shunt components are affected.

P-34

Metabolomic response of *Ulmus minor*, *Ulmus laevis*, and *Ulmus glabra* to drought stress

Alina Kalyniukova[1], Jaroslav Holuša[1], Ivana Tomášková[1], Jaromír Hradecký[1], Axel Schopf[2]
[1]*Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, CZ – 165 21 Praha 6 – Suchdol, Czech Republic*
[2]*Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, Peter-Jordan-Straße 82/1, 1190 Vienna, Austria*

The widespread European native elm species—*Ulmus minor*, *Ulmus laevis*, and *Ulmus glabra*—are significantly affected by biotic stress. This study aimed to examine the impact of summer drought stress on these tree species in the context of climate change. Utilizing LC-MS and GC-MS metabolomic analyses, the effects of drought on secondary metabolism and non-structural carbohydrates were investigated in the leaves of these three elm species under semi-field conditions and two water regimes during one season.

Metabolomic analysis revealed substantial differences in the profiles of non-volatile and volatile metabolites among the three elm species. GC-MS analysis identified typical green leaf volatiles, such as 2-hexen-1-al and 3-hexenyl-1-acetate, and terpenes in *Ulmus*, which were more pronounced in *U. glabra* and *U. laevis* than in *U. minor*. The phenolic compound ratios varied by tree species, showing the highest levels in July, but drought and irrigation did not significantly alter phenolic quantities.

Our results suggest that *U. minor* is the best adapted to drought conditions among the three elm species, while *U. laevis* exhibits the most significant changes in secondary metabolism to mitigate osmotic and subsequent biotic damages.

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P-45

Regulation of NUDX1-1a gene, a pivotal player in geraniol production in Roses

Mariana Lopez-Gordillo[1], **Corentin Conart**[1], **Saretta Paramita**[1], **Jean-Claude Caissard**[1], **Robert C. Schuurink**[2], **Sylvie Baudino**[1], **Denis Saint-Marcoux**[1]
[1]Université Lyon, Université Saint-Etienne, CNRS, UMR 5079, Laboratoire de Biotechnologies Végétales appliquées aux Plantes Aromatiques et Médicinales, Saint-Etienne, France
[2]Green Life Sciences Research Cluster, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, the Netherlands

Roses have been cherished since antiquity for its ornament and scent qualities. Rose scent originates from a complex mixture of volatile organic compounds (VOCs), geraniol being one if its main constituents. Contrary to other aromatic plants, geraniol in roses is synthetized by a non-canonical pathway involving a different enzyme family, the nudix hydrolases. Geraniol biosynthesis is not only related to key synthases but is also regulated by the activity of transcription factors (TFs). Some TFs have been identified to modulate *NUDX1.1a* gene, but its positive and tissue specific regulation remains unexplored. In the present study, a petal-specific TF was sought for, performing a comparative transcriptome analysis of different tissues, stages and varieties of roses. The action of the TF candidates on the promoter of the target gene *NUDX1.1a* was studied using tobacco dual-luciferase assays, yeast one-hybrid (Y1H) analysis and CHIP-qPCR. We determinated that one particular TF was able to transactivate the expression of the reporter gene. This result gives insights related to the transcriptional regulatory framework underlying the control of geraniol production in rose.

P-62

Pheromone biosynthesis gene silencing using RNA interference in a spruce forest pest, *Ips typographus*

Rajarajan Ramakrishnan, Jaroslav Strádal, Anna Jirošová
Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Czech Republic

Bark beetle attacks on coniferous vegetation have been recorded worldwide. In central Europe and Asia, the main pest is the Eurasian spruce bark beetle, *Ips typographus*, which has caused severe ecological and economic damage over the last decade. Bark beetles coordinate their activities using aggregation pheromones to select an appropriate host and establish a healthy colony. Understanding the genetic basis of aggregation pheromone production, and the subsequent silencing of key genes using RNA *interference* (RNA*i*), may be a future management method for these pests. Previous studies on *Ips typographus* have identified several potential genes involved in pheromone biosynthesis. Here, we describe the RNA*i* silencing of a key gene, isoprenyl diphosphate synthase (IPDS), suspected for the terminal biosynthesis step of a main pheromonal component, the hemiterpene 2-methyl-3-buten-2-ol. Initially, we optimized the delivery method by injecting the double-stranded RNA (*ds*RNA) into the beetle body. Subsequently, freshly emerged males in their host-searching stage were chosen for this method. After 3 days post-injection, males fed on spruce logs to induce pheromone biosynthesis were collected. Pheromone levels were assessed via non-invasive headspace analysis using GC-MS, while the transcripts of the targeted gene from the gut were quantified using qRT-PCR. Treated male beetles emitted four times less 2-methyl-3-buten-2-ol and showed a two-fold reduction in IPDS transcript levels compared to the controls. This RNA*i* approach provided insights into the functional characterization of the key pheromonal gene, IPDS in *Ips typographus* and could serve as the basis for future pest management methods.

P-65

Exploring chemical communication in *Dalbulus maidis*: Double choice olfactometer bioassays indicate that males are the sex-pheromone producer

Mateus Sanches[1,2], Raul Laumann[1], Miguel Borges[1], Marina Frizzas[2], Charles Martins Oliveira[3], Maria Carolina Blassioli-Moraes[1]
[1]Semiochemicals Laboratory, Embrapa Genetic Resources & Biotechnology, Brasília, Brazil. Semiochemicals Laboratory, Embrapa Genetic Resources & Biotechnology, Brasília, Brazil
[2]Graduate Program in Ecology, University of Brasília, Brasília, Brazil
[3]Embrapa Cerrados, Brasília, Brazil

The corn leafhopper, *Dalbulus maidis*, is an important corn pest due to its transmission of phytopathogens to maize. Therefore, exploring communication could aid in controlling this pest. For many insect species, pheromones are vital for intra-specific communication, especially in reproduction. Understanding how insects locate sexual partners over long distances can provide information, such as pheromones and reproductive behavior, for developing effective monitoring and control methods for pests. The aim of this study was to investigate if *D. maidis* produces pheromones to attract mating partners. The volatiles released by males and females were collected using an air-entrainment system, that consisted of a glass chambers with 100 specimens of each sex without food. A second set of volatile collection was conducted over a period of 3 weeks, with three replicates per sex, along with maize plants as food. The behavioral responses of *D. maidis* females and males was evaluated in Y olfactometer bioassays to odor emitted by 20 specimens of each sex versus pure air. The chemical analysis of sample volatiles of males and females did not show any sex specific peak. In the bioassays, males showed no preference for female volatiles or air ($p = 0.796$), while females showed a strong preference for male volatiles over air ($p = 0.001$). These results suggest that *D. maidis* males release volatile attractive compounds to females, indicating that males can be the sex pheromone producer. Studies are being conducted to identify the pheromone compounds of this species.

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P-79

All on one chromosome – Revealing the genetic underpinnings of terpene formation in the Asian ladybeetle *Harmonia axyridis*

Zarley Rebholz[1], Hailey Larose[1], Maria Doronitsyna[2], Todd Ugine[3], Jinsong Zhu[2], Paul O'Maille[4], Dorothea Tholl[1]
[1]Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA
[2]Department of Biochemistry, Virginia Tech, Blacksburg, VA, USA
[3]Cornell University, Ithaca, NY, USA
[4]SRI International, Menlo Park, CA, USA

Many insects release volatile terpenes as semiochemicals. Recent studies provided evidence for the ability of insects to produce these compounds with endogenous enzymes, which evolved from isoprenyl diphosphate synthases (IDS) by gaining terpene synthase (TPS) activity. We investigated whether this evolutionary scenario led to the formation of volatile terpenes in the invasive Asian lady beetle, *Harmonia axyridis. H. axyridis* females emit an aggregation pheromone consisting of sesquiterpenes with (*E*)-β-caryophyllene (EβC) as the predominant constituent. Males and females also contain monoterpenes of unknown function. We identified a family of six IDS-type genes on a single chromosome in the *H. axyridis* genome consisting of two canonical *trans*-IDSs (*trans*-farnesyl diphosphate synthases), a *cis*-IDS with predominant function as neryl diphosphate (NPP) synthase, and three genes with TPS function: HaTPS3 can convert NPP into monoterpenes found in the insect, and HaTPS1 and 2 are sesquiterpene synthases with TPS1 producing all female pheromone components. Subcellular localization experiments indicated a mitochondrial compartmentalization of HaTPS1 and IDSs that form the TPS substrate. Mining and synteny analysis of *H. axyridis* and other lady beetle genomes revealed the presence of IDS and TPS orthologs in related species with a conserved occurrence of *cis*-IDS and *cis*-substrate-acting monoterpene synthases. Together, our findings show that *H. axyridis* IDS and TPS genes evolved on a single chromosome to support the formation of terpene semiochemicals and suggest that *cis*-IDS enzymes might represent an evolutionary link between *trans*-IDS and TPS functional states.

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P-84

Molecular identification of a laccase that catalyzes the oxidative coupling of a hydroxycinnamic acid amide for hordatine biosynthesis in barley

Naoki Ube[1], **Atsushi Ishihara**[2], **Yasuo Kato**[1], **Taiji Nomura**[1]
[1]*Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Imizu, Japan*
[2]*Faculty of Agriculture, Tottori University, Tottori, Japan*

Plants produce dimerized phenolic compounds as secondary metabolites. Hordatine A (HA), a dehydrodimer of *p*-coumaroylagmatine (*p*CA), is an antifungal compound accumulated at high levels in young barley (*Hordeum vulgare*) seedlings. The enzyme responsible for the oxidative dimerization of *p*CA, which is the final step of the hordatine biosynthetic pathway, has not been identified. In this study, we first verified the presence of this enzyme activity in the crude extract of barley seedlings. Because the enzyme activity was not dependent on H_2O_2, the responsible enzyme was not peroxidase, which was previously implicated in HA biosynthesis. The analysis of the dissection lines of wheat (*Triticum aestivum*) carrying aberrant barley 2H chromosomes detected HA in the wheat lines carrying the distal part of the 2H short arm. This chromosomal region contains two laccase genes (*HvLAC1* and *HvLAC2*) that are highly expressed at the seedling stage and may encode enzymes that oxidize *p*CA during the formation of HA. Changes in the *HvLAC* transcript levels coincided with the changes in the HA biosynthesis-related enzyme activities in the crude extract and the HA content in barley seedlings. Moreover, *HvLAC* genes were heterologously expressed in *Nicotiana benthamiana* leaves and in bamboo (*Phyllostachys nigra*) suspension cells and HA biosynthetic activities were detected in the crude extract of transformed *N. benthamiana* leaves and bamboo suspension cells. The HA formed by the enzymatic reaction had the same stereo-configuration as the naturally occurring HA. These results demonstrate that HvLAC enzymes mediate the oxidative coupling of *p*CA during HA biosynthesis.

P-85

Beneath the surface: Revealing plant defenses against seed predators

Gwanhyeong Yu[1], **Bo Eun Nam**[2], **Jonghun Noh**[1], **Youngsung Joo**[1,3]
[1]*School of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea*
[2]*Research Institute of Basic Sciences, Seoul National University, Seoul 08826, Republic of Korea*
[3]*Institute for Data Innovation in Science, Seoul National University, Seoul 08826, Republic of Korea*

Plants have diverse secondary metabolites as a defense strategy against herbivores that attack diverse tissue of host plants. Although seeds possess unique features and are considered to have high fitness value, how plants defend their seed is still elusive. We used *Ceutorhynchus albosuturalis*, a seed specific herbivore of Brassicaceae and *Capsella bursa-pastoris* as a model system to investigate interactions between plants and seed predators. We established a laboratory colony of *C. albosuturalis* and designed the experimental egg inoculation method to manipulate plant-seed predator interactions. We found increased transcription levels of JA biosynthesis, response genes and the actual JA level was increased by the seed predator. Additionally, the seed predator induced the expression of many genes associated with the biosynthesis of secondary metabolites. To determine which secondary metabolites were induced by the seed predator, we analyzed the glucosinolate contents of attacked seeds using liquid chromatography-tandem mass spectrometry. Although total glucosinolate contents were not significantly changed by the seed predator, some aliphatic and putative glucosinolates were increased by the seed predator. To test the function of induced glucosinolates and their biosynthesis genes, we plan to compare larval performance on transgenic *C. bursa-pastoris*.

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P-86

Chemical defence and warning signal pigment evolution in an aposematic bug

Jiajia Zhao, Astrid Groot, Emily Burdfield-Steel
Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands

Insects have evolved a lot of strategies to deter predators, with the most striking being aposematism. Aposematic animals use conspicuous colour and chemical defence to advertise their unprofitability to natural enemies. However, the synthesis of warning signals and the production or sequestration of defence chemicals can be costly. Thus, there may be a trade-off between aposematic colour and chemical defence in many species. Still, the exact mechanisms underlying this trade-off have been investigated in only a few species and remain poorly understood. Here we investigate chemical and pigment variation in the aposematic bug, *Lygaeus simulans*, displaying red-black (wild type) and yellow-black (pale mutant) colours due to a recessive allele at 29 ºC. It's shown that pale mutants are temperature sensitive – yellow mutants revert to wild-type colouration when reared below 24 ºC. We found pteridines are major pigments in adults, and a candidate pigment, erythropterin (which is associated with orange and red colour in butterflies), may be underlying the colour change in the pale mutant. Then, we analyzed bug CHCs and pheromone components to investigate the relationship between their chemical signaling and colour polymorphism. Future work will focus on toxin sequestration in the two lines. We propose *L. simulans* as a new model system in which to detangle the mechanistic relationships between colour production and chemical defence.

S9: Synthesis as a key technology in chemical ecology

***Chair:** Stefan Schulz*

ORAL PRESENTATIONS

The mysterious case of sodorifen

Jeroen S. Dickschat, Houchao Xu, Lukas Lauterbach
Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Bonn, Germany

Terpene synthases catalyse the conversion of acyclic and achiral oligoprenyl diphosphate precursors into usually (poly)cyclic, chiral and enantiomerically enriched terpene hydrocarbons or alcohols. These reactions proceed with multiple carbon-carbon bond formations and changes of the hybridisation of often more than half of the precursor carbons in just one enzymatic step. The mechanisms of terpene synthases can be investigated through isotopic labelling experiments,[1] structure based site-directed mutagenesis,[2,3] computational chemistry,[4] and, ideally, combinations thereof.[5] The lecture will discuss the intricate biosynthesis of the unusual methylated sesquiterpene sodorifen.[6,7]

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The fate of custom chemical synthesis of pheromones in academia is intertwined with commercial enterprise

Robert Setter, **Mike Fraser**
Synergy Semiochemicals Corp

The commercial development of the insect pheromone market is crucial to the longevity of custom pheromone synthesis in academia. Successful commercial operations utilizing pheromones help drive the desire to expand the use pheromones to new species. However, commercial manufacturing of many small batches of unique pheromones is extremely challenging economically. Supporting a custom synthesis lab commercially demands significant revenue be generated from other sources. We are fortunate in that we can subsidize small scale custom chemical synthesis work with mid-scale product synthesis coupled to formulation, manufacturing and product sales. But we still rely on academic labs to find novel pathways for challenging syntheses. When using published academic protocols for novel compounds we often encounter great challenges with scaling up the reactions for commercial use. Minimum yields acceptable in many academic syntheses are much smaller than the yields required even for small numbers of commercial lures. Many academic syntheses utilize reagents that may not be cost effective or safe once the reaction size is scaled up. There is often a need for significant reworking of a reaction pathway. Multiply this effort by the number of requests for custom syntheses for new insects and it becomes a challenge to do the custom work without charging high fees. This results in lost commercial potential and erodes support for custom synthesis work. How can academia and industry work closer together in order to smooth the transition of novel compound synthesis from an academic exercise to commercial viability?

Identification and synthesis of macrolides from *Anagasta kuehniella* (Lepidoptera, Pyralidae) frass, attractive to the ectoparasitoid *Habrobracon hebetor* (Hymenoptera, Braconidae)

Douglas J. Melo, João Pedro de Albuquerque Souza, Arodí Prado Favaris, Paulo H. G. Zarbin
Department of Chemistry, Federal University of Parana – UFPR, Curitiba/PR, Brazil

In breeding the moth *Anagasta kuehniella*, used for mass rearing natural biological control agents, the ectoparasitoid *Habrobracon hebetor* poses a significant issue. Bioassays revealed that caterpillar frass attracts this parasitoid. To identify the kairomones involved, frass was extracted, fractionated, and tested. *H. hebetor* showed attraction to the 95:5 hexane:ethyl ether fraction. GC analysis of this fraction indicated two compounds, one major and one minor.

The major compound was identified using GC-FTIR, revealing an intense peak at 1,733 cm^{-1} (ester group) and a low-intensity peak at 3,002 cm^{-1} (Z-configuration double bond). GC-MS analysis showed a molecular ion with m/z 280, suggesting a molecular formula of $C_{18}H_{32}O_2$ and a cyclic structure. The position of the double bond was determined using DMDS, resulting in peaks at *m/z* 136 and 122, indicating unsaturation at position 9. The kairomone was identified as (*Z*)-oxacyclononadec-9-enone.

The kairomone was synthesized in seven steps, starting from nonane-1,9-diol and using metathesis with a Grubbs II catalyst, yielding a 9:1 mixture of *Z* and *E* isomers. The structure was confirmed by coelution with the *Z* isomer. The minor compound is still being identified and synthesized.

Identification and synthesis of a male-specific compound from *Leucoptera sinuella* (Lepidoptera: Lyonetiidae) and study of its role in intraspecific communication

Laura Sánchez-Aros[1], Wilson Barros-Parada[2], Jorge Guajardo[3], Abel Queiroz[4], Glenn Svensson[5], Jan Bergmann[1]
[1]*Institute of Chemistry, Pontificia Universidad Católica de Valparaíso, Chile*
[2]*Agronomy School, Pontificia Universidad Católica de Valparaíso, Chile*
[3]*Universidad de Talca, Chile*
[4]*Centro Universitario Ages, Brazil*
[5]*Biology Department, Lund University, Sweden*

The poplar moth *Leucoptera sinuella* (Lepidoptera: Lyonetiidae) is native to the palearctic region but was introduced accidentally into Chile about 10 years ago, where it is causing direct damage in poplar nurseries and affects export of fresh fruit due to the presence of pupae.

Recently, our group has identified the main and two minor components of the female sex pheromone. Additionally, we detected a male-specific compound in the abdominal hairpencil structure. These structures are known as dissemination structures for male pheromones in Lepidoptera, and the aim of this work was to identify the unknown compound present in males and to evaluate its role in attracting and/or accepting females in mate selection.

Pupae were collected from poplar trees in central Chile and were kept in the laboratory until adult emergence. Extracts were prepared from hairpencil glands and analyzed by GC-MS. Through chemical microderivatization reactions and comparison of mass spectra and retention indices of the natural compounds with synthetic standards, two compounds were identified: (*Z*)-3-decenyl hexanoate (major) and (*Z*)-3-decen-1-ol (minor). Although EAG recordings using the synthetic compound on both male and female antennae were inconclusive, behavioral laboratory bioassays point to a role in the mating behavior. Pairs with intact males and intact females generally copulated successfully, but pairs where either the male was lacking the hairpencil or the female was lacking antennae, generally did not mate. For males without hairpencils, mating success was at least partially restored when male extract or synthetic (Z)-3-decenyl hexanoate was present.

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Benzooxathiols and aryl methyl sulfides: New compound classes in the chemical weapon arsenal of Collembola

Anton Möllerke, Stefan Schulz
Institute of Organic Chemistry, TU Braunschweig, 38106 Braunschweig, Germany

Collembola are soil-inhabiting arthropods and closely related to the Insecta, from which they diverged about 400 mya. They are commonly referred to as springtails, due to their ability to catapult themselves out of danger using their furca, a tail-like appendage. However, soil-dwelling or soil-inhabiting species have a reduced or absent furca and rely more on chemical defenses for protection. We aimed to clarify the composition of this chemical defense system.

A combination of GC/MS and GC/IR analysis was utilized to elucidate the structures of compounds from crude extracts of *Ceratophysella denticulata*. This approach allowed us to identify a group of aryl sulfides that demonstrated a deterrent effect on predatory insects in bioassays. Additionally, we developed a total synthesis to provide access to the natural compounds. Although aryl methyl sulfides have been reported as natural products in bacteria and marine invertebrates, there have been no previous reports of benzooxathiols or aryl methyl sulfides in arthropods, best to our knowledge. The high abundance of these compounds in *C. denticulata* indicates their importance for the springtail.

Total synthesis of oligomeric ascarosides from the hermaphroditic nematode *Caenorhabditis tropicalis*

Célia P. Bergame,[1] Chuanfu Dong,[2] Stephan H. von Reuss[1]
[1]Laboratory for Bioanalytical Chemistry, University of Neuchatel, Neuchatel, Switzerland
[2]Center for Biological Sciences and Technology, Beijing Normal University, Zhuhai City, China

Chemical signaling in nematodes involves a diverse class of glycolipids called ascarosides that are based on the 3,6-dideoxysugar L-ascarylose connected to (ω – 1)-linked homologous acyl aglycones ranging from 5 to 15 carbons. While these basic ascarosides are highly conserved in nematodes, they also serve as building blocks for the biosynthesis of species-specific derivatives that carry additional moieties from various primary biosynthetic pathways attached to the L-ascarylose sugar or the acyl aglycone. The resulting modular ascarosides regulate species-specific interactions and are biosynthesized in trace quantities only, which renders their identification by isolation and NMR spectroscopy quite challenging [1].

For example, various species-specific ascaroside dimers have previously been identified in *Caenorhabditis* [2] and *Pristionchus* [3] species. Application of highly sensitive HR-MS/MS screens also revealed a series of related ascaroside homo-oligomers based on a basic asc-C5 building block in the hermaphroditic *Caenorhabditis tropicalis*. Because biosynthesized amounts are far to small for structure elucidation via isolation and NMR spectroscopy, we utilized MS/MS fragmentation to generate a structure proposal and subsequently developed a total synthesis for the dimeric, trimeric, tetrameric and pentameric ascarosides in order to unambiguously establish their structure assignments and to generate authentic material for bioassays.

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Mapping out potential roles of natural products driven by synthetic chemistry

Karl Gademann
Department of Chemistry, Universität Zürich, CH-8057 Zurich, Switzerland

Synthetic organic chemistry constitutes a powerful scientific approach in addressing fundamental questions in biology. The creation of new matter allows to probe molecular function of natural products when combined with detailed biological studies. In this presentation, we will discuss recent work on microbiome metabolites and their potential biological role.

The obligate bacterial leaf symbiosis of *Burkholderia* with *Psychotria* plants has been studied for over a century, yet the main drivers for success remain unclear. We provide evidence that C7 cyclitols such as streptol and kirkamide produced by bacteria can serve dual roles in both herbicidal and insecticidal properties. Mechanism of action studies demonstrate an unusual target for these compounds.

Diazenium diolates such as valdiazen and leudiazen were isolated from *Burkholderia* and *Pseudomonas* bacteria and demonstrated to serve as signaling molecules mediating bacterial communication related to pathogenicity and virulence. Given their low concentrations, lack of a strong chromophore, high polarity, high volatility, and limited stability, chemical synthesis was key in establishing correct structures and delineating activity. In addition, synthesis allowed for the generation of antagonists that suppress bacterial pathogenicity.

POSTERS

P-40

Synthesis of sex pheromones of mealybug and scale species and their field application

Takeshi Kinsho, Miyoshi Yamashita, Naoki Ishibashi, Yasuhiko Kutsuwada, Akihiro Baba, Yuki Miyake, Erina Ohno, Takeru Watanabe
Specialty Chemicals Research Center, Shin-Etsu Chemical Co., Ltd., Joetsu City, Niigata 942-8601 Japan

Sex pheromones of mealybug species of the genus *Crisicoccus*, *Dysmicoccus, Maconellicoccus, Nipaecoccus, Phenacoccus, Planococcus* and *Pseudococcus* (Hemiptera: Pseudococcidae) and scale species of the genus *Aonidiella*, *Comstockaspis* and *Pseudaulacaspis* (Hemiptera: Diaspididae) have been synthesized, aiming for industrial scale up and practical application. These acyclic and cyclic terpenoid esters were successfully manufactured to supply sufficient amounts of the active ingredients for field attraction and mating disruption trials. Trials have been conducted in several countries in Africa, North and South Americas, Asia and Europe so as to demonstrate powerful use of these pheromones and their efficacy in pest identification and population control.

P-43

Ethyl-branched sesquiterpenes: Chemical signals in tropical frogs?

Johanna Kuhn, **Stefan Schulz**
Institute of Organic Chemistry, Technical University of Braunschweig, Braunschweig, Germany

It has been known for several years that frogs communicate not only through visual and acoustic signals, but also through the use of volatile semiochemicals. Male-specific semiochemicals can be released from gular or femoral glands. The volatile compounds are often macrocyclic lactones within the Mantellidae and Hyperoliidae, whose function is not yet well understood. Determining the structure of the components using NMR spectroscopy is challenging due to the difficulty of collecting animals in the rainforest or cultivating them in the laboratory, resulting in limited material. GC/MS enables highly sensitive and powerful separation, allowing for the analysis of complex mixtures with minimal material. GC/MS analysis of a femoral gland extract from the tropical frog *Gephyromantis granulatus* revealed a complex mixture, including some unknown macrocyclic lactones that were further investigated with GC/HRMS and GC/IR.

Analysis of the mass spectrum of the main component suggested it to be a sesquiterpene macrocyclic lactone with ethyl groups instead of the usual methyl branches. In addition, other macrolides with similar mass spectra were discovered; they probably represent a class of substances. The biosynthesis of these unusual terpenoids is still unclear and requires further research.

The structure proposal of the main component and its absolute configuration was confirmed by enantioselective total synthesis. The synthesis uses flexible building blocks and the Julia-Kocienski olefination and macrolactonization as key steps. A silver chromatography allowed the isolation of pure material and the determination of the absolute configuration by GC on a chiral phase.

P-51

Synthesis of pheromones of two processionary moths, *Thaumetopoea pityocampa* and *processionea*, and their application in forest

Yuki Miyake, Takeshi Kinsho, Erina Ohno, Miyoshi Yamashita, Tatsuya Hojo, Takeru Watanabe
Specialty Chemicals Research Center, Shin-Etsu Chemical Co., Ltd., Joetsu City, Niigata 942-8601 Japan

The Pine processionary moth (PPM), *Thaumetopoea pityocampa* Denis & Schiffermüller, and the Oak processionary moth (OPM), *Thaumetopoea processionea* Linnaeus, are the most destructive defoliators of pine trees (*Pinus*) and oak trees (*Quercus*) in Europe. These caterpillars have tiny hairs containing an irritating protein called thaumetopoein and the hairs cause an allergic reaction in people and animals. Insecticide application against both PPM and OPM has been examined, however, it is sometimes difficult because pesticide affect animals and other insects. Pheromone-based control methods against these serious pests have been strongly desired. Female produced sex pheromone of PPM is (13*Z*)-13-hexadecen-11-ynyl acetate and that of OPM is a mixture of (11*Z*,13*Z*)-11,13-hexadecadienyl acetate and (11*Z*,13*E*)-11,13,15-hexadecatrienyl acetate. Wittig reaction of the C-3 and C-13 building blocks enables industrial production of the PPM sex pheromone. One of the OPM pheromone components was synthesized from a conjugated diyne by Hydroboration-Protonation process. The field mating disruption (MD) trials for PPM and OPM have been conducted in France by INRAE-UEFM. In MD trial for PPM, we have deployed tube-type dispensers to pine forest canopy. MD efficacy was demonstrated through suppression of male trap capture and reduction of nest numbers. In MD plots, the trap shutdown was almost perfect and nest numbers were lower than those in control plots. We will also mention the field trial for OPM.

P-57

Biosynthetic strategies in plant defense and plant interactions to assess the impact of plant chemicals on selected natural enemies of whiteflies

Isiaka A. Owolabi[1], Petrina McKenzie-Reynolds[2], Gaelen R. Burke[3], Amnon Levi[2], Alvin M. Simmons[2]
[1]*Department of Entomology, University of Georgia, Charleston, SC, 29414, USA*
[2]*USDA-ARS, U.S., Vegetable Laboratory, Charleston, SC 29414, USA* [3] *Department of Entomology, University of Georgia, GA 30602 Athens, USA*

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera Aleyrodidae) is one of the most destructive insect pests of many agricultural crops including vegetables. It damages plants of cultivated crops through its feeding activities and by transmission of many plant viruses. The sweetpotato whitefly had developed resistance to different categories of several synthetic chemicals. Many non-traditional approaches have been proposed for pest management on crops including the application of biopesticides because they tend to be more environmentally friendly than conventional synthetic pesticides. However, conventional synthetic pesticides for vegetable production typically require costly inputs of non-renewable resources that contribute to environmental degradation, undesirable insecticide residues, soil erosion, or results in adequate pest control. Therefore, there is need to evaluate and determine the impact of chemicals for their effectiveness in reducing target organism without having a negative impact on natural enemies that may be present in the system. Moreover, there are many factors to be considered in determining the best fit for any insect control tactic within an integrated pest management program including efficacy against target pests, safety to applicators and farm workers, minimal residues at harvest, effects on beneficial target and non-target organisms, environmental persistence, and unique mode of action (resistance management). In this study, GC-MS and HPLC are tools to confirm the identification of specific organic compound contents in plants for insect control. This information will be useful in the development of improved pest management.

P-71

New macrocyclic lactones as cuticular lipids in butterflies

Ozan Solak, **Stephanie Ehlers**, **Stefan Schulz**
Institute of Organic Chemistry, TU Braunschweig, Braunschweig, Germany

The identification and synthesis of a new type of insect cuticular lipids selectively found in butterflies of the genus *Eueides* (Heliconiinae) is described.

During the analysis of wing extracts of *Eueides*, unknown compounds were found. Based on GC/MS data, their structure was proposed to be large macrocyclic lactones. All macrolides are saturated and have a chain length between C24 and C31, being cyclized at different positions. They occur in complex mixtures and mostly replace the usual hydrocarbons found on the insect cuticle. To prove the structure of these unprecedented compounds, a simple synthesis was developed starting from enantiomers of commercially available epoxides with a terminal alkene group. Cu-mediated ring opening of the epoxide leads to chiral secondary alcohols that can be esterified with ω-unsaturated carboxylic acids. A ring-closing metathesis reaction produces unsaturated macrolides that are hydrogenated to the target compounds. Comparison of GC/MS data of a series of synthesized macrolides confirmed the structural proposal. Analysis of additional species revealed species-specific mixtures of the macrolides. In contrast, other closely related basal heliconiine species did not contain these macrolides. In conclusion, we describe here a new type of cuticular compounds not previously reported from other arthropods.

S10: Chemical ecology and its role in social organization in social insects

Chairs: *Abdullahi Yusuf, Christian Pirk*

ORAL PRESENTATIONS

Origin of the queen pheromone in the termite *Embiratermes neotenicus*

Jitka Šťáfková, Natan Horáček, Jan Křivánek, Lenka Ulrychová, Pavlína Kyjaková, Robert Hanus
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

Queen primer pheromones are fundamental communication components in colonies of advanced eusocial insects. They advertise the presence of fertile queens, ensure their reproductive monopoly, and generate social cohesion of the colony. Queen pheromone chemistry is only known for two species of termites, the Japanese subterranean termite *Reticulitermes speratus* and the South-American soil-feeding termite *Embiratermes neotenicus*. In the latter species, we recently ascribed the queen primer pheromone function to the sesquiterpenoid alcohol (3*R*,6*E*)-nerolidol, which is secreted on the body surface and to the headspace of primary and neotenic queens and is also present on the surface of eggs. Exposure of female nymphs to (3*R*,6*E*)-nerolidol prevents their differentiation into neotenic queens. Discovery of *E. neotenicus* queen pheromone generated a number of questions that we try to answer in our current research, such as what is the biosynthetic origin and production organ of (3*R*,6*E*)-nerolidol in queens. In my talk, I will report the identification in *E. neotenicus* of the terpene synthase EneoC, which converts farnesyl pyrophosphate into enantiomerically pure (3*R*,6*E*)-nerolidol. Expression of EneoC is greatly upregulated in queens compared to other castes and the same applies to Eneo protein abundances. Silencing of EneoC expression reduces the quantity of (3*R*,6*E*)-nerolidol secreted by the queens. Using indirect immunofluorescence we determined the localization of EneoC protein in epidermal cells underlying the cuticle of the whole body. Likewise, the tissue-specific Eneo expression analysis confirmed almost exclusive EneoC expression in the queen's epidermis.

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A complex alarm system: Heterogeneity and dynamics of the venom gland composition in the invasive hornet *Vespa velutina nigrithorax*

Mélissa Haouzi[1,2], Laurence Berville[1], Christophe Lucas[1], Florian Bastin[1,3], Alix Khalil[1], Jérémy Gévar[4], Anne-Geneviève Bagnères[5], Eric Darrouzet[1]
[1]IRBI, UMR CNRS 7261, University of Tours, France
[2]LEF, Institute of Biology, University of Neuchâtel, Switzerland
[3]CRA-W, Belgium
[4]iEES Paris, INRAE, France
[5]CEFE UMR CNRS 5175, University of Montpellier, France

In insect societies, pheromones, through chemical communication, play a key role in interspecific interactions and are therefore essential for maintaining social cohesion by regulating colonial behaviour. Among them, the alarm pheromone is at the heart of colonial survival, by enabling conspecifics recruitment and defensive behaviours in the event of danger. Accidentally introduced in France around 2004, the invasive hornet *Vespa velutina nigrithorax* has spread across a large part of the Western Europe, causing a great deal of economic, ecological and public damages. Understanding their chemical communication system is therefore essential for developing effective and selective trapping methods. In this study, the effects of intrinsic factors such as the caste, age and colonial origin were studied on the chemical composition of the hornets' venom gland. We found a heterogeneity between castes, with quantitative and qualitative differences between females, but also a colonial signature of the alarm pheromone. Results highlighted the dynamics of the alarm signal in fertile females and in workers as function of their age. This pheromone could therefore be perceived as a recognition signal and transmit different types of information within the colony.

The two-way street to eusociality: Larval hunger signals shape sociality and sociality shapes brood care in primitively-eusocial bumble bees

Sarah K. Spence, **Etya Amsalem**
Department of Entomology, Center for Chemical Ecology, Center for Pollinator Research, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA 16802, USA

The brood of social insects serves as an extended phenotype of the queen and can therefore shape the social structure by emitting signals about hunger and regulating worker reproduction. In primitively-eusocial bumble bees, as in honeybees and some ants, the brood has profound effects on worker reproduction and sexual production, but whether these effects are mediated via pheromones and the identity of these pheromones remain elusive. Here we hypothesize that bumble bee (*Bombus impatiens*) larvae use a brood pheromone to signal starvation. Six potential chemical compounds from fed and starved larvae extracts were tested against a solvent control in a two-choice bioassay. Of these, hentriacontane (C31) and ethyl linoleate were significantly more attractive to workers. Workers' response to larval signals coincided with the age and the stage of the colony from which they were sampled, with high responsiveness by workers from young colonies where the queen is the sole reproducer of eggs, and a lack of differentiation between signals and solvent in workers from gyne-rearing colonies. Overall, our data show that bumble bee larvae not only produce hunger signals, but that workers' response to these signals are context-dependent on their social structure, resulting in the larvae both shaping the social structure and being cared for depending on the social structure.

The secret life of little fire ants

Carmel M.L. Herold Lozover, Abraham Hefetz, Netta Dorchin
School of Zoology, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel

The little fire ant (LFA), *Wasmannia auropunctata*, is one of the world's worst invasive species. Worldwide attempts to manage or contain the LFA have been only partially successful, mostly because of the unusual biology and ecology of this species.

Ants rely heavily on chemical communication, with trail pheromones playing a pivotal role in foraging. The LFA prefers trail following over diffused individual foraging, yet the components of its trail pheromone are unknown. We aim to decipher the trail pheromone components, then recreate an artificial trail and observe the reaction of individuals encountering it, in lab and later field conditions. Utilizing artificial trails may help disrupt LFA foraging and attract workers to insecticide-laden baits, targeting mostly the LFA and reducing the effect of harmful chemicals on other arthropods and the environment.

In Israel and other places where the LFA invaded, queens reproduce clonally and are genetically identical. Yet, it is unclear whether Israeli colonies exhibit true polygyny or do queens manifest differences in secreted pheromones, cuticular hydrocarbon (CHC) profiles, fertility potential or other phenotypic traits despite genetic uniformity. We examined cuticular extract of queens, workers and males and found a caste-specific cuticular composition. Males and workers have a similar profile, and queens' profile is disparate. This unique profile may play a role in worker attraction and may be correlated to queens' fertility status. We investigate potential correlations between queen preference by workers to traits that may differentiate the queens from each other, and may explain a queen's dominance and reproductive success.

Functional evolution of cuticular hydrocarbons in social insects

Abraham Hefetz
School of Zoology, Tel Aviv University, Israel

Cuticular hydrocarbons are important communication agents in ants, functioning as mediators of nestmate recognition and queen signaling. Originally, hydrocarbons evolved as a part of a protective cuticular layer preventing excess evaporative water loss. For that, long chain saturated linear alkanes are optimal. However ants, for example, are endowed with mono- and dimethyl branched hydrocarbons, which decrease the cuticular impermeability. Moreover, biosynthetically they use a novel pathway rather than deriving from the linear alkane pathway. This indicates that they serve as communicative signals, but how did they evolve as such? I hypothesize that they derived from branched fatty acids, which in turn evolved as disinfectant agents. Long chain branched fatty acids, in particular antesio fatty acids, are membrane disruptors and therefor toxic to microorganisms that lacks cell walls such as mycoplasma. Chromato-probe analysis of head extracts of *Cataglyphis niger* indeed revealed an abundance of antesio fatty acids accompanied by a homologous series of their heptyl esters. These are not found in the hydrocarbon storage postpharyngeal glands but are seemingly produced elsewhere in the head. It was a simple evolutionary leap to derive from them the homologous hydrocarbons for communicative purposes. Moreover, the shift in branch position towards the middle of the molecule that occurred later in evolution imposed a handicap on the impermeability capacity of the hydrocarbon reinforcing their role as communicative signals. While the role of the cuticular hydrocarbons in nestmate recognition is well established, their role as queen pheromones is still debatable.

The empress's new clothes: Similar shifts in queen cuticular profiles occur across different bumblebee species

Margarita Orlova[1], **Etya Amsalem**[2]
[1]State University of New York Polytechnic Institute, Utica, NY, USA
[2]Pennsylvania State University, University Park, PA, USA

Chemical signals are instrumental in queen-worker communication in eusocial species. However, in many species their nature is not well understood. This is especially true for species with an annual life cycle where queen physiology and colony environment differ vastly at different time points. Bumblebees are an example of such a system. In our current study we analyzed the cuticular hydrocarbon profiles of spring queens and late summer gynes of several wild bumblebee species to assess how they change throughout the queen's life cycle and whether these changes follow the same trajectory in different species. Our findings suggest that life stage determines the cuticular lipid composition more significantly than species, and similar shifts associated with the life cycle occur between different species. One such shift is the decrease in proportions of acetate esters in spring queens compared to summer gynes. The chain length profile also changes with life stage – in an unexpected way. These findings suggest that conserved mechanisms regulate the composition of CHC profiles and provide the links between queen physiology and chemical signals.

Pathogen detection in ant colonies

Michaela Hoenigsberger, Jinook Oh, Anna V. Grasse, Sylvia Cremer
Cremer Group, ISTA (Institute of Science and Technology Austria), Klosterneuburg, Austria

Members of social groups often face an elevated risk of infectious disease transmission. To combat this threat, many social species, particularly social insects such as ants, honeybees, and termites, have developed intricate collective cooperative defenses against diseases. An effective hygiene measure employed by social species involves the removal of infectious particles from the body surfaces of contaminated individuals through grooming. This grooming response can be triggered by a single chemical compound found in the membranes of fungal spores known as ergosterol. Due to its low volatility, ergosterol likely serves as a short-range cue for ants, allowing detection only in close proximity. To investigate whether ants can also detect pathogen presence through long-range-acting volatile compounds emitted by exposed colony members, we conduct two analyses. Firstly, we examine the volatile fraction of fungal spores using gas chromatography-mass spectrometry (GC-MS). Secondly, we observe the behavioral reactions of ants to the presence of fungus-contaminated brood within the nest, which are spatially separated, thus allowing only volatiles to pass. Using self-developed software, we quantify the collective activity patterns of ants in response to potential airborne pathogen cues that may serve as an early warning system to the ants. This investigation aims to shed light on how the interplay between short- and long-range pathogen detection mechanisms enables a fast and efficient anti-pathogen defense at the colony level.

Why do bumblebees prefer flowers of male-fertile cultivars?: Examining the pollen macronutrient factor protein/lipid ratio as a determinant of bee-visitation rate in male-fertile/sterile systems

Shinnosuke Mori[1], **Masahiro Mitsuhata**[2], **Tomoyuki Yokoi**[3]
[1]Department of Biology, Keio University, Yokohama, Japan
[2]Arysta LifeScience Corporation, Tokyo, Japan
[3]Graduate School of Science and Technology, University of Tsukuba, Tsukuba, Japan

In orchards of self-incompatible plant species such as peach, pear, and apricot, they primarily depend on bee pollinators to achieve cross-pollination with a different cultivar. In such mixed orchards, balanced visitation of bees to flowers of all cultivars is favorable; however, biased bee-visitation towards male-fertile cultivars over -sterile ones is often observed. A previous study demonstrated that pollen protein/lipid (P/L) ratio influences host-plant choice behavior of bumblebees; as P/L increased, bees exponentially increased their visitation rates, implying that foragers are trying to balance their nutrition. Here, we hypothesized that the macronutrient factor is involved in the bee preference for male-fertile cultivars. We examined the preference of flower-naïve bumblebees (*Bombus ignitus*) to six leading male-fertile/sterile cultivars of the Japanese pear (*Pyrus pyrifolia*) by two caged-behavioral assays using (1) whole flowers and then (2) pollen-bearing anthers without all other organs. In both assays, bee-visitation rate was significantly higher in male-fertile cultivars than male-sterile ones, indicating that pollen is involved in the preference. Pollen nutrition analyses showed that the male-fertile cultivars had richer proteins than the male-sterile ones, while there were little inter-cultivar differences in lipid content. The P/L was significantly higher in the male-fertile cultivars than in the sterile ones and correlated with the bee-visitation rate in the Poisson regression model. The P/L influences the foraging behavior of bumblebees likely by serving as a chemotactile and/or gustatory cue, and the biased bee-visitation would be due to the inter-cultivar differences in the P/L.

The chemical evolution of Dufour’s gland reproductive signals across bees

Nathan Derstine, **Sujay Paranjape, Cameron Murray, Etya Amsalem**
Pennsylvania State University, USA

Bees provide an excellent system to explore how chemical signals evolve because of the extensive use of pheromones in social bees and the versatility of social organizations in Anthophila. Signals regulating reproductive division of labor in social bees often communicate the reproductive status of the emitter and are hypothesized to evolve as byproducts from cues associated with reproductive physiological processes in solitary bees. However, studies examining the evolution of reproductive signals across lineages are scarce. Here, we examined the evolution of Dufour’s gland reproductive signals by analyzing the gland chemistry and ovary activation of reproductive and non-reproductive females in 19 bee species comprising 5 families across a gradient of social organizations consisting of solitary, subsocial, facultatively eusocial, and eusocial species. The gland’s compounds were identified, quantified, and analyzed using multiple indexes to assess complexity, diversity, richness, molecular weight, and volatility across lineages and between conspecific females. We found that complexity, specificity and volatility of the Dufour’s gland compounds increased along the gradient of sociality, but varied significantly in solitary species. We also found significant differences in the quantity and quality of compounds between reproductive and non-reproductive females. This study demonstrates how pheromones mediating social behavior may have evolved in bees and emphasizes the changes in pheromone chemical properties in the transition from a cue to a signal.

How the Western honey bee *Apis mellifera* supply their larvae with essentilal sterols

Abdullahi Yusuf[1], **Anja Buttstedt**[1,2]
[1]Social Insects Research Group, Department of Zoology and Entomology, University of Pretoria, South Africa
[2]Institute of Evolution and Ecology, Comparative Zoology University of Tubingen, Germany

Western honey bee (*Apis mellifera*) workers feed their larvae with food jelly that is secreted by specialized glands in the worker bees' heads – the hypopharyngeal and the mandibular glands. Food jelly contains all the nutrients allowing the larvae to develop into adult honey bees, including essential dietary sterols. The main sterol in food jelly, 24-methylenecholesterol (24MC), is delivered to larvae in a complex with two proteins, major royal jelly protein 1 (MRJP1) and apisim which upon addition of the fatty acid 10-Hydroxydecenoic acid further assembles into fibril like structures thereby shielding the hydrophobic sterol from the environment. Whereas the proteins are secreted from the hypopharyngeal glands, the glandular source of 24MC is so far unknown. We identified by direct detection of the four main honey bee sterols (24MC, campesterol, β-sitosterol and isofucosterol) using gas chromatography, that the mandibular glands are the source of sterols in food jelly. In addition, 24MC seems to be specifically enriched in the mandibular glands, thereby likely ensuring that food jelly contains the necessary amounts of 24MC to enable complex formation of MRJP1, apisimin and 24MC.

Impact of ozone pollution on the learning and memory of bumblebees

Benjamin Prat[1], Maxence Gérard[2], Emily Baird[3], Maryse Vanderplanck[4]
[1]*Master DARWIN, Univ Montpellier, Montpellier, France*
[2]*Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium*
[3]*INSECT Lab, Division of Functional Morphology, Department of Zoology, Stockholm University, Stockholm, Sweden*
[4]*CEFE, CNRS, Univ Montpellier, EPHE, IRD, Montpellier, France*

For the last decades, major drivers of pollinator decline have been identified such as habitat loss and degradation, spread of pathogens, soil and air pollution, and climate change. Especially, it has been recently shown that elevated temperatures alter bee cognition. Besides, little is known about how reduced air quality impacts pollinator cognition. However, this is a major issue that needs to be addressed as global warming is increasing the tropospheric ozone concentration that can alter the cognitive ability of pollinators. These cognitive skills are essential for pollinators to feed and to remember the location of the most suitable floral resources. Ozone pollution has recently been shown to have a significant impact on the olfaction and behavior of pollinators (i.e., fig wasps and bumblebees). Here we aim to investigate whether cognitive impairments related to ozone pollution could be a factor in pollinator decline by assessing under controlled conditions the effect of realistic ozone episodes on learning and memory in bumblebees. To do this, we will conduct learning and memory tests by associating visual signals with rewarding or repellent molecules. These tests will be carried out on different groups of bumblebees exposed to several realistic concentrations of O_3 during various exposure times. The results will allow us to determine whether ozone pollution can alter the cognitive performance of pollinators. If this assumption is verified, these cognitive impairments could have a role in the future structuring of plant-pollinator interactions.

Keywords: behaviour, bumblebees, cognition, memory, ozone pollution

S11: Plant-plant communication in both above- and below-ground environments

Chairs: *Kaori Shiojiri, Junji Takabayashi*

ORAL PRESENTATIONS

Plant volatile-mediated communication and the integration of environmental information

André Kessler[1], Alexander Chautá[1,2], Michael B. Mueller[1,2]
[1]Dept. Ecology & Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA
[2]Dept. Entomology, Cornell University, Ithaca, NY 14853, USA

Plant defense chemistry affects herbivore performance and can thus influence insect population dynamics both via affecting their fecundity as well as their movement through a plant population. In particular, plant induced responses to herbivory and volatile-mediated plant communication represent temporal changes in plant secondary metabolism that affect food quality as well as the chemical information space that facilitates herbivore host choices. The functionality of induced responses that can affect interactions with other organisms necessitates the integration of environmental cues that allow plants to optimize responses, for example, to herbivory for a maximal investment in reproduction. Here we present new data on how tall goldenrod plants, *Solidago altissima*, integrate chemical and spectral cues to optimize plant metabolism to deal with interacting neighboring plants and herbivores. The findings suggest that plants utilize environmental cues to optimize responses to integrate past experience and predict future circumstances.

Do initial herbivory and exposure to herbivory-induced volatiles affect arthropod community and species richness?

Kinuyo Yoneya
Faculty of Agriculture, Kindai University, Nara, Japan

Plant ecological traits influence the species identity of plant-colonizing arthropods, which in turn induces species-specific trait changes in plants and establishing feedback between the two. This feedback mechanism has the potential to amplify initial differences in species composition, leading to large variations. Additionally, herbivory-induced plant volatiles induce the changes of traits in neighboring plants. We hypothesized that differences in initial plant conditions have sustained effects on arthropod community composition and species richness. To investigate this, we monitored arthropod community assembly on *Salix eriocarpa*, a willow tree subjected to three initial treatments: undamaged (in chamber 1); damaged by the specialist leaf beetle, *Plagiodera versicolora* (chamber 2); and "exposed" plants that were undamaged but were exposed to volatiles from damaged plants. The community composition on damaged and exposed plants became significantly different on day 32. In addition, the divergence in composition between plant individuals was significantly smaller in undamaged plants (from chamber 1) than in damaged and exposed plants (from chamber 2) on day 60. The compositional variations (⌧ diversity) between chambers, between treatments, and between days, comprised a large proportion of the total species richness (⌧ diversity) in the whole community of arthropods. These results suggest that plant initial condition is a key driver of community assembly and the maintenance of species diversity. To elucidate the impact of initial exposure to plant volatiles on community composition and species diversity, another experiment is currently being conducted to exclude chamber effects. The forthcoming results will also be presented in this study.

Microbe-induced plant volatiles

Jin-Soo Son[1], Geun Cheol Song[1], Hyun Gi Kong[2], Je-Seung Jeon[3], Choong-Min Ryu[1,4]
[1]Molecular Phytobacteriology Laboratory, KRIBB, Daejeon, 34141, Republic of Korea
[2]Department of Plant Medicine, Chungbuk National University, Cheongju, 28644, Republic of Korea
[3]Industrial Crop Utilization Division, National Institute of Horticultural and Herbal Science, Rural Development Administration (RDA), Eumseong, 27709, Republic of Korea
[4]Biosystems and Bioengineering Program, University of Science and Technology, Daejeon, 34242, Republic of Korea

Plants emit a plethora of volatile organic compounds (VOCs) in response to beneficial microbes and pathogens. These compounds act as infochemicals for ecological communication in the phytobiome. Plant VOCs are affected by the taxonomic position of the microbe, the identity of the plant and the type of interaction. Susceptible plants can respond to MIPVs from resistant plants and become resistant. Taken together, we proposed a new concept microbe-induced plant volatiles (MIPVs). Here, we provide two examples of MIPVs. First, we investigated the effect of MIPVs on the rhizosphere microbiome of tomato plants inoculated with a plant growth-promoting rhizobacterium *Bacillus amyloliquefaciens* GB03 and that of their neighbouring plants. Leaves of the tomato plant treated with strain GB03 on root released β-caryophyllene as a signature MIPV, which elicited the release of a large amount of salicylic acid (SA) in the root exudates of a neighbouring tomato seedling. The exposure of tomato leaves to β-caryophyllene resulted in the secretion of SA from the root. Secondly the infection of lima bean with the aboveground bacterial pathogen *Pseudomonas syringae* pv. tomato led to the production of MIPVs. Surprisingly, the bacterial type III secretion system, which injects effector proteins directly into the plant cytosol to subvert host functions, was found to prime both intra- and inter-specific defense responses in neighbouring wild tobacco plants through a C8 volatile 1-octen-3-ol emission. This knowledge will help broaden our understanding of plant-plant communications through MIPVs and should facilitate the development of new emerging techniques for sustainable plant disease management.

Interplant flowering communication

Ariel Novoplansky
Mitrani Department of Desert Ecology, Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, Israel

Plants display various forms of phenotypic plasticity in anticipation of changing conditions, many of which are influenced by information obtained from neighboring plants. We studied the hypotheses that neighboring plants emit and eavesdrop on flowering cues that affect both flowering timing and flower type. We show that soil leachates from various plant taxa growing under flowering-inducing conditions accelerate flowering in conspecific target plants growing under non-inductive conditions. In addition, we have demonstrated that the cleistogamic *Lamium Amplexicaule* plants can adaptively modify the production of chasmogamous (CH) and cleistogamous (CL) flowers based on the perception of conspecific neighbors via inter-root cueing. The findings demonstrate novel modes of interplant communication enabling plants to adaptively adjust both flowering time and the production of CH and CL flowers in response to the presence of potential reproductive partners. Further research is needed to explore the identity of the involved root exudates, and the broader ecological implications of these modes of reproductive interplant cueing.

Blumeria hordei affects volatile emission of susceptible and resistant barley plants and modifies the defense response of recipient plants

Silvana Laupheimer[1], Andrea Ghirardo[2], Baris Weber[2], Jörg-Peter Schnitzler[2], Ralph Hückelhoven[1]
[1]Chair of Phytopathology, TUM School of Life Sciences, Technical University of Munich, Emil-Ramann-Str. 2, 85354 Freising, Germany
[2]Research Unit Environmental Simulation (EUS), Institute of Biochemical Plant Pathology, Helmholtz Center Munich, Ingolstädter Str. 1, 85764 Neuherberg, Germany

The powdery mildew disease caused by the biotrophic fungus *Blumeria hordei* (*Bh*) poses enormous risks in cereal crop production due to yield and quality losses. Plants and fungi can produce and release volatile organic compounds (VOCs) that serve as signals in plant communication and defense signaling. The present study aims to identify volatile profiles of barley (*Hordeum vulgare*) during infection with *Bh* and VOC-induced disease resistance in receiver plants. VOC profiles of susceptible *MLO* wild type (*MLO* WT) and a resistant near-isogenic backcross line (*mlo5*) were characterized over time (1 day or 3 days after *Bh* inoculation) using TD-GC/MS. Comparative analysis revealed genotype-dependent VOC profiles and significant differences in emission rates for β-caryophyllene, linalool, (*Z*)-3-hexenol, and methyl salicylate, respectively. Furthermore, susceptible barley plants were exposed to the complex VOC bouquet of *MLO* WT or *mlo5* sender plants in plant-to-plant communication. Evaluation of fungal infection success in receiver plants supported sender genotype-dependent VOC-induced resistance. Gene expression analysis showed upregulation of *BARLEY CHEMICALLY INDUCED-4* but not *JASMONATE INDUCED PROTEIN 23* marker genes in response to VOCs of infected *MLO* WT. Exogenous application of methyl salicylate resulted in a similar gene expression pattern and induced resistance in the receiver plant. The findings suggest genotype-dependent alterations in barley VOC profiles during a biotrophic plant-fungus interaction and show a VOC-mediated resistance that shares components with salicylic acid-related pathways. VOC signals might serve as noninvasive markers to detect disease progression in barley-powdery mildew interactions and as signals of resistance or susceptibility induction in recipient plants.

Searching for the plants' *E*-2-Hexenal receptor

Leonora Kurdow, Silke Allmann, Kresimir Sola, Juliette Silven
Department of Plant Physiology, Swammerdam Institute for Life Sciences, University of Amsterdam, the Netherlands

Plants can use a wide range of volatile compounds to interact with other organisms in their vicinity. Upon herbivory or wounding, green leaf volatiles, small, lipid-derived C-6 compounds, are rapidly emitted. Among these compounds, *E*-2-Hexenal (E2AL) has emerged as an important volatile cue exhibiting signalling functions for both insects and plants. While the biosynthesis pathway and the physiological effects of E2AL in plants are well documented, the mechanism by which plants perceive E2AL remains elusive. We therefore aim to close this gap by identifying the receptor responsible for E2AL perception.

One of the earliest responses to E2AL exposure is an influx of cytosolic calcium, occurring within seconds. Using an *Arabidopsis thaliana* EMS mutant population carrying a reporter for cytosolic Ca^{2+}, we identified two allelic mutant plants (ncre; No Calcium Response to E2AL) which failed to exhibit this characteristic calcium burst while reacting normally to other Ca^{2+} inducing stimuli, such as wounding. Subsequent analysis revealed a locus containing 164 genes potentially associated with the ability to perceive and/or respond to E2AL. Additionally, we developed a high-throughput method with ImageJ for quantifying the intensity of calcium bursts, thereby facilitating the identification of ncre phenotype plants in new mapping populations. This integrated approach provides a systematic framework for further elucidating the genetic basis of E2AL perception in plants.

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Plant–plant communications both above- and belowground in osmotic stress

Kai Ito[1], Haruna Ohsaki[2], Akira Yamawo[3]
[1]Department of Biology, Faculty of Agriculture and Life Science, Hirosaki University, 1 Bunkyo-cho, Hirosaki 036-8560, Japan
[2]Mitrani Department of Desert Ecology, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer Campus 84990, Israel
[3]Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan

Plants transmit information about stress not only aboveground but also belowground. Genetic relatedness of conspecific neighbours may affect information transmission among individuals through kin-selection. Here, we tested the effects of a combination of aboveground versus belowground communication and genetic relatedness (siblings, same haplotype [different population] or different haplotype) of osmotic stress signals in *Plantago asiatica*. We placed three *P. asiatica* plants within a glass box. The central plant was placed under osmotic stress. The side plants shared either soil or air with it. At 60 min after the imposition of osmotic stress on the central plant, soil-sharing plants had a larger proportion of closed stomata when the central plant was a sibling or of the same haplotype than when it was of a different haplotype. In contrast, all air-sharing plants increased the proportion of closed stomata when the central plants were exposed to osmotic stress, regardless of relatedness. These results suggest that aboveground information transmission is a form of the eavesdropping phenomenon, whereas belowground signalling is a form of discreet active signalling.

Plant genetic diversity – Exploring the role of intraspecific trait variation on growth, chemistry, and plant-insect-microbe interactions in white clover (*Trifolium repens* L.) populations

Jennifer Gabriel[1,2], **Nicole M. van Dam**[2,3], **Harald Auge**[4], **Henriette Uthe**[1,2]
[1]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Jena, Germany
[2]Institute of Biodiversity, Friedrich Schiller University, Jena, Germany
[3]Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany
[4]Helmholtz Centre for Environmental Research (UFZ), Halle, Germany
[5]Leibniz Institute of Plant Biochemistry (IPB), Halle, Germany

The global decline of genetic diversity threatens ecosystem functioning. Previous studies have highlighted the role of plant genetic diversity in shaping ecological interactions, especially through variations in functional traits. However, gaps persist in understanding the extent to which intraspecific genetic diversity affects growth, the plant metabolome, and direct or plant-mediated interactions.

Our study aimed to investigate how intraspecific variation in two chemical defense traits affects plant-insect-microbe interactions in white clover. We examined morphological and chemical plant traits, shoot herbivory, and symbiotic root associations with arbuscular mycorrhiza fungi and *Rhizobium*. Four white clover genotypes were selected based on their production of hydrogen cyanide (HCN) and leaf flavonoid content. Genotypes producing (cyanogenic) or not-producing HCN (acyanogenic) and with high or low flavonoid levels served to establish populations in the field. These were either genetically diverse or diverse on the trait level resulting in genotypic monocultures, trait monocultures, and trait mixtures.

Plot-level analyses revealed decreased shoot biomass production and arthropod richness in mixtures, alongside reduced leaf herbivory compared to monocultures. At the genotype level, cyanogenic mixtures exhibited decreased root and shoot growth as well as leaf herbivory. Colonization with AMF and *Rhizobium* was unaffected by the diversity level. The root metabolome varied between mixtures and monocultures, driven by the compound superclasses of benzoids, organoheterocyclic, and organonitrogen compounds.

In conclusion, white clover mixtures showed reduced plant growth and herbivory, likely linked to shifts in the root metabolome. Further experiments including other plant species may elucidate the role of plant metabolites in genetically diverse populations.

Plant-to-plant signaling between relatives or strangers

Triinu Remmel, Bin Liu
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia

Plants change their odour bouquet in times of stress, such as herbivory. This odour change is public information, available to all organisms in the vicinity that have relevant receptors. This includes the neighbouring plants, which often react to such stress signals. It has long been debated whether this plant-to-plant signalling might be a two-way communication or rather an ‘eavesdropping’ by the neighbouring plant. It is generally assumed that the latter is the case, as the neighbouring plant is a competitor and it makes more sense to hide useful information from it. However, this does not exclude deliberate signalling to close relatives. To reveal possible selective signalling to a neighbour and/or concealment of information, we grew two species of willow plants (*Salix cinerea* and *S. triandra*) in pots in pairs, either paired with a close relative (a clone) or with a competitor from the other species. We measured odour bouquets before and after simulated herbivory. *S. cinerea* emitted significantly lower amounts of compounds known as stress signals (monoterpenes and benzenoids) when growing with *S. triandra* compared to when it shared a pot with a close relative or grew alone. This may be the first finding of plants hiding stress-related information from a competitor but not from a clone. Other possible interpretations will be discussed.

Stemborer-induced rice plant volatiles boost direct and indirect resistance in neighboring plants

Yunhe Li[1,2], **Chengcheng Yao**[1,2], **Ted C. J. Turlings**[1,3]
[1]State Key Laboratory of Crop Stress Adaptation and Improvement, State Key Laboratory of Cotton Bio-breeding and Integrated Utilization, Henan University, Kaifeng, 475004, China
[2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[3]Laboratory of Fundamental and Applied Research in Chemical Ecology, University of Neuchâtel, Neuchâtel 2000, Switzerland

Herbivore-induced plant volatiles (HIPVs) are known to be perceived by neighboring plants, resulting in induction or priming of chemical defenses. There is little information on the defense responses that are triggered by these plant-plant interactions and the phenomenon has rarely been studied in rice. Using chemical and molecular analyses in combination with insect behavioral and performance experiments we studied how volatiles emitted by rice plants infested by the striped stem borer (SSB) *Chilo suppressalis* affect defenses against this pest in conspecific plants. Compared to rice plants exposed to the volatiles from uninfested plants, plants exposed to SSB-induced volatiles showed enhanced direct and indirect resistance to SSB. When subjected to caterpillar damage, the HIPV-exposed plants showed increased expression of jasmonic acid (JA) signaling genes, resulting in JA accumulation and higher levels of defensive proteinase inhibitors. Moreover, plants exposed to SSB-induced volatiles emitted larger amounts of inducible volatiles and were more attractive to the parasitoid *Cotesia chilonis*. By unraveling the factors involved in HIPV-mediated defense priming in rice we reveal a key defensive role for proteinase inhibitors. These findings pave the way for novel rice management strategies to enhance the plant's resistance to one of its most devastating pests.

The exposure of maize seedlings to weed volatiles affects their resistance, growth and seed quality

Yusuke Sakurai[1], Satomi Ishizaki[1], Shu Murayama[1], Nami Minato[2], Yasuko Hayashi[1], Shota Izumi[3], Takuma Yoshida[3], Kaori Shiojiri[3], Junji Takabayashi[4]
[1]*Graduate School of Science and Technology, Niigata University, Niigata, Japan*
[2]*Faculty of Agriculture, Niigata University, Niigata, Japan*
[3]*Department of Agriculture, Ryukoku University, Otsu, Japan*
[4]*Center for Ecological Research, Kyoto University, Otsu, Japan*

Plants exposed to volatiles emitted from damaged conspecific or heterospecific plants exhibit increased resistance to herbivorous insects. We examined whether volatiles from artificially damaged weeds can be used in maize cultivation by affecting their resistance, growth and reproduction. Seven days after germination, maize seedlings were exposed to volatiles emitted by artificially damaged mugwort or tall goldenrod plants either separately, or as a mixture of the two, for seven days. Treated and control (unexposed) seedlings were cultivated in an experimental field. Plants exposed to either of the three volatile treatments sustained significantly less damage than controls. Seedlings exposed to either goldenrod or mixed volatiles produced more leaves and tillers than control plants. Furthermore, a significant increase in the number of ears was observed in plants exposed to the volatile mixture. In all treated plants, ear sugar content was significantly higher than that in the controls. In the laboratory experiments, a significant reduction in the growth of common armyworm larvae was observed when maize plants were exposed to the volatile mixture. In addition, cucumber mosaic virus (CMV) infection was also suppressed. Exposure to the volatile mixture increased salicylic acid content in the seedlings, although the amount of jasmonic acid was not affected. Our results suggest that seedling exposure to the weed volatiles can increase the seed quality and resistance against herbivores and pathogens of maize plants.

Exploring the impact of long-term push-pull cropping on belowground microbiome diversity, phytochemistry, and *Spodoptera frugiperda* resistance: Paving the way for resilient farming systems

Abdul A. Jalloh[1,2], **Fathiya Khamis**[1], **Abdullahi Ahmed Yusuf**[2,3], **Sevgan Subramanian**[1], **Daniel Munyao Mutyambai**[1,4]
[1]*International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100 Nairobi, Kenya*
[2]*Department of Zoology and Entomology, University of Pretoria, Private Bag x20 Hatfield, Pretoria, South Africa*
[3]*Forestry and Agricultural Biotechnology Institute, University of Pretoria, Private Bag x20 Hatfield, Pretoria, South Africa*
[4]*Department of Life Sciences, South Eastern Kenya University, P.O. Box 170-90200 Kitui, Kenya*

Background: The soil biota, comprising diverse microbial communities and organisms, varies across farming systems, affecting soil health, phytochemistry, and productivity. Despite its significance, there's limited exploration of how cropping systems influence belowground microbiomes and plant-insect interaction. We studied soil physicochemical properties and belowground microorganisms, along with phytochemistry, in push-pull technology (PPT) compared to maize-monoculture (Mono) cropping.

Results: PPT cropping system changed the composition and diversity of belowground microbial communities, led to notable improvements in soil physicochemical characteristics compared to Mono. Distinct bacterial and fungal genera played a crucial role in influencing the variation in microbial diversity within these cropping system. The abundance of these genera were more enriched in PPT compered to Mono. These microbial communities are associated with essential ecosystem services. Conversely, pathogenic associated bacterial and fungal genus were more enriched in Mono. Maize-plants cultivated in PPT-soil-conditioned exhibited higher biomass and growth rates. These plants produced a greater diversity of volatiles, and higher levels of non-volatile-metabolites, compared to Mono. The observed soil-mediated effects showed that *Spodoptera frugiperda* neonates fed less on maize-leaves from PPT-soil-conditioned plants, compared to Mono-soil-conditioned plants.

Conclusion: PPT positively influences soil and maize-root microbiomes and enhances soil physicochemical properties, maize growth, reduces *Spodoptera frugiperda* larval feeding, alters phytochemistry, and subsequently confers direct and indirect resistance to *Spodoptera frugiperda*. This highlights its potential for agricultural and environmental sustainability. These findings contribute to our understanding of the diverse ecosystem services offered by this cropping system where it is practiced regarding the system's resilience and functional redundancy.

POSTERS

P-70

Airborne and soilborne cues of herbivory reduce damage and accelerate flowering

Kaori Shiojiri[1], Richard Karban[2]
[1]*Ryukoku University Faculty of Agriculture, Japan*
[2]*Department of Entomology and Nematology University of California, Davis, USA*

Many studies have found that volatile cues from damaged plants can induce resistance to herbivores in undamaged neighbors. Fewer studies have examined effects of soilborne cues. Few studies have considered effects of induced resistance resulting from plant-plant communication on plant reproduction. We conducted experiments with tomato seedlings exposing them to airborne and soilborne cues from experimentally damaged neighbors. These plants were then transplanted to the field and the level of damage by chewing and sucking herbivores was observed in the field. We also recorded the time before flowering was initiated by these plants. We found that both airborne and soilborne cues reduced the proportion of leaves that were damaged for plants exposed to cues relative to controls that were near undamaged neighbors. These two cues were not found to interact synergistically. Plants that had been exposed to soilborne cues flowered sooner than controls, consistent with theoretical expectations. Plants exposed to airborne cues did not differ from controls with respect to flowering time.

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Silent treatment: The role of *S. indica* in *Arabidopsis* communication and defence against infection

Liza Zhyr[1], Michael Reichelt[2], Axel Mithöfer[1]
[1]*Research Group Plant Defense Physiology, Max Planck Institute for Chemical Ecology, Germany*
[2]*Department for Biochemistry, Max Planck Institute for Chemical Ecology, Germany*

For all living organisms, survival requires exchanges of energy, matter, and information. The exchange of the latter might be considered as communication. Nowadays, the existence of plant-plant communication is widely accepted, since it was repeatedly confirmed that plants are able to recognize signals from aggressors or neighbours and respond to them. One of the most widespread mechanisms of aboveground plant-plant communication is the release of volatile compounds. Belowground communication, additionally, might be mediated by employing mutualistic fungi (e.g., mycorrhizae). Such symbiosis can provide nutrients and facilitate the transport of active molecules. Moreover, during colonization, fungi can prime plants making them more resilient. However, there is not much knowledge about physiological changes in plant-fungus-plant interaction upon infection.

In our study, we aimed to find out whether primary and secondary metabolites are affected by the presence of fungal hyphae connection between plants in the first days of infection. Also, we are interested in the unique metabolites for plant-fungus interaction. In order to reach these goals, we measured primary and secondary metabolites in *Arabidopsis thaliana* plants colonized by *Serendipita indica* during the first days of *Alternaria brassicae* infection and analysed metabolic profiles of the infected donor and the non-infected receiver plants. While it became clear that colonisation by *S. indica* affects both the primary and secondary metabolism of plants with the presence of compounds unique for fungal colonisation in roots and leaves, we found no significant differences in the measured parameters that could indicate that the fungus plays a role in plant-plant communication.

S12: Chemical ecology of insect vector-plant interactions

***Chair:** Jonathan Bohbot*

ORAL PRESENTATIONS

Molecular detection of plant volatiles in vector mosquitoes

R. Jason Pitts, Robert M. Huff, Heidi Pullmann-Lindsley, John Boyi
Department of Biology, Baylor University, Waco, USA

Vector insects respond to plant volatiles for nectar acquisition and sheltering, among other possible reasons. While some studies have identified compounds emitted by preferred host plants, little is known about the molecular events of chemical reception that mediate behavior, especially in mosquitoes. In the present study, we have characterized the response profiles of Odorant Receptors (ORs) to a suite of compounds including terpenes, green leaf volatiles, indoles, and other plant-emitted odors. Moreover, we have identified functional homologs across diverse species, suggesting evolutionary conservation for common ecological purposes. Our findings establish a baseline for further investigations into the chemical ecologies of vector insects and the evolution of insect chemoreceptors. Conceptually, potent activators of ORs could be exploited for improved vector surveillance or bait-and-kill technologies.

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Evolution of monoterpenoid-mediated repellency in the palp of culicine mosquitoes

Robert M. Huff[1], Yuri Vainer[2], Yinliang Wang[2,7], Esther Yakir[2], Dor Perets[2], Evyatar Sar-Shalom[2], Majid Ghaninia[5], Iliano V. Coutinho-Abreu Gomes[3], Carlos Ruiz[6], Alon Warburg[4], Omar S. Akbari[3], Philippos A. Papathanos[2], Rickard Ignell[5], Jeffrey A. Riffell[6], Ronald J. Pitts[1], Jonathan D. Bohbot[2]
[1]*Department of Biology, Baylor University, USA*
[2]*Department of Entomology, The Hebrew University of Jerusalem, Israel*
[3]*Division of Biological Sciences, University of California, USA*
[4]*Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel*
[5]*Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Sweden*
[6]*Department of Biology, University of Washington, USA*
[7]*Northeast Normal University, China*

The ancient practice of using aromatic plants rich in monoterpenoids to repel mosquitoes originated in Southeast Asia and gradually spread across the world. However, the molecular mechanisms of action of these fragrant compounds remain poorly understood. The odorant receptor neurons located in the capitate peg sensillum of the maxillary palp are activated by CO_2-sensitive gustatory receptors and odorant receptor OR8, which are crucial for sensing human-hosts. The third and enigmatic component of this sensory system is the orphan odorant receptor OR49. Through a pharmacological screen, we show that OR49 is activated by plant-released bicyclic monoterpenoids. Using electrophysiological and functional brain imaging techniques, we identified the neural pathway responsible for monoterpenoid-induced repellency. Understanding the functional role of the maxillary palp is essential as a preliminary step to investigating host signal integration and its influence on host preference.

OR49-borneol: The key to study olfactory integration

Yuri Vainer, Esther Yakir, Jonathan Bohbot
Department of Entomology, The Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

The mosquito olfactory system consists of the antennae and maxillary palps. The capitate peg sensillum found on the maxillary palp is considered an animal-host detector due to its sensitivity to CO_2 and octenol. This sensillum houses three sensory neurons, each expressing one type of olfactory receptor thereby providing a simple model to study pre- and post-synaptic olfactory integration predicated that the functional identity of all three neurons is known. In this study, we used terpene mixtures derived from different *Cannabis* chemovars to deorphanize the mosquito odorant receptor OR49, the remaining orphan receptor in the capitate peg sensillum. The bicyclic monoterpenoid (+)-borneol was the most efficacious and potent ligand. Moreover, the functional orthology of OR49s in culicine mosquitoes suggests that borneol fulfils a conserved ecological role in these mosquitoes. These findings lay the foundation to study olfactory integration in the peripheral and central nervous system and they challenge the notion that the capitate peg sensillum is a strict human sensor.

Mapping *Anopheles* mosquito nutritional intake: Metabolomic and molecular perspectives

Balvina Leyva, Felipe Yon, Emily Motta, Gabriela Cáceres
Alexander Von Humboldt Institute of Tropical Medicine, Universidad Peruana Cayetano Heredia, Lima, Peru

Malaria, an infectious disease transmitted by *Anopheles* mosquitoes, need diverse nutrients for their physiological functions, including parity, longevity, survival, and vectorial capacity. The mosquito's crop, part of its digestive system, acts as a reserve for digesting consumed sugars. In Peru, the research about the nectarivores diet is limited and does not exist data about the metabolomic profile of the Anophelines species by ultra high-performance liquid chromatography mass spectrometry, which can separate chemical compounds and identify metabolites with high sensitivity, resolution and accuracy. This study aimed to establish a metabolomic database of *Anopheles* species' diets. Samples included crops from *An. pseudopunctipennis* collected in the field and those exposed to five field-collected plants in bioassays. Methanolic extracts from these plants and internal sugar standards were used for comparison and quality control. UHPLC-MS analysis revealed a correlation between plant compounds and mosquito crop metabolites, identifying sugars, amino acids, pesticides, lipids, and other natural plant compounds. Additionally, molecular network analysis unveiled interconnected metabolic pathways within the mosquito's nutritional ecology, shedding light on the complex interactions among metabolites. These findings offer insights into feeding preferences, metabolic pathways, and potential pesticide resistance mechanisms, providing a basis for future research on malaria control strategies such as designing more efficient traps and understanding metabolic pathways and pesticide resistance.

Attraction and repellency: Olfactory effects on *Aedes aegypti* (Diptera: Culicidae) nectar foraging behavior

Evyatar Sar-Shalom, Jonathan Bohbot
Department of Entomology, The Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Mosquito survival, fertility, and offspring production are critically dependent on carbohydrate sources such as floral nectar, extra-floral nectars, and honeydew. To locate these nectar sources, mosquitoes rely on multiple senses, including olfaction, vision, and taste. Although the interaction between mosquitoes and animal hosts has been extensively studied, the interaction between mosquitoes and plant hosts is not well understood. This study introduces a novel monitored setup to track and explore the olfaction-driven interaction between mosquitoes and plant sugar volatiles. Using a custom object detection model, we quantified attraction behavior parameters and differentiated between male and female mosquitoes. Our system measures behavioral parameters such as the number of visits, duration, and distance traveled within the region of interest. Our results indicate that sucrose-starved mosquitoes of both sexes demonstrate attraction to date palm extract odor, which is diminished when lemongrass oil, a known repellent, is added. This study aims to explore olfaction-driven behaviors when mosquitoes are exposed to plant sugar scents. Expanding knowledge of mosquito-plant host interactions could lead to novel, eco-friendly strategies for mosquito management.

The olfactory basis of *Ae. albopictus* dominance over *Aedes aegypti* at the larval stage

D. Perets, E. Sar-Shalom, D. Zaada, V. Mackevičius, F. Krsticevic, E. Yakir, J.D Bohbot, P.A Papathanos
Department of Entomology, The Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Larvae of the invasive Asian tiger mosquito (*Aedes albopictus*) outcompetes the yellow fever (*Aedes aegypti*) mosquito in aquatic environments. *Aedes* larvae are detritivores that live in spatially-small and often temporary water bodies. The potential role of the larval olfactory system in this interspecies competition has not been explored. We compared olfactory gene expression patterns between the larval antennae of *Ae. aegypti* and *Ae. albopictus*. Despite their 57 million years of evolution, we show that 80% of expressed *Ae. albopictus* larval odorant receptor genes are shared with *Ae. aegypti*. The remaining *Ae. albopictus* odorant receptor genes are species-specific and may reflect their ecological advantage. Among those receptors, indole-sensitive ORs are highly expressed in *Ae. albopictus* larvae and exhibit identical functions as their *Ae. aegypti* counterparts. Behavioral analyses indicate conserved roles of indoles in *Aedes* larvae. The comparison of the olfactory gene repertoire of these two medically significant mosquito species is fundamental step to unveil the genetic basis driving the ecological dominance of *Ae. albopictus*.

Exploring a natural alternative for repelling mosquitoes: Nootkatone

Merybeth Fernandez Triana[1,2], Felippe Andreazza[3], Benjamin Fabian, Nadia Melo[1], Dan-Dan Zhang[1], Rickard Ignell[2,5], Ali Afify[6,7], Christopher J Potter[6], Silke Sachse[2,4], Ke Dong[3], Marcus C. Stensmyr[1,2]
[1]*Department of Biology, Lund University, Lund, Sweden*
[2]*Max Planck Center next Generation Chemical Ecology*
[3]*Department of Biology, Duke University, Durham, USA*
[4]*Research Group Olfactory Coding, Max Planck Institute for Chemical Ecology, Jena, Germany*
[5]*Disease Vector Group, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Lomma, Sweden*
[6]*The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, USA*
[7]*Department of Biology, Drexel University, Philadelphia, USA*

Mosquitoes play a pivotal role in the transmission of pathogens that give rise to significant diseases and result in numerous casualties annually. Presently, they thrive in tropical and sub-tropical urban environments across the globe, with their geographic distribution anticipated to expand due to ongoing global phenomena such as climate change and urbanization. Consequently, mosquitoes demand substantial attention from the scientific community and health authorities, necessitating the development of effective methods for managing insect vectors.

Repellency stands as the predominant strategy for averting mosquito bites and, consequently, curtailing the propagation of arboviruses. Nevertheless, the escalating availability of synthetic repellents has raised concerns regarding their toxicity and environmental sustainability. In this context, botanical-based products have emerged as a promising alternative to synthetic mosquito repellents.

Nootkatone, a compound found in the essential oil of grapefruit peel and Alaskan yellow cedar heartwood, has exhibited potential as an insecticide and repellent against various arthropod species in controlled laboratory settings. However, the precise molecular and cellular mechanisms underlying its action remain undisclosed. Our research efforts centered on the use of genetically modified mosquitoes in behavioral bioassays and a heterologous expression system, with the primary objective of elucidating the molecular targets of nootkatone in *Aedes aegypti*. This endeavor aims to uncover the mode of action of nootkatone, particularly in relation to its repellent properties.

Innovative selective vector feeding-based trap offers a solution for a safer future

S. Noushin Emami
NRI, University of Greenwich, London, UK

Recent data show that parasites manipulate the physiology of mosquitoes and human hosts to increase the probability of transmission. We demonstrated that the male mosquito has also a critical role in vector-control strategies. We have focused on chemical ecology, behaviour, and transcriptional variation in this sex. We also investigated the phagostimulant activity of *Plasmodium*-metabolite, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), in the primary vectors of multiple human-diseases, *Anopheles coluzzii*, *An. arabiensis*, *An. gambiae* s.s., *Aedes aegypti*, and *Culex pipiens*/*Culex torrentium* complex species. We showed that it is possible to mimic a blood meal so close that mosquitoes naturally prefer by using HMBPP supplementation of plant juice. This so-called pink juice is an artificial toxic solution. Furthermore – the effect of feeding on the toxic cocktail on mosquitoes was within 100-350 minutes post-feeding; all tested were found dead. We are just beginning to unravel the complex mosquito to mosquito and parasite to mosquito interactions leading to key opportunities to destabilise anopheline populations. Altogether, we provide a proof-of-concept for a specialised and eco-friendly feeding trap that can be deployed where needed. Our recent findings on an uncharted aspect of vector-borne diseases—the chemical cues governing transmission-related vector behaviour—point to novel strategies for disrupting pathogen-transmission.

Comparative osmoregulation strategies and host plant adaptation in *Bemisia tabaci*

Yonatan Sadeh[1], Rekha Swamy[2], Dor Wintraube[1], Yehudit Amor[3], Shai Morin[1], Osnat Malka[1,4]
[1]The Hebrew University of Jerusalem, Rehovot, Israel
[2]The Natural Resources Institute (NRI), Chatham, UK
[3]Savyon Diagnostics LTD, Israel
[4]Katif Research and Development Center, Israel

Phloem sap is a nutrient-rich food source and the exclusive source of nutrients for most Hemipterans. These insects must overcome the high osmotic potential to exploit phloem sap, primarily due to its high sucrose concentration. The cryptic, polyphagous, phloem-feeding whitefly *Bemisia tabaci* can feed on a wide range of host plants. However, the role of osmoregulation strategies in plant adaptation remains poorly understood. In this study, we examined the honeydew secretions of two *B. tabaci* species: Sub-Saharan Africa (SSA), which infests cassava, and Middle East-Asia Minor1 (MEAM1), which does not. Our goal was to elucidate differences in osmoregulation strategies between these species and their relationship to host plant utilization. After feeding on a common host plant, both species managed osmotic pressure similarly in the adult stage, with no significant differences in honeydew sugar profiles. Both species primarily produced sucrose isomers, such as trehalulose, to cope with osmotic pressure. However, when we tested the nymphs' sugar profiles, we found that SSA nymphs excreted sucrose more efficiently and had a higher ratio of oligosaccharides to isomers than MEAM1 nymphs. Furthermore, we found that plant secondary metabolites significantly impact honeydew sugar profiles. For instance, adding linamarin to artificial diets increased the production of oligosaccharides in adult honeydew. In addition, transcriptomic analysis revealed differential expression of osmoregulation genes in the two species, including glycoside hydrolase13, sugar transporters, and aquaporins. These results suggest that SSA's ability to produce high ratios of oligosaccharides, and efficient sucrose excretion might confer a selective advantage when adapted to cassava.

S13: Plant chemodiversity and its evolutionary driving forces

Chairs: *Shuqing Xu, Gabriela Anjos De Stefano Escaliante, Omer Nevo, Linh M. N. Nguyen*

ORAL PRESENTATIONS

Allopolyploidy-mediated chemodiversity enhancement and innovation of gain-of-function defenses in a plant-insect arms race

David Elser[1,2], Aura Navarro-Quezada[2], Remigius Groening[2], Laurence Herrgott[1,2], Monika Kupke[2], Ludivine Malherbe[1], Emmanuel Gaquerel[1,2]
[1]*Institut de Biologie Moléculaire des Plantes du CNRS, Université de Strasbourg, France*
[2]*Centre for Organismal Studies, University of Heidelberg, Germany*

Defensive specialized metabolite diversification is a core process to plants' adaptation to diverse ecological niches. Specialized herbivorous insects respond actively to this process by evolving biochemical and behavioral counter-adaptations, imposing additional selection pressure on their host plants. The *Nicotiana* genus, with half of its species being allopolyploids, offers a compelling framework to explore the role of allopolyploidization as an evolutionary driving force for defensive specialized metabolite diversification. A recent evolutionary metabolomics analysis from our group has delved into chemodiversity innovations linked to allopolyploidization in *Nicotiana*, highlighting *N*-acyl-nornicotines (NANNs) derived from the nicotine ancestral state of the genus as defenses strongly modulated by allopolyploidy events (Elser et al. Science Advances, 2023: 10.1126/sciadv.ade8984). Nicotine is well known to act a potent defense, although certain herbivores, notably the tobacco hornworm, have developed tolerance, likely through efficient nicotine excretion. Using the evolutionary trajectory of NANN as a case study, I will illustrate during my talk how *Nicotiana* section *Repandae* allopolyploidization has turned, through tissue- and chemotype-level shifts, trichome-based NANN production into a gain-of-function defense against specialized insects. In particular, I will present findings on the discovery of NANN biosynthetic genes, their evolutionary origin in light of allopolyploidy events, and their original mode of action in circumventing nicotine tolerance.

Floral scent diversity is related to the diversification of cyclocephaline beetle – angiosperm pollination interactions

Gibernau Marc[1], Artur Campos Dalia Maia[1,2,6], Yann Quilichini[3], Diane Dabir-Moghaddam[4], Tony Robillard[4], Romain Nattier[4], Pascaline Chifflet-Belle[4], Paschoal Coelho Grossi[5], Daniela do Amaral Ferraz Navarro[6], Ariadna Valentina Lopes[2]
[1]CNRS-University of Corsica Pascal Paoli, UMR 6134 SPE, Equipe Chimie et Biomasse, Route des Sanguinaires, 20000 Ajaccio, France
[2]Departamento de Botânica, Universidade Federal de Pernambuco, Recif, Pernambuco, 50670-901, Brazil
[3]CNRS-University of Corsica Pascal Paoli, UMR 6134 SPE, Equipe Gestion et Valorisation des Eaux en Méditerranée, Campus Grimaldi, 20250 Corte, France
[4]MNHN-Museum National d'Histoire Naturelle, UMR7205 ISYEB, 57 rue Cuvier 75005 Paris, France
[5]Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros, s/n, Dois Irmãos, Recife, Pernambuco, CEP 52171-900, Brazil
[6]Departamento of Fundamental Chemistry, Universidade Federal de Pernambuco, Recife, 50740-560, Brazil

Neotropical cyclocephaline beetles serve as highly specialized pollen vectors for night-blooming species of five angiosperm families (Nymphaeaceae, Araceae, Magnoliaceae, Cyclanthaceae, Annonaceae). Referred to as the 'cyclocephaline beetle pollination syndrome,' this phenomenon represents a convergence of morphological and physiological plant reproductive traits. These traits include intensely fragrant and thermogenic flowers, nocturnal and protogynous anthesis, large pollination chambers, and nutritious floral structures. This intricate pollination system comprises 115 documented species across 9 genera and 151 plant species across 22 genera and 5 families.

The occurrence of cyclocephaline beetle-plant pollination interactions exclusively at night suggests the primacy of the olfactory communication channel. Notably, the floral scent compositions of approximately 110 species from the five angiosperm families are well-documented. These scents are characterized by high absolute emissions, reduced compositional complexity, and unique compounds. To date, 18 volatile organic compounds (VOCs) have been identified as the main constituents attracting anthophilous cyclocephaline beetles. One noteworthy finding is the extensive chemical repertoire of these primary floral VOCs and, consequently, the diverse sensory capacity of cyclocephaline beetles to respond. This rich chemical and sensory diversity is likely linked to the evolutionary success of these specialized plant-insect interactions, resulting in a high diversity of both pollinating cyclocephaline beetles (approximately 500 species) and their host plants (around 900 species).

Flavor over fragrance? Gustation guides pollinators along a chemical roadmap to floral nectar

Robert A. Raguso[1], Kim C. Heuel[2], Emma Coogan[3], River Mallick[3], Kirsten J. Keleher[1], Manfred Ayasse[2], Hannah Burger[2], Robert J. Gegear[3]
[1]*Department of Neurobiology and Behavior, Cornell University, NY 14853 Ithaca, New York, USA*
[2]*Institute of Evolutionary Ecology and Conservation Genomics, Ulm University, 89081 Ulm, Germany*
[3]*Department of Biology, University of Massachusetts Dartmouth, MA 02747 Dartmouth, Massachusetts, USA*

How do plants with structurally complex flowers promote pollination? The spatial partitioning of volatile organic compounds (VOCs) may function as a 'chemical roadmap' to help flower-naïve pollinators learn the location of concealed nectar rewards while benefiting plants through increased constancy and efficiency. Here we construct a VOC roadmap for *Impatiens capensis* and *I. pallida*, two North American plants that have a similar complex floral architecture and are pollinated by the same bumblebee species, *Bombus impatiens*. Although VOCs are well-known to elicit olfactory responses in bee pollinators, the presence of unique compounds in floral nectar coupled with reports of inexperienced bees probing different flower parts as they learn to handle flowers suggest that VOCs might also influence bee behavior on flowers through gustatory channels. Accordingly, we found that bumblebee responses differed when VOCs were presented as odorants than when they were dissolved in sugar solutions presented to bees as rewards. VOCs present in nectar (vanillin) or at the corolla (α-pinene) did not reduce sugar consumption by the bees, whereas VOCs that were emitted by the nectar spur and other floral tissues but were excluded from nectar (benzaldehyde) were repellent when sampled as gustatory cues. Thus, spatial partitioning of floral VOCs in *Impatiens* appears to manipulate bumblebee behavior in ways that encourage pollination and discourage nectar robbing. Our findings highlight the non-olfactory functions of floral VOCs as flavors or contact chemical stimuli, and their potential to structure plant-pollinator networks and filter their interactions with other organisms, including larcenists and nectar-utilizing microbes.

An overlooked syndrome of deceptive pollination: Mimicry of food sources for attracting females of anautogenous flies

Valentin Vrecko[1], Magali Proffit[1], Aroonrat Kidyoo[2], Doyle McKey[1], Rumsais Blatrix[1]
[1]CEFE, University of Montpellier, CNRS, EPHE, IRD, CEDEX 5, 34293 Montpellier, France
[2]Plants of Thailand Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand

Traditionally recognized pollination syndromes are often too broadly defined to represent the diversity of plant-pollinator interactions, particularly in plants that deceptively attract insects by mimicking resources they seek. Within such interactions, we document a distinct but previously ill-defined deceptive pollination strategy in which plants exploit the protein-seeking behavior of females of anautogenous Diptera by olfactorily mimicking their insect prey. We show that this strategy characterizes at least 97 plant species from seven families, pollinated by females of three dipteran families, Ceratopogonidae, Chloropidae and Milichiidae. Comparison of floral odours based on compound identity and structural similarity revealed floral traits that characterize a hitherto undescribed pollination syndrome. Interestingly, related plants of three of these families emit a different set of floral volatiles to attract flies of other families in brood-site mimicry pollination systems. Our findings open new avenues, from both methodological and conceptual perspectives, for understanding the evolution of specialized pollination systems.

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Natural rubber reduces herbivory and alters the microbiome below ground

Laura Böttner[1,2,3], Antonino Malacrinò[2,4], Christian Schulze Gronover[5], Nicole van Deenen[1], Boje Müller[5], Shuqing Xu[2,3], Jonathan Gershenzon[6], Dirk Prüfer[1,5], Meret Huber[1,3]
[1]*Institute of Plant Biology and Biotechnology, University of Münster, Münster, Germany*
[2]*Institute for Evolution and Biodiversity, University of Münster, Münster, Germany*
[3]*Institute of Organismic and Molecular Evolution, Johannes Gutenberg University Mainz, Mainz, Germany*
[4]*Department of Agriculture, Universita degli Studi Mediterranea di Reggio Calabria, Reggio Calabria, Italy*
[5]*Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Münster, Germany*
[6]*Department of Biochemistry, Max-Planck Institute for Chemical Ecology, Jena, Germany*

Laticifers are thought to have evolved to protect plants against both herbivores and pathogens. However, there is little evidence for this dual function. We investigated whether a latex polymer, *cis*-1,4-polyisoprene, which is the major constituent of natural rubber, alters plant resistance and the root microbiome of the Russian dandelion (*Taraxacum koksaghyz*) under attack of a root herbivore, the larva of the May cockchafer (*Melolontha melolontha*). Rubber-depleted transgenic plants lost more shoot and root biomass upon herbivory than normal rubber content near-isogenic lines. *Melolontha melolontha* preferred to feed on artificial diet supplemented with rubber-depleted rather than normal rubber content latex. Likewise, adding purified *cis*-1,4-polyisoprene in ecologically relevant concentrations to diet deterred larval feeding and reduced larval weight gain. Metagenomics and metabarcoding revealed that abolishing biosynthesis of natural rubber alters the structure but not the diversity of the rhizosphere and root microbiota (ecto- and endophytes) and that these changes depended on *M. melolontha* damage. However, the assumption that rubber reduces microbial colonization or pathogen load is contradicted by four lines of evidence. Taken together, our data demonstrate that natural rubber biosynthesis reduces herbivory and alters the plant microbiota, which supports the notion that multiple biotic factors shape the evolution of laticifers and plant chemical diversity.

Increased secondary defense metabolite production by *Pinus* spp. is linked to greater susceptibility to pitch canker disease

Lente van Zyl[1], **Almuth Hammerbacher**[2], **Sanushka Naidoo**[1], **Emma Steenkamp**[1], **Brenda Wingfield**[1]
[1]Department of Biochemistry, Genetics, and Microbiology, University of Pretoria, Pretoria, South Africa
[2]Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

Fusarium circinatum is a destructive necrotrophic fungal pathogen of *Pinus* spp. Colloquially known as pitch canker disease, *F. circinatum* infection causes the exudation of resin (or 'pitch') from cankerous wounds in infected mature trees. In young pines, infection can often be asymptomatic, and high post-planting mortality rates occur when seemingly healthy plants are used for plantation establishment. The presence of this pathogen in pine nurseries is therefore responsible for significant economic losses in the South African pine industry. As a defense mechanism against *F. circinatum*, pine trees produce secondary defense metabolites such as terpenes in specialized structures throughout their stems and needles. Some species of pines are significantly more successful in resisting *F. circinatum* infection than others. Although research on the transcriptomic differences between resistant and susceptible pines provide important insight into this phenomenon, little is known about the role of chemical defenses against this pathogen. We used GCMS and LCMS analysis to compare the production of secondary defense metabolites between resistant and susceptible pine species and hybrid cultivars. We found that pines that are more susceptible to *F. circinatum,* with higher death rates and more severe disease symptoms, also produce higher concentrations of secondary defense metabolites. We discuss how these results shape our understanding of chemical defense strategies against fungal pathogens such as *F. circinatum.* Ultimately, identification of chemical markers for *F. circinatum*-susceptibility can assist in the development of more successful targeted breeding programmes.

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Fruit scent evolution in plant-seed disperser interaction

Linh M. N. Nguyen[1,2,3], **Diary Razafimandimby**[4], **Rebekka Sontowski**[5], **Jana Ebersbach**[6], **Renske Onstein**[1,7], **Philipp Schluter**[8], **Nicole van Dam**[1,2,3,5], **Omer Nevo**[1,2]
[1]German Centre for Integrative Biodiversity Research (iDiv) Halle- Jena- Leipzig, Germany
[2]Institute of Biodiversity, Friedrich- Schiller Universitat Jena, Jena, Germany
[3]Max Planck Institute for Chemical Ecology, Jena, Germany
[4]Faculty of Sciences, Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar
[5]Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany
[6]Department of Molecular Evolution and Plant Systematics and Herbarium (LZ), Institute of Biology, Leipzig University, Leipzig, Germany
[7]Naturalis Biodiversity Center, Leiden, the Netherlands
[8]Department of Plant Evolutionary Biology, Institute of Biology, University of Hohenheim, Stuttgart, Germany

Fruits have evolved to be attractive to seed dispersers, and fruit traits evolved in response to selection pressures by frugivores. Fruit scent has evolved as a signal for frugivores, signalling ripeness and potentially fruit quality. However, it is still unclear how and what parts of fruit scent have evolved as signals for frugivores and what information they contain. Unlike most chemical classes, aliphatic esters are found to be concentrated in species that communicate with seed dispersers via chemical cues and only in ripe fruits. Some evidence and theoretical basis have indicated a positive correlation with sugar content. This means that there might be a link between chemical signals and where chemical coevolution is expected for fruit dispersed in lemurs. Our research aims to identify whether aliphatic esters are indeed an honest signal for fruit quality and test whether it is an adaptation acquired by plants. We test this hypothesis on a model system of up to 20 fig species (*Ficus* spp; Moraceae) growing in Madagascar. With collections of fruit and leaf, we intend to (1) establish an ecological network, (2) reconstruct phylogeny, (3) identify the link between chemical signal and reward by using thermal desorption gas chromatography-mass spectrometry (TD-GCMS) and high-performance liquid chromatography (HPLC) to test whether it is the unique link between species and dispersal ecology, and (4) to sequence alcohol acyltransferase (AAT) to check if the selection regimes corresponding to the dispersal mode.

Do frequency dependent selection processes contribute to the maintenance of steroidal glycoside chemodiversity in *Solanum dulcamara*?

Nicole M. van Dam[1,2,3], **Redouan Adam Anaia**[1,2]
[1]*Molecular Interaction Ecology, German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany*
[2]*Institute of Biodiversity, Friedrich Schiller University, Jena, Germany*
[3]*Leibniz Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany*

Bittersweet nightshade (*Solanum dulcamara*) produces a large variety of steroidal glycosides (SG), including alkaloids (SGAs). Total leaf SGA levels as well as structural differences and diversity in SG(A)s are associated with herbivore preference and microbial resistance in other Solanaceae. Plants with unsaturated SGAs were found to be more resistant to slugs and a pathogen than those expressing *GLYCOALKALOID METABOLISM25* (*GAME25*), coding for a short-chain dehydrogenase/reductase leading to a chemotype with saturated SGAs. Natural populations of *S. dulcamara*, however, contain individuals with both saturated and unsaturated SGA leaf chemotypes. We experimentally tested whether frequency-dependent selection pressures may contribute to the maintenance of this chemical polymorphism. In a common garden experiment, we manipulated the frequencies of two different *S. dulcamara* SGA leaf chemotypes to create plots with different levels of chemodiversity. We found chemotype-specific effects on the level of herbivory by Colorado potato beetle, seed production and germination rates as well as plot chemodiversity effects on fruit production. Interestingly, the total number of fruits produced per plant and per plot was the highest in plots with balanced mixtures of unsaturated and saturated SGA chemotypes. The number of visits by bumble bee pollinators were not significantly different among chemotypes, except for the third most abundant species, *Bombus lapidarius*, which slightly preferred to visit flowers of chemotypes with unsaturated SGAs. This suggest that both frequency-dependent selection processes and chemotype-specific effects on plant-insect and plant-pollinator interactions may contribute to the maintenance of SG chemodiversity in natural *S. dulcamara* populations.

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How does hybridization affect chemical profiles and performance against biotic and abiotic factors in willows?

Sofian A. Renoult[1,2], **Priscila Mezzomo**[1,2], **Jing vir Leong**[1,2], **Martin Moos**[1], **Pavel Šebek**[1], **Václav Pokorný**[1,2], **Shristee Panthee**[3,4], **Nicole M. van Dam**[3,4], **Martin Volf**[1,2]
[1]*Biology Centre CAS, Institute of Entomology, Ceske Budejovice, Czech Republic*
[2]*University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic*
[3]*Leibniz Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany*
[4]*Institute of Biodiversity, Friedrich-Schiller-Universität Jena, Jena, Germany*

Hybridization occurs in ca 40% of plant families and 16% of plant genera, and has important consequences on their chemical profiles. Changes in genotype impact metabolic pathways, creating novel metabolic profiles in hybrids, which allows them to cope with environmental pressure differently from their parents. Thanks to recent advances in metabolic analysis and modelling methods, we can link changes in hybrid's chemical α–diversity and β-diversity to their performance against abiotic constraints and herbivory. We use genome repetitive sequences to identify hybrids, and determine their chemical profiles using UHPLC-ESI-MS/MS. We also measure several abiotic factors and identify the insect herbivores to which the plants are subjected in various systems. We work with different *Salix* species and their hybrids to identify patterns and generalize our results. We aim to identify clear links between the performance of hybrids in their environment compared to their parental species depending on the variation of their metabolic profiles. This study ultimately will allow us to confirm that plant hybridization is fuelled by the adaptive value of hybrids in the face of environment pressure as well as driven by their natural enemies.

Plant chemical defence to soybean cyst nematodes in wild soybean (*Glycine soja*)

Hengyou Zhang[1], **Neha Mittal**[1], **Xu Li**[2,3], **Zenglu Li**[4], **Chunying Li**[5], **Eric Davis**[5], **Jianfeng Chen**[1], **Changbao Li**[6], **Han-Yi Chen**[2,3], **Qijian Song**[7], **Yong-qiang Charles An**[8], **Bao-Hua Song**[1]
[1]*Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC 28223, USA*
[2]*Plant for Human Health Institute, North Carolina State University, Kannapolis, NC 28081, USA*
[3]*Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA*
[4]*Institute of Plant Breeding, Genetics and Genomics and Department of Crop and Soil Sciences, University of Georgia, GA 30602, USA*
[5]*Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607, USA*
[6]*Syngenta Research Triangle Park, Durham, NC 27709, USA*
[7]*United States Department of Agriculture, Agricultural Research Service, Soybean Genomics and Improvement Laboratory, Beltsville, MD 20705, USA*
[8]*US Department of Agriculture, Agricultural Research Service, Midwest Area, Plant Genetics Research Unit, Donald Danforth Plant Science Center, St. Louis, MO, 63132, USA*

Soybean cyst nematode (SCN) is the most damaging soybean pest worldwide, causing over $1.5 billion yearly yield loss in US soybean production. Deploying SCN-resistant soybean varieties is the most efficient and environmentally friendly strategy for managing SCN damage. However, this strategy is challenging because SCN populations evolve rapidly, and the current SCN-resistant soybean varieties are losing resistance. To solve this significant agriculture problem, the critical first steps are to identify new genetic resources with broad-spectrum SCN resistance and to uncover its underlying molecular mechanisms. We leveraged the high genomic diversity of the wild soybean *Glycine soja*, which harbors untapped genetic resources for novel SCN resistance. We identified a *G. soja* genotype, WSRG, showing resistance to both HG types 2.5.7 and 1.2.5.7, and further analysis showed the current known molecular mechanisms cannot explain its resistance. We further investigated the underlying resistance mechanism by integrating transcriptomics and metabolomics analyses. This integrative analysis identified a core set of differentially expressed genes (e.g., Ca^{2+}- and salicylic acid (SA)-related signaling genes, isoflavonoid pathway genes) and metabolites (e.g., phenolic acids and isoflavonoids) that commonly responded to the two HG types. Two phenolic compounds, 4-hydroxybenzaldehyde and 2,3-dihydroxybenzoic acid, exhibited nematocidal activities against SCN. This study showed that Ca^{2+}-SA signaling and enhanced phenolic biosynthesis contribute to the enhanced resistance of wild soybean WSRG, which shed light on the novel mechanism of SCN resistance and provided a new perspective on plant chemical defence.

Real-time evolution of plant chemical defenses in natural communities

Sara Nouere[1], Antonino Malacrinò[2], Martin Schäfer[1], Shuqing Xu[1]
[1]Institute of Organismic and Molecular Evolution, Johannes Gutenberg University Mainz, Germany
[2]Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Reggio Calabria, Italy

Herbivores are generally considered to reduce plant fitness and can drive the evolution of plant chemical defenses. However, as in natural communities, herbivores can also increase plant fitness via indirect effects, such as by reducing competing species. Currently, it remains unclear how herbivores drive plant resistance evolution in natural communities. To address this challenge, we performed several outdoor real-time evolution experiments using the giant duckweed and its natural herbivores as a model system. Our results demonstrated that while direct effects of herbivory can impose strong selection on the plant and drive rapid adaptive evolution of induced defenses, indirect effects of herbivory, such as via changes in species competitions in natural communities, can act in the opposite direction and slow down the evolution of plant defenses. These data suggest that a realistic understanding of plant evolution requires quantifying indirect ecological effects in natural communities.

Volatile responses to branch-localized induction of *Quercus petraea* in relation to neighboring tree diversity

Gabriela Anjos De Stefano Escaliante[1,2], **Julia Piko**[1,4,5], **Alexander Weinhold**, **Nicole M. van Dam**[2,3]
[1]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany
[2]Friedrich Schiller University Jena, Institute for Biodiversity, Germany
[3]Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Theodor-Echtermeyer-Weg 1,14979 Großbeeren, Germany
[4]University of Göttingen, Department of Forest Nature Conservation, Büsgenweg 3, 37077 Göttingen, Germany
[5]Martin Luther University Halle-Wittenberg, Department of Geobotany and Botanical Garden, Germany

Plants produce specific volatile organic compounds (VOCs) to protect themselves against biotic and abiotic stresses. When herbivores damage plant tissues, plants release signals, which attract natural enemies of the herbivores and inform neighboring plants about a possible attack. Intensity of plant-herbivore interactions and plant diversity could shape VOC emissions resulting in the production of specific compounds, possibly over different resource availability due to a diverse community. How exactly plant defense mechanisms and diversity influence the VOCs emissions is unknown. We hypothesized that branch-localized VOC emissions after induction are influenced by treatment repetition and tree diversity, ultimately affecting predation rates. To test this, we studied VOCs from *Quercus petrea* in the MyDiv BEF experiment during leaf flush and predation rates by arthropods using clay caterpillars before, during, and after treatments. We selected oak trees from monocultures, two and four species mixtures. Branches from five oaks per plot were repeatedly treated with methyl jasmonate to simulate herbivory or with water as control. VOCs were passively adsorbed onto PDMS tubes and analyzed by TD-GC-MS. Sesquiterpenes were up-regulated in MeJA-treated branches when compared to control branches, particularly α-farnesene, known for attracting natural enemies of herbivores. Repeated MeJA treatment intensified branch-localized induction, positively affecting arthropod predation. Green leaf volatile emissions decreased with time in treated branches, possibly due to lower mechanical damage. The interaction between plot diversity and treatment was significant on GLVs and linalool. These results show that localized VOCs induction is affected by plot diversity and amplify *Q. petraea*'s indirect defense potential.

Variation in induced volatile and non-volatile metabolites: Do willow species share responses to herbivory?

Priscila Mezzomo[1,2], Jing V. Leong[1,2], Petr Vodrážka[1], Martin Moos[1], Leonardo R. Jorge[1], Tereza Volfová[1,2], Jan Michálek[1,4], Paola de L. Ferreira[1,3], Petr Kozel[1,2], Brian E. Sedio[5,6], Martin Volf[1,2]
[1]*Biology Centre CAS, Institute of Entomology, Ceske Budejovice, Czech Republic*
[2]*University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic*
[3]*Aarhus University, Department of Biology, Aarhus, Denmark*
[4]*Algatech Centre, Institute of Microbiology, Trebon, Czech Republic*
[5]*Department of Integrative Biology, University of Texas at Austin, Austin, USA*
[6]*Smithsonian Tropical Research Institute, Balboa, Republic of Panama*

Chemical variation is one of the key aspects affecting the performance of co-occurring plants. High chemical variation in metabolites directly affecting herbivores can reduce the number of herbivores shared by closely related plant species. In contrast, low intraspecific variation in induced volatile organic compounds (VOCs) and their high specificity to insect herbivores may improve the attraction of specialist predators or parasitoids. We explore if the induced chemical variation following herbivory by various insect herbivores differs between VOCs and non-volatile metabolites (non-VOCs) in six closely related willow species. Willow species identity explained most variation in VOCs and non-VOCs. The amount of variation explained by the independent effect of the herbivore treatment was higher in the case of VOCs. Contrastingly, the herbivore treatment explained much less of the observed variation in the case of non-VOCs. We found pronounced differences in induced responses among the studied willow species, particularly in the case of non-VOCs. We also observed similarities in induced VOC responses among some willow species, which may affect how they attract predators and parasitoids of their herbivores. Our results suggest that largely divergent responses between different chemical defences may further contribute to flexible plant responses against herbivory and environmental stimuli. At the same time, our findings shed light on the direction future research on plant-herbivore interactions should focus on.

Carrot seed crops and honey bee pollinators – A Chemistry conundrum

Björn Bohman[1,2], **Stephen R Quarrell**[3], **Gavin Flematti**[1]
[1]School of Molecular Sciences, University of Western Australia, Perth, Australia
[2]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Lomma, Sweden
[3]Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Australia

Studies investigating the chemistry of pollinator attraction have traditionally focused on specialised systems of high natural history interest. Although these specialised plant-insect interactions are key to natural ecosystems, much less progress has been made investigating economically important insect pollinator crops. Many agricultural species struggle with limitations in pollinator attraction, impacting on crop yields, causing higher costs for production. Instead, agricultural plant-breeding programs often focus on traits that appeal to growers and consumers instead of pollinators, leading to further declines in pollinator attraction and yield. Using hybrid carrot seed production as a model, we investigated high and low-yielding carrot varieties by analyzing sugars, minerals in nectar and floral volatile composition. While the analysis of nectar sugars and minerals did not reveal any key differences and therefore reward between the carrot varieties, the floral volatiles did. Numerous differentiating sesquiterpenes were identified in floral solvent extracts, and subsequent behavioral assays showed that β-ocimene from higher-yielding carrot varieties stimulated nectar feeding (attractant), while α- and β-selinene from lower-yielding lines decreased feeding (deterrents). It is therefore likely that the interplay between these attractive and repulsive sequiterpines mediate pollinator attraction in these crops. Sesquiterpenes have previously been implicated in plant defense, suggesting a trade-off between pollination and protection. Our results highlight the importance of volatiles as regulators of pollinator attraction in agricultural settings and suggest these will be important in other economically important crop species.

POSTERS

P-11

Chemical diversity in hostplants of tenthredinid sawflies

Jean-Luc Boevé, **Gontran Sonet**, **Carl Vangestel**
OD Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Rue Vautier 29, 1000 Brussels, Belgium

When phytophagous insects are feeding, they ingest plant secondary metabolites (PSM) that they can exploit as "insect secondary metabolites" (ISM). Here, the aim was to explore a large lineage of insects, the sawfly family *Tenthredinidae*, and to compare among their species which types of PSM they ingest. Screenings were performed by linking lists of hostplant genera with a dedicated databank of organic compounds. Principal component analyses revealed that among all known hostplants of the tenthredinids, the *Ranunculaceae* and *Lamiaceae* are two important plant families because they contain diterpenoids. Within each of the seven known tenthredinid subfamilies, the species were screened and then monophagous species were compared with non-monophagous species (i.e., feeding on one versus more than one plant genus). The chemical diversity of the hostplants was similar between species with a narrow versus larger diet breath in sawfly subfamilies mainly feeding on herbaceous plants, whereas diversity of the PSM was significantly lower for monophagous than non-monophagous species in sawfly subfamilies generally feeding on shrubs and trees. Furthermore, major types of PSM (e.g., glucosinolates, terpenoids, steroid alkaloids, steroid saponins) were those chemicals that are sequestered by tenthredinid larvae to defend themselves against predators. Overall, using one comprehensive dataset about PSM may help to understand several facets of plant-insect relationships, among which the determinants of defensive ISM.

P-33

A multiomics approach of the scent in *Pelargonium* species: Analysis of terpene diversity and functional characterization of sTPSa genes

C. Bihanic[1], L. Martinelli[1], T. Barrit[1], A. Rius-Bony[1], F. Jeanpierre[1], B. Blerot[2], G. Chietera[2], J-L. Magnard[1], D. Saint-Marcoux[1], F. Jullien[1]
[1]Laboratoire BVpam – UMR 5079, Université Jean Monnet Saint-Étienne, CNRS, Saint-Étienne 42023, France
[2]IFF-LMR naturals, 126 av L. Bobet 06130 Grasse, France

The genus *Pelargonium* (*Geraniaceae*) is an example of evolutionary radiation, with more than 280 species, mostly originating from South Africa. Approximately 30 botanical species, mainly belonging to a same section, exhibit a remarkable diversity of scents. Breeding led to many hybrids showing a large range of scents, still unexploited. Today, rose-scented pelargoniums are the only hybrids cultivated and are mainly used for the production of essential oils and absolutes. The scent of pelargonium is mainly due to mono- and sesqui-terpenes. As of yet, two main pathways involved in monoterpene biosynthesis have been described in rose-scented pelargonium; one leading to citronellol and derivative esters and the other producing *p*-menthanes. Only one sesquiterpene synthase is currently described in pelargonium.

To complete our understanding of the terpene biosynthesis in pelargonium, we present metabolomic analysis of 30 botanical species and 50 hybrids providing description of several terpene profiles allowing us to cluster terpenes likely produced through the same pathway.

We also correlated transcriptomic data of 10 different accessions to their sesquiterpene composition and assessed the likely functions of orthologous gene clusters. Subsequently, sesquiterpene synthase candidates of interest were selected and six were characterized; among them, a 6,9-guaiadiene synthase was reported for the first time in plants.

P-72

Unravelling the genetic basis of phytochemical variation in wild soybean

Bao-Hua Song[1], **Farida Yasmin**[1], **Hengyou Zhang**[1], **Larry Leamy**[1], **Jason Winnike**[2], **Robert Reid**[3], **Cory Brouwer**[3]
[1]Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte NC 28223, USA
[2]Metabolon, Inc. Morrisville NC 27560, USA. [3]Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, Charlotte NC 28223, USA

Phytoalexins, a group of induced plant specialized metabolites, play critical roles in plant response to various environmental stresses. They are also a source of sustainable human nutrition and potential novel pharmaceuticals. Glyceollin is one kind of phytoalexin that is induced in legume species with biotic and abiotic stresses, and has well-documented anticancer, antioxidant, and neuroprotective properties. However, few studies have investigated the genetic basis of glyceollin induction with biotic stimuli. In this study, we used a metabolite-based genome-wide association study (mGWAS) to identify genes and gene clusters involved in glyceollin induction from genetically diverse and underexplored wild soybean plants with soybean cyst nematode infection. Our analyses found eight SNPs significantly associated with variation in glyceollin induction, and four SNPs on Chromosome nine. Six candidate genes on chromosome nine, belonging to two gene clusters, encode glycosyltransferase in the phenylpropanoid pathway. Further analyses suggested epistatic interactions of these genes. These findings indicate that these genes and their interactions might play critical roles in glyceollin induction. We also found transcription factors, such as WRKY and NAC, within the LD blocks of the significant SNPs, indicating they might be involved in regulating glyceollin accumulation. Further functional analyses will shed light on the roles of these candidate genetics in glyceollin induction. Our study provides a fundamental basis for the long-term goal of developing biofortified soybean cultivars rich in glyceollin, which would benefit plant and human health to meet current and future global challenges.

S14: Chemosensory system in Acarines and other non-insect arthropods

Chairs: *Nicoletta Faraone, Kirk Hillier*

ORAL PRESENTATIONS

Chemosensory systems in small arachnids: State of the art, knowledge gaps and challenges in mesostigmatic mites

Lise Roy
CEFE, Université Paul-Valéry Montpellier 3, Montpellier, France

Chemoreception in general, and olfaction in particular, have been much less studied in non-insect arthropods than in insects. However, the majority of insect olfactory receptors are located on the antennae, an appendage unique to Mandibulata, and belong to a group of receptors (OR) that constitute an evolutionary innovation specific to insects. Arachnids belong to Chelicerata arthropods, and have neither antennae nor OR. Clearly, they detect volatile compounds at a distance thanks to other appendages and receptors, since they exhibit attraction or repulsion responses in the absence of contact. But knowledge is still incomplete. Furthermore, among arachnids, the relationship between anatomical structures and olfactory response is particularly difficult to study in tiny taxa such as mites. I'll provide an overview of the state of knowledge on behavioral responses to odors, the anatomical structures bearing sensillae and the molecular supports involved in chemoreception in mites, with a focus on mesostigmatic mites. By the way, I'll emphasize the importance of developing specific tools, or adapting existing ones, to advance our understanding of chemosensory behaviour in these tiny animals. To illustrate this overview and provide food for thought, I will present some results on the role of different pairs of legs in the perception of volatile compounds, on inter-individual and inter-population variation in behavioral responses or on the difficulties of using an attractant to control a mite.

Effect of repellent exposure on attractant detection in infected and non-infected *Ixodes scapularis* (Say) (Arachnida: Ixodidae) ticks

Nicoletta Faraone[1]; **Kayla Gaudet**[2]; **Luis Adriano Anholeto**[1]; **N. Kirk Hillier**[2]
[1] *Chemistry Department, Acadia University, Wolfville (NS), Canada*
[2] *Biology Department, Acadia University, Wolfville (NS), Canada*

Ixodes scapularis Say (Arachnida: Ixodidae) is a growing health concern for humans as vector of the causative agent of Lyme disease, *Borrelia burgdorferi*, and many other pathogens. Given the potential health threat presented by *I. scapularis*, and the need to find effective strategies to prevent tick bites, it is pivotal to understand the chemosensory system of ticks and their host-seeking behaviours when exposed to repellents. In this study, we investigated whether exposure to synthetic and plant-derived repellents impair the ability of *I. scapularis* to detect attractants and host volatiles (such as butyric acid), and ultimately how these repellents interfere with host-seeking behaviour in both wild and lab-reared ticks. Furthermore, we screened wild ticks used in electrophysiology and Y-tube behavioural assays for presence of pathogens to evaluate if the bacterial infection status would affect the detection of butyric acid under the exposure to repellents. We determined that the exposure to *N,N*-diethyl-3-methyl benzamide (DEET), lemongrass essential oil, citral, and geraniol significantly inhibited the ability of both lab-reared and wild *I. scapularis* adult females to detect and respond to butyric acid. We also found that tick infection status does not significantly impact host-seeking behaviour in *I. scapularis* adult female. The knowledge gained from our study contributes to advance our understanding of host-seeking behaviour in ticks and the impact that the exposure to repellent has on the tick chemosensory system. These findings will be important for elucidating the mechanism of repellence in ticks and for the development of effective tick repellent management tools.

Blacklegged ticks reduce predation risk by eavesdropping on communication signals of ants

Claire Gooding, Charlotte Pinard, Regine Gries, Anand Devireddy, Gerhard Gries
Department of Biological Sciences, Simon Fraser University, British Columbia, Canada

Blacklegged ticks, *Ixodes scapularis*, are obligatory blood feeders and transmit more disease-causing microbes than any other blood-feeding arthropod. Despite their reputation as blood-feeders, ticks spend most of their lifespan off hosts. Off-host ticks are highly susceptible to predation, particularly by ants, but the mechanisms underlying ant avoidance behavior have not yet been studied. Because foraging ants deposit semiochemicals to communicate with nestmates, and because blacklegged ticks have poor defenses against ant predation, we tested the hypothesis that ticks avoid ant-frequented areas by sensing the ants' semiochemical deposits. In two-choice still-air olfactometers, we show that semiochemical deposits of thatching ants, *Formica rufa,* deter adult blacklegged ticks. The deterrent semiochemicals originate from both the poison and Dufour's glands. Extracts of both glands combined, but not of either gland alone, proved deterrent to ticks. Additionally, we test synthetic gland extracts for potential future use in tick management.

Olfactory acid detection in acarines

N.K. Hillier[1], K. Gaudet[1], J. Comeau-Ouellette[1], N. Faraone[2]
[1] *Biology, Acadia University, Wolfville, Canada*
[2] *Chemistry, Acadia University, Wolfville, Canada*

Acari (mites and ticks) are economically and medically important arachnids with significant impacts on human health and agriculture. Species such as black-legged ticks, *Ixodes scapularis*, and Varroa mites, *Varroa destructor*, are of public health and veterinary importance, transmitting disease and impacting agricultural production. *Tetranychus urticae*, the red-spotted spider mite, is a major global pest of many plant species, ranging from houseplants and horticultural products to greenhouse vegetables. This presentation will outline recent studies investigating chemical ecology of these acarines, using combined approaches of chemical analyses, electrophysiology, and behavioral assays, with an emphasis on the importance of acids as behaviorally-relevant cues. Using this knowledge, we plan to develop new, environmentally sustainable technologies for managing these acarine pests.

Changes in leaf volatile emissions, profile of primary metabolites, and leaf damage of tomato plants after infestation by *Tetranychus urticae* and *Tetranychus evansi*

Stamatia Sakavala, George Tziotzios, Xanthoula Eirini Pantazi, Dimitrios Koveos, Katerina Karamanoli
Faculty of Agriculture, Aristotle University of Thessaloniki, Thessaloniki Greece

In this study, the level of leaf damage, and the composition of emitted volatile organic compounds (VOCs) and primary metabolites after infestation of tomato plants with either *Tetranychus urticae* (Tu) or *Tetranychus evansi* (Te) at different time points, were determined. According to our results, feeding damage has been clearly illustrated by the employment of three different machine learning (ML) models on the hyperspectral data, leading to the successful classification of the infested leaves in distinct groups according to the damage caused by the different mite species. GC-MS analysis revealed differences in the synthesis of VOCs emitted by tomato plants infested by either Tu or Te compared to control plants. Terpenoids such as α-pinene, ocimene, 4-carene, and γ-terpinene were found in high concentration in infested plants, 72h after mite deposition. Among VOCs emitted from Te plants in high concentration were hexanal, α-pinene, isolimonene, myrcene, trans-β-ocimene, p-cymene, p-cymen-8-ol, isocaryophyllene, humulene and 3,7,7-trimethyl-1,3,5-cycloheptatriene, especially 10 and 20 days after mite infestation. According to leaf metabolic profile, accumulation of amino acids such as isoleucine, and threonine was increased in infested plant tissues, while the level of sugars was decreased compared to control plants, which may indicate metabolic regulation in the damaged plants towards defense and signaling. Changes of plant metabolic profile were more pronounced after prolonged infestation by Te than Tu. Our results show that there is a differential response of tomato plants to Te and Tu infestation with concern to the emission of VOCs, metabolic profile, and leaf injury.

Aging virgin females of the false widow spider (*Steatoda grossa*) engage in dishonest pheromone signaling

Andreas Fischer[1,2], **Andrea C. Roman-Torres**[2], **Beatrice Chee**[2], **Gerhard Gries**[2]
[1]*General and Systematic Zoology, University of Greifswald, Greifswald, Germany*
[2]*Biological Sciences, Simon Fraser University, Burnaby, Canada*

The terminal investment concept proposes that decreased expectation of future reproduction (e.g., arising from increasing age) induces greater investment in current reproduction including greater efforts to locate or attract mates. Typically, aging female animals become less fecund and less attractive to males, who achieve greater reproductive fitness by mating with younger females. Here, we tested the hypothesis that aging females of the false widow spider, *Steatoda grossa*, engage in dishonest signaling, thus 'lying about their age'. Female *S. grossa* are known to deposit on their webs courtship-inducing contact pheromone components which pH-dependently hydrolyze, giving rise to airborne mate-attracting pheromone components. Females can regulate their webs' pH, thus manipulating the rate of hydrolysis and thereby the release rate of mate-attractant pheromone components. Using high-performance liquid chromatography-mass spectrometry, we quantified the contact pheromone components that virgin females deposit on their web over their multi-year lifetime, and we recorded the females' fecundity and attractiveness to males. As females aged, they produced fewer viable offspring and deposited less contact pheromone components but altered their webs' pH to elevate the release of mate-attracting pheromone components equivalent to levels of young females. Essentially, this tactic made old females as attractive as young females, as shown in mate-choice experiments with males, despite diminished fecundity. Our data support the conclusion that female *S. grossa* engage in dishonest signaling, thereby increasing their reproductive fitness to the detriment of male spiders that would achieve greater reproductive potential by mating with younger females.

Epicuticular chemistry – Differences and similarities between insects, spiders, and springtails

Stefan Schulz, Moritz Gerbaulet, Anton Möllerke
Institute of Organic Chemistry, Technische Universität Braunschweig, Braunschweig, Germany

The epicuticular lipid layer of insects is well understood in composition and function. Long-chain linear alkanes, often methyl-branched, and alkenes dominate, occurring usually in complex mixtures of many components. These layers prevent the desiccation of insects but also carry information used by many species for various traits such as species, or individual recognition. We were interested, in how other arthropod lineages that separated early such as Collembola, basal hexapods, or Arachnida, who share only crustacean ancestors with insects explore this trait. Our analyses show that both spiders and springtails employ specific chemistry for this purpose. Arachnids use hydrocarbons similar to those of insects but also use unique compounds, long-chain alkyl methyl ethers, and carboxylic esters. These compounds occur either as mixtures, as in insects, but also as mixtures of only a few compounds, e. g. in *Tetragantha* spiders. Recent results showed that cuticular ethers are used in species recognition and are important for species radiation. In contrast, a survey on collembola revealed a completely different set of compounds. Usually, only a few compounds are used, often with unique structures. While long-chain hydrocarbons are found as in insects, their structures differ, including unusual structural elements such as cyclopropanes. Even more surprising is the large amount of terpenes occurring as cuticular lipids of Collembola. These are often linearly connected long-chain terpene precursors which are either cyclized to complex head groups or are prenylated, resulting in highly branched compounds. We will discuss these results from an evolutionary perspective.

Sesquiterpene volatiles from *Trichoderma virens* repel the fungivorous Collembola *Folsomia candida*

Matthäus Slonka[1], **Ilka Vosteen**[1], **Artemio Mendoza-Mendoza**[2], **Michael Rostás**[1]
[1]Agricultural Entomology, Department of Crop Sciences, University of Göttingen, Grisebachstr. 6, 37077 Göttingen, Germany
[2]Faculty of Agriculture and Life Sciences, Department of Wine, Food and Molecular Biosciences, Lincoln University, Lincoln, New Zealand

The volatile profile *Trichoderma virens* Gv29.8, an endophytic biocontrol strain, is uniquely made up of mostly sesquiterpene terpenoids. The volatile blend of *T. virens* Gv29.8 suppresses pathogenic fungi, and mediates growth promotion and enhanced pathogen resistance in plants. However, no information existed on how these volatiles might affect direct interactions between *T. virens* and fungivores in the soil.

We hypothesized that sesquiterpenes emitted by *T. virens* Gv29.8 play a key role in the direct interactions of the fungus and these fungivorous soil arthropods.

Using gas chromatography-mass spectronomy, we showed that deletion of the terpene cyclase *vir4* in this strain led to a drastic reduction of sesquiterpene synthesis, while random reintroduction of this gene into the genome of the knock-out mutant, resulting in the complementation strain, led to an intermediate level of sesquiterpene emissions. Using these three mutants in behavioural experiments, we demonstrated that the soil Collembola *Folsomia candida* was significantly more attracted to the knock-out mutant in comparison to wild-type *T. virens* Gv29.8, while no significant difference could be observed in attraction between the wild-type and the complementation strain. Functionality of *vir4* and sesquiterpene emission had no impact on the three fitness parameters growth, survival and reproduction of *F. candida* when it was feeding on fungal mycelium.

We conclude that sesquiterpenes emitted by *T. virens* Gv29.8 are repellent, but not toxic to fungivores such as *F. candida* and play an important part in the defence against fungivore grazing in this fungus.

POSTER

P-46

Influence of selected essential oils on the behavior of female wolf spiders *Pardosa hortensis* (Araneae: Lycosidae)

Marek Chajduk[1], Marek Gołębiowski[1], Marzena Stańska[2], Christophe Lucas[3]
[1]*Department of Environmental Analysis, Laboratory of Analysis of Natural Compounds, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland*
[2]*Institute of Biological Sciences, Faculty of Sciences, Siedlce University, Siedlce, Poland*
[3]*Institut de Recherche sur la Biologie de l'Insecte (UMR7261), CNRS – University of Tours, Tours, France*

Spiders play a crucial ecological role as predators, regulating insect populations and promoting biodiversity. Their integration into biocontrol strategies has led to reduced pesticide use and pest management in agroecosystems. However, spiders can also pose psychological and health risks to humans, particularly those suffering from arachnophobia. This study explores the potential of essential oils (EO) as natural repellents against the synanthropic spider *Pardosa hortensis*. Wild-caught spiders from France were subjected to binary choice tests using 1% EO solutions from ten plants, analyzed by GC-MS. The EOs of catnip, cedarwood, cinnamon, citronella, and clove exhibited significant repellent effects, whereas peppermint, lavender, eucalyptus, tea tree, and lemon did not. The chemical composition of the effective EOs suggests that compounds such as eugenol, ☒-caryophyllene, geraniol, and cis-thujopsen may be highly attributed to their repellent properties. These findings contribute to the understanding of EO efficacy as spider repellents, highlighting the potential for natural management of human-spider interactions and minimizing the reliance on chemical pesticides. Further research is needed to explore the variability in EO effectiveness across different spider species and environmental conditions.

S15: Chemical ecology in the Anthropocene

Chair: *Robbie Girling*

ORAL PRESENTATIONS

Climate change vs. natural fluctuations – environmental impacts on the chemical communication process across temporal and spatial scales

Christina C Roggatz[1,2]
[1]Dynamic Ecological Chemistry, Faculty of Biology & Chemistry, University of Bremen, Bremen, Germany
[2]UFT – Center for Environmental Research and Sustainable Technology, University of Bremen, Bremen, Germany

The process of chemical communication is essential for life on our planet from bacteria to complex multicellular organisms. Across realms, communication or interaction processes follow a series of distinct steps and take place under system-specific environmental conditions. First, I will provide an overview on how climate change affects chemical communication in different realms in similar patterns, from molecular to ecosystem-wide levels. I will illustrate the importance of different stressors for terrestrial, freshwater, and marine ecosystems and propose a systematic cross-disciplinary approach to address identified knowledge gaps. With a focus on one of these current gaps – the relevance of climate change stressors in light of much larger naturally fluctuating conditions – I will further assess the impact of temperature and pH changes on the degradation of bacterial autoinducers (acyl-homoserine lactones) employing a numerical physical-chemical model. The substantial effects of climate change on these important bacterial signals shown by the model will be discussed in the context of effects caused by daily and seasonal natural fluctuations in marine photosynthetic biofilms. This talk will highlight the importance of taking realistic environmental conditions and their temporal and spatial dynamics into consideration when studying chemical interactions today and in the future.

Climate change alters specialist and generalist herbivore performances through ontogeny-dependent response of *Aristolochia contorta*

Bo Eun Nam[1,2], Hyun Jun Park[1], Gisuk Lee[3], Sang-Gyu Kim[3], Youngsung Joo[2], Jae Geun Kim[1]
[1]Department of Biology Education, Seoul National University, Seoul, Republic of Korea
[2]School of Biological Sciences, Seoul National University, Seoul, Republic of Korea
[3]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

Plants interact with their environment differently as they grow and age, impacting herbivorous insects who feed on them. Herbivorous insects, with varying diet breadth, might be affected by these changes differently. The research focused on how the growth stages of a host plant (*Aristolochia contorta*) influence its relationships with an obligate specialist herbivore (*Sericinus montela*) and a generalist herbivore (*Spodoptera exigua*) under conditions simulating climate change. The plants were exposed to two CO_2 concentrations and varying watering regimes. The climate's impact shifted across the plant's life stages. High CO_2 impeded the growth and survival of the specialist herbivore, but more frequent watering lessened this negative impact. Performance of the generalist herbivore was slightly better with increased CO_2, especially in later plant ontogenetic stage. Second-year juvenile plants were less affected by climate changes in comparison to the 1st-year ontogenetic stages. Elevated CO_2 levels also negatively affected the nutritional value of host plants, as indicated by the C:N ratio. It is possible that climate change could disrupt the herbivore community, as the growth and survival of generalist herbivores during late plant ontogenetic stages may compete with the growth and survival of specialist herbivores. This result indicates that assessing plant-insect interactions at a single stage of plant growth may not accurately reflect the potential population dynamics in a changing climate.

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Chemotypic variation in drought tolerance of wild cotton populations

Marine Mamin[1], Carlos Bustos-Segura[2], Biiniza Pérez Niño[3], Teresa Quijano-Medina[3], Luis Abdala-Roberts[3], Ted CJ Turlings[1], Mary V Clancy[1]
[1]University of Neuchâtel, Institute of Biology, Fundamental and Applied Research in Chemical Ecology, Neuchâtel, Switzerland
[2]Sorbonne Université, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, CNRS, Institut de Recherche pour le Développement, Université Paris-Est-Créteil-Val-de-Marne, Université Paris Cité, Institut d'Ecologie et des Sciences de l'Environnement de Paris, Versailles 78026, France
[3]Departamento de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, México

Gossypium hirsutum (upland cotton) is one of the most economically important industrial crops worldwide, and is grown across wide ranges of climates. Although cultivated cotton is considered to have a relatively high tolerance to water stress, global challenges such as climate change and population growth have led to an urgent need to improve drought tolerance in cotton cultivars. One such way of improving tolerance to adverse conditions, such as drought, is by exploring genetic variation that exists in the wild relatives of domesticated crops. In this study, we evaluated the tolerance of wild cotton populations from the Yucatán peninsula (likely the centre of origin of *G. hirsutum*) to two varying levels of drought stress, primarily focusing on the links between chemotypic and regional variation to drought stress tolerance. Initial findings indicate that wild cotton exhibits diverse adaptive strategies to drought.

The use of sensory cues in aquatic pest management – evaluating the complexity of controlling crustaceans in a changing world

Jorg D. Hardege, **Jonathan W. Burnett, Hannah Ohnstad, Steph Templeman, John Terschak, Helga Bartels-Hardege**
School of Natural Sciences, Biology, The University of Hull, Hull, UK

The marine ecosystem has recently seen a rise in crustaceans as major invasive pest species. This includes the European Green Crab, *Carcinus maenas* in the US as well as the Blue Crab, *Callinectes sapidus* in the Mediterranean. Trapping crustaceans for fisheries or pest control currently utilises natural baits, including fish such as herring, mackerel or tuna. These types of lures have seen little change in centuries, lacking species specificity / leading to by-catch which represents a major ecological and economic problem. Here we evaluate the use of chemosensory cues to be employed in trapping efforts or to repel crabs from specific sites such as bivalve aquacultures. Our data on the use of shore crab sex pheromones and odours from predators shows that there is huge potential for the development of odour-based lures and repellents, but also that a wide range of complexities impact their success. These include not only 'obvious' factors such as population density, availability of shelter, interaction with other non-target species, cue dispersal, concentration and specificity, local differences in cues, learning and adaptation to cues etc, but also climate change effects. Aquatic sensory cues have been shown to be impacted by ocean acidification with pH altering animal behaviour, signal structure and reception, highlighting that odour cues can play a key role as management tools in marine systems but do not represent a simple magic 'silver bullet'.

The genetic mechanism of diquat tolerance reveals insights into the evolution of non-targeted site resistance to herbicides

Martin Höfer[1], **Martin Schäfer**[1], **Yangzi Wang**[1], **Samuel Wink**[2], **Shuqing Xu**[1]
[1]*Institute for Organismic and Molecular Evolution (iomE), Johannes Gutenberg University, 55128 Mainz, Germany*
[2]*Institute for Evolution and Biodiversity, University of Münster, 48148 Münster, Germany*

Since the introduction of herbicides into modern agriculture the global emergence of herbicide resistance cases has increased exponentially over time. Very often these resistance traits underly complex quantitative genetic and molecular principles, which is why they are categorized as non-targeted site resistance (NTSR). Aiming to understand the rapid evolution of NTSR, we investigated the physiology of resistance to the herbicide diquat, for which no target site mechanisms are known, using the giant duckweed *Spirodela polyrhiza* as a model system. A screening of 138 genotypes revealed an intraspecific variation of diquat resistance by a factor of 8.5. Increased resistance was associated with reduced herbicide uptake kinetics and an increased activity of the redox hub, indicating a joint contribution of uptake and radical quenching activities in diquat resistance. With a GWAS-guided gene expression study, we associated several candidate genes involved in stress-response pathways with herbicide resistance. Our results suggest, that herbicide resistant plants benefit from a constitutively upregulated general stress-response to environmental factors such as herbivory, UV-light, high salt and heavy metal exposure. Since most of our genotypes had no history of diquat-based weed control, we suggest, that diquat resistance originates from an adaption to multiple environmental stress factors, which select for resistant phenotypes in an indirect way.

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Chemical ecology of invasive plants

Evans Effah[1,2], D. Paul Barret[1], Andrea Clavijo McCormick[1,2]
[1]College of Sciences Massey University, Palmerston North, New Zealand
[2]Biosecurity New Zealand, Ministry for Primary Industries, Wellington, New Zealand

Alien invasive species pose a threat to native species and ecosystems worldwide. Environmental weeds are of particular concern, as these often grow unmanaged on disturbed landscapes becoming rapidly dominant and spreading widely. The ability to produce allelopathic compounds is a ubiquitous trait of invasive plants. These compounds can have direct impacts on neighbouring plants (e.g., affect native plant seed germination and growth through root exudates), but also indirect impacts (i.e., through changes on the environment or the behaviour of other species). Many of these impacts (direct and indirect) remain poorly documented, despite their relevance to invasive species management. Another aspect that requires attention is how the environment and species present in the invaded range impact the chemical behaviour of invasive plants. This talk will document key findings of nearly a decade of work on invasive weeds in the Central Plateau of New Zealand's North Island, which includes the Tongariro National Park, a dual UNESCO world heritage site due to its natural features and cultural value to indigenous Māori communities. Topics covered include 1) the environmental drivers that influence invasive plant chemistry, 2) communication between native and invasive plant and insect species, and 3) implications for species conservation and biological control.

Studying the effects of semiochemicals in Scots pine (*Pinus sylvestris*) using a dynamic chamber system

Muhammad Usman Rasheed[1], **Stefanie Döll**[2], **Nicole van Dam**[3], **James Blande**[1]
[1]*University of Eastern Finland (UEF), Kuopio, Finland*
[2]*German Center for Intigrative Biodiversity Research (iDiv), Leipzig, Germany*
[3]*Leibniz Institute of Vegetables and Ornamental Crops (IGZ), Grossbeeren, Germany*

Over the past forty years, it has become well established that plants interact by releasing semiochemicals such as volatile organic compounds (VOCs) above- and belowground; and non-volatile organic compounds released into the soil, i.e. root exudates. Such semiochemicals have been shown to mediate plant-plant interaction effectively over short distances. The nature of responses to semiochemicals is poorly understood. We designed a dynamic chamber system where potted plants can be placed proximally across a permeable window. This allows live herbivore infested emitter plants to interact with receiver plants without the risk of herbivores causing direct damage to the receiver plants. This dynamic chamber system allows the emitter and receiver plants to be potted in the same tray, so they share the soil, allowing for interaction not only via aboveground VOCs but also via belowground VOCs and root exudates. We conducted an experiment to study the responses of one-year-old Scots pine (*Pinus sylvestris*) receiver seedlings to above- and belowground exposure to semiochemicals from one-year-old Scots pine seedlings infested with large pine weevils (*Hylobius abietis*). We studied the volatile and non-volatile secondary metabolites' profile in the receiver plants in response to the semiochemicals from the weevil infested Scots pine seedlings. Non-targeted analysis was performed and the emission profiles of the VOCs from shoots and rhizosphere were studied using GC-MS and secondary metabolites from needles, stem, roots and exudates were studied using LC-qToF-MS. We report that the receiver plants' root exudate profile is affected by the semiochemicals.

Insect chemical communication in a polluted world

Nanji Jiang, Bill S. Hansson, Markus Knaden
Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Jena, Germany
Next Generation Insect Chemical Ecology, Max Planck Centre, Max Planck Institute for Chemical Ecology, Jena, Germany

Most insects use chemical cues like pheromones for their sexual and social behavior. During the Anthropocene we are facing increasing amounts of oxidant pollutants like nitric oxides and ozone in the atmosphere. Due to their oxidizing power these pollutants can degrade many of the compounds the insects rely upon. Sex pheromones for example often contain carbon double bonds that make these compounds sensitive to degradation. Here we show that behavioral effects of such a pollutant-induced degradation can be dramatic. Female flies lose interest in their conspecific mates, after the latter have lost most of their pheromones due to exposure to slightly increased levels of ozone. Apart from sexual communication also social communication in ants can become affected. We found that ants after being exposed to increased levels of ozone become attacked by their nestmates. Nestmate recognition of ants basically relies upon saturated cuticular hydrocarbons that are not easily degraded by ozone. However, after ozone exposure we observed that the few unsaturated hydrocarbons (i.e. hydrocarbons containing carbon double bonds) in the colony-specific blends became diminished by ozone exposure, which obviously was sufficient to corrupt nestmate recognition. As all the described effects were observed already at ozone levels that have been repeatedly reported for urban areas, the corruption of insect sexual and social behavior might be another factor that contributes to the ongoing insect decline.

Mapping innate odor preference in the bumblebee *Bombus impatiens*

Paige Henderson, Jordannan Sprayberry
Muhlenberg College, USA

Bumblebees are essential pollinators who use odor cues to find resources. Odor valence, the degree to which an odor is appetitive or aversive, has been peripherally observed but not concretely tested in this taxon. We use a forced-choice proboscis extension reflex (PER) paradigm for testing odor valence. The free-moving proboscis extension reflex (FMPER) has been used in many studies to study associative learning and generalization behavior in bumblebees; we remove the learning trials to test for innate odor-preference. Bees were tested for preference between floral (clover, bee-balm, and hydrangea), vegetative (leaf mixture, walnut), and non vegetative (coyote urine, skunk extract) odors. The majority of odors did not elicit aversion or attraction. Skunk extract, however, was a notable exception, eliciting aversion in every comparison. SPME and odor analysis will look for structural correlates of aversion. Elucidating the structural boundaries of aversive odors is likely a critical step in understanding bumblebee responses to odor pollution. Given the proliferation of research indicating negative impacts of odor pollution on pollinator foraging behavior in the past decade, this research could have broad conservation applicability.

Effects of man-made chemical pollutants on the perception and attraction to floral odors in bumblebees

Marion Javal[1,2,3], Maryse Vanderplanck[1], Lilian Roux[1], Thibaut Bachelet[1], Marie Dolfi[1], Juliette Fernandez[1], Bruno Buatois[1], Benoit Lapeyre[1], Virginie Cuvillier[2]*, Magali Proffit[1]*
**equal contributions*
[1]CEFE, University of Montpellier, CNRS, EPHE, IRD, Montpellier, France
[2]University of Lille, CNRS, UMR 8198 – Evo-Eco-Paleo, Lille, France
[3]CBGP, IRD, INRAE, CIRAD, Institut Agro, University of Montpellier, Montpellier, France

Pollinators are subject to a variety of pressures whose relative importance in the global decline crisis remains largely unknown. Among environmental stressors, exposure to man-made chemical pollutants could play a significant role, either by directly causing toxic effects, or by triggering sublethal effects. Among these chemicals, phthalates are of particular concern. Present in both rural and urban environments, these molecules can easily penetrate insect cuticles due to their lipophilic nature, and their endocrine-disrupting properties have been confirmed in numerous animal models, both vertebrate and invertebrate. They are therefore likely to strongly affect the selective value of pollinators, as well as their interactions with flowering plants, for example by disrupting olfactive ecdysteroid-regulated pathways. In this study, we tested under controlled laboratory conditions the impact of two phthalates prevalent in the atmosphere, DnBP and DEHP, on the sensory response and their attraction to floral odors of worker bees of *Bombus terrestris*. Electroantennographic recordings indicate that, at environmental doses, these two phthalates, tested alone and in mixtures, significantly modify the ability of bumblebees to detect volatile organic compounds (VOCs) commonly emitted by flowering plants. Moreover, olfactometry attraction tests show that these same exposures affect the bumblebees' behavioral response towards VOCs. These results suggest that phthalate exposure has the potential to affect the attraction of pollinators to plants, and possibly plant-pollinator interaction networks.

POSTERS

P-04

The effect of temperature on olfaction in a moth revealed by its interaction with body mass

Thibaut Bagni, David Siaussat, Thomas Chertemps, Nicolas Montagné, Annick Maria, Annabelle Fuentes, Philippe Couzi, Manuel Massot
Institute of Ecology and Environmental Sciences of Paris, Sorbonne University, Paris, France

There is a growing interest in the effects of climate warming on olfaction, as temperature may affect this essential sense. In insects, the response of the olfactory system to developmental temperature might be mediated by body size or mass because body size and mass are negatively affected by developmental temperature in most ectotherms. We tested this hypothesis of a mass-mediated effect of developmental temperature on olfaction in the moth *Spodoptera littoralis*. We measured the olfactory sensitivity of males to female sex pheromone and five plant odors using electroantennography. We compared males reared at an optimal temperature (25ºC with a daily fluctuation of ± 5ºC) and at a high temperature (33 ± 5ºC) close to the upper limit of *S. littoralis*. On average, the olfactory sensitivity of males did not differ between the two developmental temperatures. However, our analyses revealed an interaction between the effects of developmental temperature and body mass on the detection of the six chemicals tested. This interaction is explained by a positive relationship between antennal sensitivity and body mass observed only with the high developmental temperature. Our results show that the effect of developmental temperature may not be detected when organism size is ignored.

P-10

Fungi in stored grain: Contamination detection at an early stage by application of an insect antenna as a biosensor

Laima Blažytė-Čereškienė[1], Violeta Apšegaitė[1], Irena Nedveckytė[1,2], Vincas Būda[1]
[1]Laboratory of Chemical and Behavioral Ecology, Nature Research Centre, Akademijos 2, 08412 Vilnius, Lithuania
[2]Institute of Biosciences, Life Sciences Centre, Vilnius University, Sauletekio Avenue 7, 10257 Vilnius, Lithuania

Mould contamination of stored grain spoils products and can be harmful to human health. Early detection of mould infection remains an important problem. Egg-laying tests revealed that mated females of *Plodia interpunctella* distinguished between *Aspergillus flavus* infected and uninfected wheat grains. GC-FID/EAD and GC-MS analysis revealed that olfactory receptors of *P. interpunctella* females responded to four VOCs emitted by fungi-uninfected grain (1-hexanol, nonanal, phenylacetaldehyde and 4-oxophorone), and to a single compound, 3-methyl-1-butanol, when grain were infected by a different fungi. 3-Methyl-1-butanol became detectable by GC-FID on the 3rd day after fungus inoculation, while the females avoided to lay eggs directly on inoculated grain as early as the 1st day after the grain inoculation. On the 3rd day after fungus inoculation oviposition suppression was recorded. The present data suggested a simple, rapid and highly sensitive method for determining mould contamination in grain (EP 3400438 B1). A comparative sensitivity study on 8 insect species of different taxonomic groups demonstrated that sensitivity of *P. interpunctella* to the 3-methyl-1-butanol was similar to that of the honeybee, *Apis mellifera*, while the highest sensitivity was noted in fruit fly *Drosophila melanogaster*. In view of this, of the 8 insect species tested, *D. melanogaster* antennae were the most suitable biodetector for revealing 3-methyl-1-butanol, marker volatile of grain contamination with fungi.

The study was funded by grant (Contract No SVE – 10/2014) from the Research Council of Lithuania.

P-63

Alarm calls of sagebrush coverage when herbivory is high

Richard Karban[1], Muhammad Usman Rasheed[2], Mikaela Huntzinger[1], Patrick Grof-Tisza[3], James D. Blande[2]
[1]University of California, Davis, USA
[2]University of Eastern Finland, Kuopio, Finland
[3]University of Neuchâtel, Neuchâtel, Switzerland

Herbivory is a major threat to survival and reproduction for virtually all plants, so adaptations to avoid herbivory will generally be selected for. One potential adaptation is the ability to "listen in" on the volatile cues emitted by plants that are experiencing herbivory and to then respond by ramping up defenses. The nature of these volatile cues is poorly understood. Sagebrush plants that are exposed to cues of experimentally damaged neighbors experienced less herbivory over the growing season; this induction was most effective if emitter and receiver plants had similar volatile emission profiles, termed chemotypes. Previously, we observed that sagebrush populations that were in locations with high herbivory exhibited little diversity of volatile cues compared to populations with low herbivory. Two different hypotheses could explain this result. First, high risk of herbivory could select for individuals that converged on a common "alarm cue" that all individuals would respond to. In this case, individuals of rare chemotypes that were less able to eavesdrop would experience more damage than common chemotypes. Alternatively, low chemotypic diversity among sagebrush could allow herbivore populations to increase. In this case, rare chemotypes would experience less damage than common chemotypes when herbivores were common. We examined the chemotypes of sagebrush individuals from sites on the east side of the Sierra Nevada range and found that rare chemotypes experienced more damage than common chemotypes when herbivores were abundant. This result is consistent with the hypothesis that herbivory selects individuals that are effective communicators and shapes the communication system.

S16: Chemical and molecular ecology of multitrophic interactions

Chairs: *Ricardo A. R. Machado, Christelle A. M. Robert*

ORAL PRESENTATIONS

Ecology and evolution of bacterial bioluminescence in belowground ecosystems

Arthur Muller[1*], Patricia Morales-Montero[1*], Anja Boss[1*], Alexandre Hiltmann[1], Carlos Castaneda-Alvarez[1], Aashaq H. Bhat[1], Carla C. M. Arce[2], Gaetan Glauser[3], Susan A. Joyce[4,5], David J. Clarke[4,6], Ricardo A. R. Machado[1*]
[1]Experimental Biology Group, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland
[2]Laboratory of Fundamental and Applied Research in Chemical Ecology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland
[3]Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Neuchâtel, Switzerland
[4]APC Microbiome Ireland, University College Cork, Cork, Ireland
[5]School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland
[6]School of Microbiology, University College Cork, Cork, Ireland
**These authors contributed equally to this study*

Apart from its entomopathogenic abilities, an intriguing biological trait of the bacterial genus *Photorhabdus* is the production of bioluminescence. Bioluminescence is the chemical production and emission of light by living organisms. This trait has evolved multiple independent times 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 prey. 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 soil ecosystems.

Induction of leaf necrosis by molecules of butterfly egg-associated secretions

Nina E. Fatouros[1], **Lotte Caarls**[1,2]
[1]Biosystematics Group, Wageningen University and Research, Wageningen, the Netherlands
[2]Laboratory of Plant Breeding, Wageningen University and Research, the Netherlands

Plants perceive and respond to herbivore insect eggs. Upon egg deposition on leaves, a strong hypersensitive response (HR)-like necrosis can be activated leading to egg desiccation and/or dropping. In *Brassica* spp., including many crops, the HR-like mechanism against butterfly eggs (Pieridae) is poorly understood. We studied the cellular and molecular plant response to *Pieris* eggs and characterized potential insect egg-associated molecular patterns (EAMPs) inducing HR-like necrosis. We found that eggs induce typical hallmarks of early immune responses, such as callose deposition, production of reactive oxygen species and cell death in *B. nigra* and *B. rapa* leaf tissue, also in plants that did not express HR. Only eggs and egg wash from *Pieris* spp. and the close relative *Anthocharis cardamines* contain compounds that induce cell death, but eggs of non-brassicaceous feeding Pieridae or distantly related butterflies and moths did not. Furthermore, wash made from hatched *Pieris* eggs, egg glue, and accessory reproductive glands (ARG) that produce this glue, induced HR, whereas washes from unfertilized eggs dissected from the ovaries or removal of the glue from eggs resulted in no or a reduced response. This suggests that there is one or multiple egg-associated molecular patterns (EAMPs) located in the egg glue. Lastly, our results indicate that the EAMP is neither lipidic nor proteinaceous. Chemical analysis is still ongoing and points to an organic compound of low molecular weight.

Is oilseed rape specific glucosinolates content a key cue for larval infestation of *Psylliodes chrysocephala* in field conditions?

Laurie Magnin[1], **Alexandra Jullien**[2], **Alice Baux**[1], **Ivan Hiltpold**[3]
[1]Cultivation Techniques and Varieties in Arable Farming, AGROSCOPE, Nyon, Switzerland
[2]EcoSys, UMR INRAE AgroParisTech, Saclay, France
[3]Entomology and Nematology, AGROSCOPE, Nyon, Switzerland

The cabbage stem flea beetle (CSFB – *Psylliodes chrysocephala*) is among the main pests in winter oilseed rape (OSR – *Brassica napus*) across Europe. Specific glucosinolates compounds found mainly in *Brassicacea* plants are feeding stimulant for the CSFB specialist insect and have an impact on their larval development. However, it remains unclear if glucosinolates profile (GP) is a key cue for females' host acceptance to lay their eggs in proximity of a chosen plant. Increasing evidence of the reduction of CSFB attacks by OSR-mixed cropping systems were provided in recent years. The impact of genetic variability and companion plant on OSR's GP and its possible implication in CSFB host acceptance were investigated.

Three OSR varieties were grown in microplots with or without Fababeans intercropping, in a field trial with four replicates. GP of leaves from three samples per microplots were analyzed before CSFB infestation and Berlese traps were done on the sampled plants in winter to evaluate the number of larvae.

The number of larvae per plant was positively correlated with the concentration of glucoraphanin and of butyl-glucosinolate and negatively correlated with glucobrassicanapin's concentration. The varietal factor was the main driver of the variability in GP among the surveyed plants. Mixed cropping had an effect on some specific glucosinolate concentrations and its effect was variety-dependent. However, only the genetic factor had a significant influence on the OSR infestation. We brought evidence of the impact of specific glucosinolates on host acceptance of CSFB in field conditions and its variability among cultivars.

Impact of faba bean on oilseed rape glucosinolates profile and cabbage stem flea beetle foraging behavior

Léa Bolis[1,2], **Sergio Rasmann**[1], **Ivan Hiltpold**[2]
[1]*Faculty of Biology, University of Neuchâtel, 2000 Neuchâtel, Switzerland*
[2]*Entomology and Nematology, Plant Protection Strategic Research Division, Agroscope, 1260 Nyon, Switzerland*

Psylliodes chrysocephala, commonly referred to as the cabbage stem flea beetle (CSFB) is one of the most important pest of oilseed rape (OSR). Neonicotinoid bans in central Europe coupled with multiple emergences of resistance to other classes of insecticides in this pest drives the need for efficient alternative management measures. Recent studies have shown that intercropping OSR with faba bean helps decrease CSFB infestation and damage under field conditions. Underlying mechanisms behind such effects are not yet understood but could potentially be the source of novel environmentally friendly management strategies against this pest. Glucosinolates (GLS) are secondary metabolites mainly found in brassicaceous plants which breakdown products act as defense compounds against a wide variety of pests and pathogens. However, GLS can also act as phagostimulants in specialist insects such as CSFB, which have evolved resistance against their toxicity and use them as foraging cues. In this study, we investigated how GLS levels and profiles are altered before and after herbivory in OSR plants grown alone or intercropped with different faba bean varieties under laboratory and field conditions. We further assessed how intercropping treatments affected CSFB foraging behavior in a choice-test experiment with leaf discs. We show that intercropping OSR with faba bean tends to generally decrease GLS amounts present in OSR plants, whilst also being associated with reduced damage caused by CSFB feeding adults. Our data suggest that GLS profiles can be manipulated by intercropping OSR, which negatively affects CSFB behavior and offer sustainable pest management options.

Wound excretions in *Solanum atropurpureum*: A multifaceted defense against *Manduca sexta* herbivory

James W. Sims[1], Rupesh Kariyat[2], Mark Mescher[1], Consuelo De Moraes[1]
[1]Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland
[2]Entomology and Plant Pathology, University of Arkansas, Fayetteville, AR, USA

Plants employ diverse defenses against insect herbivory, encompassing both physical and chemical strategies. This study investigates the defensive mechanisms of *Solanum atropurpureum* (Purple Devil) against the herbivory of *Manduca sexta*, a Solanaceae specialist. Previous observations revealed that the spined stem of the Purple Devil impedes defoliation by *M. sexta*. We observed caterpillar feeding induces the formation of sticky exudate globules at wound sites. These excretions, primarily composed of monosaccharides and sucrose, provide an additional layer of physical defense by binding the herbivore's mouthparts and appendages. This response is instar-specific, with early instars inducing exudate formation while late instars do not unless plants are treated with salicylic acid (SA). Additionally, early instar caterpillars reared on an artificial diet supplemented with wound excretions had reduced weight gain, suggesting a potential chemical defense mechanism. Our findings suggest that in addition to the physical defense of spines, *S. atropurpureum* employs an instar-specific inducible defense mechanism, combining both physical and chemical strategies, when challenged with *M. sexta*.

Contradictory effect of foliar extracts of a wild Brassicaceae species on the feeding behavior of *Psylliodes chrysocephala* larvae and adults

Thomas Giguère[1,2], Christophe Lunel[1], Théo Cariou[1], Maël Lepage[1], Maxime Hervé[1], Anne Marie Cortesero[1]
[1]IGEPP (Institute for Genetics, Environment and Plant Protection), University of Rennes, Rennes, France
[2]De Sangosse, Pont-du-Casse, France

The cabbage stem flea beetle, *Psylliodes chrysocephala* (Coleoptera: Chrysomelidae), is an insect specialized on the Brassicaceae family that causes significant damage to oilseed rape crops in Europe. To develop a new control strategy for *P. chrysocephala*, one interesting approach is to identify deterrent or stimulant compounds through the study of the chemical diversity within the Brassicaceae family. Through the screening of multiple brassicaceous species from various lineages, we identified a wild Brassicaceae species that is rejected by both adults and larvae of *P. chrysocephala*. We therefore investigated the relationship between this species' chemistry and *P. chrysocephala* feeding, both at the larval and adult stage. A bioguided-fractionation approach was followed: foliar compounds were extracted and fractionated, and then tested in a series of feeding tests performed on agar disks. Foliar extracts were found to have conflicting effects on adults and larvae. Indeed, they act as a phagostimulant for larvae while being deterrent for adults. We then tested the effect of surface extracts on feeding in both stages, excluding any potential effect of glandular trichomes and surface compounds. Based on these results, it can be inferred that larvae primarily reject this plant due to its physical defenses (i.e. non-glandular trichomes or cuticle), which is not the case for adults. Overall, this study offers novel insights into the potential differences in the mechanisms driving host preference patterns between larval and adult stages in specialist insects.

Dual nematode infection in *Brassica nigra* affects shoot metabolome and aphid survival in distinct contrast to single-species infection

Jessil Ann Pajar,[1,2,3,4] **Pius Otto**,[3] **April Lyn Leonar**,[3] **Stefanie Döll**[3], **Nicole M. van Dam**[1,3,4]
[1]Leibniz Institute for Vegetable and Ornamental Crops (IGZ) e.V., Großbeeren, Germany
[2]Max Planck Institute for Chemical Ecology, Jena, Germany
[3]Molecular Interactions Ecology, German Centre for Integrative Biodiversity Research (iDiv), Jena-Halle-Leipzig, Germany
[4]Institute of Biodiversity, Friedrich-Schiller-University, Jena, Germany

Previous studies showed that aphid performance was compromised on *Brassica nigra* infected by root-lesion nematodes (*Pratylenchus penetrans*, Pp), but less, or positively influenced by root-knot nematode (*Meloidogyne* spp., Mi) infection. These experiments were on single-nematode infections, yet in nature roots are infected with several nematode species simultaneously.

We performed greenhouse assays to assess the effects of single (Mi, Pp) and concurrent (MP) nematode infections on aphid performance. Using targeted biochemical analyses and untargeted profiling of leaf and phloem metabolomes, we addressed the question how single- and concurrent-nematode infections affect leaf and phloem metabolomes, and elucidated their consequence on aphid performance. We found that the metabolic response towards double infection is different from single-species infections. Moreover, Mi- and Pp-infections triggered discrete changes in *B. nigra* leaf and phloem metabolic profiles. The nematode-induced metabolic profile shifts in plant shoot distinctively influenced aphid survival. Both Pp and MP-infection reduced aphid survival, suggesting that the biological effect was primarily dominated by Pp-induced changes. This concurred with increased indole glucosinolates and hydroxycinnamic acid levels in the leaves, in particular the involvement of salicylic acid-2-O-β-D-glucoside.

This study provides evidence that concurrent infection of different nematode species, as is common in natural environments, distinctly alters aboveground plant metabolomes, thereby affecting the survival of an aboveground herbivore.

The polyvalent sequestration ability of an economically important beetle

Carla C. M. Arce[1], **Ricardo A. R. Machado**[2], **Marine Mamin**[1], **Gaétan Glauser**[3], **Pamela Bruno**[1], **Betty Benrey**[4], **Matthias Erb**[5], **Christelle A.M. Robert**[6], **Ted C. J. Turlings**[1]
[1]*University of Neuchâtel, Institute of Biology, FARCE laboratory, Neuchâtel, Switzerland*
[2]*University of Neuchâtel, Institute of Biology, Experimental Biology Group, Neuchâtel, Switzerland*
[3]*University of Neuchâtel, Neuchâtel Platform of Analytical Chemistry, Neuchâtel, Switzerland*
[4]*University of Neuchâtel, Institute of Biology, E-vol laboratory, Neuchâtel, Switzerland*
[5]*University of Bern, Institute of Plant Science, Biotic Interaction Group, Bern, Switzerland*
[6]*University of Bern, Institute of Plant Science, Chemical Ecology Group, Bern, Switzerland*

Many specialized herbivorous insects sequester toxic secondary metabolites from their host plants as protection against natural enemies. Polyphagous herbivores are not known to sequester toxins, but rather to avoid them or otherwise neutralize their effects. We here show that the polyphagous beetle *Diabrotica virgifera virgifera* is uniquely different. Adult beetles were found to sequester benzoxazinoids, cucurbitacins and glucosinolates when respectively fed on maize, cucumber, and cabbage plants, but not cyanogenic glucosides from bean plants. The beetles transferred the sequestered defense metabolites to their eggs, and in choice experiments, two predators, *Dalotia coriaria* and *Orius laevigatus*, consumed more toxin-free than toxin-containing eggs and the combined presence of the metabolites showed a synergistic effect on egg protection. Survival assays confirmed the toxicity of sequestered benzoxazinoids and glucosinolates for the predators but cucurbitacins had little effect. The extraordinary capacity of the beetle to sequester multiple plant metabolites as a defense against higher trophic levels may contribute to its remarkable success as an invasive pest.

Reinterpreting the use of anti-predator chemicals as parasite protection in aposematic monarch butterflies

Jacobus C. de Roode
Emory University, Atlanta, Georgia, USA

Monarch butterflies are a textbook example of an aposematic species: with their conspicuous orange, black and white coloration, they warn would-be predators that they are distasteful and toxic. Monarchs do not produce toxins themselves, but derive them from the milkweed host plants on which they feed as larvae. The sequestration of these chemicals, called cardenolides, has long been interpreted as a predator defense alone. However, recent work has shown that these same chemicals provide protection against a highly detrimental and common protozoan parasite as well. In addition, when given a choice, monarch butterflies preferentially lay their eggs on milkweeds with higher concentrations and toxicity of cardenolides, thereby providing protection against infection in their offspring. I will summarize the medication behaviors of monarchs, and present experiments that show that cardenolides may directly interfere with parasites or indirectly increase parasite resistance by modulating monarch immunity and the gut microbiome. I will discuss how these findings, combined with the protection against parasites by toxic chemicals in many other aposematic animals, may provide new insights into the evolution of warning coloration.

Sequestration of prey chemicals for defence by ladybird beetles (Coleoptera: Coccinellidae)

John J. Sloggett
Maastricht Science Programme, Maastricht University, Maastricht, the Netherlands

Most ladybird beetles chemically defend themselves against natural enemies using autogenously produced alkaloids. In a few cases, it has been suggested that predatory ladybirds may sequester, for defence, additional chemicals from aphid or coccid prey, and ultimately from the prey host plant. However, known cases may be observational or partial, and open questions remain about the balance of benefits (i.e. whether such compounds confer additional defensive benefit over autogenous defence) and costs (e.g. toxicity of such compounds to the ladybird). I here discuss this conundrum and present pertinent new data from two potential examples of sequestration by ladybird beetles from prey. Is sequestration a valuable part of the ladybird chemical defensive armoury or a side effect of eating marginally suitable, chemically defended prey?

Honest Signalling? Testing the relationship between chemical and visual components of antipredator defence

Emily Burdfield-Steel[1,3], **Cristina Ottocento**[2,3], **Miriam Furlanetto**[3], **Bibiana Rojas**[3,4], **Ossi Nokelainen**[2,3], **Johanna Mappes**[2,3]
[1]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands
[2]Organismal and Evolutionary Biology Research Program, University of Helsinki, Helsinki, Finland
[3]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[4]Department of Interdisciplinary Life Sciences, Konrad Lorenz Institute of Ethology, University of Veterinary Medicine Vienna, Vienna, Austria

Insects use chemical defences to deter a large variety of potential predators. Often these defences are advertised with bright warning signals. However, the relationship between visual signal and chemical defence can vary, stronger signals may be an honest indicator of stronger defences, but equally the two traits may trade-off – particularly if they draw on the same resource pool. Here, we test this hypothesis using the aposematic wood tiger moth *Arctia plantaginis*, which produces methoxypyrazines known to deter bird predators. The moths' red hindwings act as a signal to predators but show considerable variation within populations. Using dietary manipulations, GC-MS, and experiments with ecologically-relevant predators we demonstrate that protein availability during development can influence the strength of both the warning signal and the secondary defence. Female moths raised on high-protein diet and ad libitum natural diet had more distasteful defensive fluids to predators than those raised on low-protein diet, or periodically food deprived. The visual appearance of the warning signal on the hindwings was not sensitive to food deprivation, but was reduced in moths raised on a low-protein diet. Finally, resource availability influences the relationship between signal and defence: moths from the high-protein diet showed a positive correlation between warning signal strength and the unpalatability of their defensive fluids, while in moths raised on a natural diet this correlation was not observed. These findings show the complex interactions between chemical and visual defences.

Volatile organic compound patterns emitted by *Halyomorpha halys*, and herbivory interact to alter volatile emissions in plant hosts

Ali Karimi, Jürgen Gross
Institute for Plant Protection in Fruit Crops and Viticulture, Julius Kühn-Institut, Federal Research Institute for Cultivated Plants, Dossenheim, Germany

The brown marmorated stink bug (BMSB), *Halyomorpha halys*, is a polyphagous plant pest with negative effects on agricultural crops. A strategy to detect the BMSB in shipping containers or field sites is monitoring this species using volatile organic compound (VOC) emitted by the insect and its plant hosts. This study aimed to investigate VOCs from BMSB and its plant hosts to identify species-specific signatures. Using an innovative dynamic headspace collecting device, VOCs emitted by BMSB from its different developmental stages including egg, nymphal stages, nymphal exuvia, and adults were collected on thermal desorption tubes and analyzed using ATD-GC-MS. Besides, we assessed the effects of BMSB feeding on VOCs emitted by pear and apple trees. According to our results, forty-five VOCs were identified in the BMSB samples, mainly consisted of tridecane, *E*-2-decenal, 2-undecenal, and *E*-4-*oxo*-2-hexenal. The random forest analysis revealed three different chemical patterns among BMSB life stages samples. Furthermore, our results indicate that feeding by BMSB adults induced changes in specific amounts of VOCs emitted by plant hosts. Pear trees exposed to the BMSB emitted copaene and α-muurolene at a higher concentration than control trees. Methyl salicylate and α-farnesene were emitted by apple trees in higher concentrations after BMSB feeding. In conclusion, this information may be used for identifying specific chemical signatures as biomarkers for on-site detection of this pest e.g. in shipping containers or agricultural areas.

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Herbivore-induced plant volatiles of maize: Signaling previous host plant colonization by *Dalbulus maidis* and *Spodoptera frugiperda*

Mateus Sanches[1,2], **Raul Laumann**[2], **Miguel Borges**[2], **Marina Frizzas**[1], **Charles Martins Oliveira**[3], **Maria Carolina Blassioli-Moraes**[2]
[1]*Semiochemicals Laboratory, Embrapa Genetic Resources & Biotechnology, Brasília, Brazil*
[2]*Graduate Program in Ecology, University of Brasília, Brasília, Brazil*
[3]*Embrapa Cerrados, Brasília, Brazil*

The corn leafhopper, *Dalbulus maidis*, and the fall armyworm, *Spodoptera frugiperda,* are considered important corn pests. Both prefer establishing in the maize whorl, although it is uncommon to find both insects on the same plant. This raised the hypothesis that herbivory induced plant volatile (HIPVs) could mediate interactions between these two pests, indicating the presence of potential competitors for feeding and oviposition sites. To check this hypothesis, volatiles compounds were collected from healthy maize and maize submitted to herbivory by *D. maidis* or *S. frugiperda.* Chemical analyses of the sample volatiles from the maize showed that there was a difference in the profile of volatile emitted by healthy and *D. maidis* feeding damaged maize plants ($p < 0.001$; PERMANOVA), and from maize with *S. frugiperda* herbivory ($p < 0.001$; PERMANOVA). In behavioral bioassays, *D. maidis* females showed a preference for volatiles from healthy maize over maize damaged by conspecifics ($p = 0.01$; chi-square) or by the fall armyworms ($p = 0.001$; chi-square). Caterpillars of *S. frugiperda* also prefer healthy maize over maize with herbivory by *D. maidis* ($p = 0.014$; chi-square). The results demonstrated that insects use plant volatiles to select suitable host, avoiding already colonized plants by other herbivores, and *D. maidis* females also seem to avoid intra-specific competition. The HIPVs released by maize could play a role in deterring colonization by other herbivores, thus reducing damage to the plant.

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Tritrophic interactions modulated by herbivore-induced coffee plant volatiles under single and multiple herbivory by phloem-feeding insects

Enggel Carmo[1], Arodí Favaris[2], José Mauricio Simões Bento[2], Maria Fernanda Peñaflor[1]
[1]Departamento de Entomologia, Universidade Federal de Lavras, Minas Gerais, Brazil
[2]Escola Superior de Agricultura Luiz de Queiroz, Departamento de Entomologia e Acarologia, Universidade de São Paulo, Piracicaba, Brazil

Plants are commonly attacked by multiple herbivores, altering the composition of herbivore-induced plant volatiles (HIPVs) compared to single infestations. The literature has shown that multiple herbivore infestations have unpredictable effects on the third trophic level, depending on the herbivores and their interactions with natural enemies. We hypothesized that co-infestation of plants with two phloem-feeding would increase the attractiveness of HIPVs to the natural enemy. We examined the prey preference of the ladybug *Cryptolaemus montrouzieri* between the white mealybug *Planococcus minor* and the green scale *Coccus viridis*, and which of the two is more likely to colonize first the coffee plant. We found that the ladybug preys on both species, however, it preferred *P. minor* in dual-choice assay. Moreover, neither insect demonstrated a preference for uninfested or non-conspecific infested plants, indicating a random arrival sequence on plant. In dual-choice olfactometer assays, *C. montrouzieri* preferred volatiles from singly-infested plants over uninfested plants, but did not differentiate between the mealybug and scale-infested plants. Surprisingly, HIPVs from multiple-infested plants were not attractive to the ladybug. GC-MS analysis revealed differences in volatile composition between mealybug-infested plant and other treatments, and multiple-infested emitted a similar blend as scale-infested plant. Thereby, our results refute our hypothesis, as multiple infestation did not increase the attractiveness of HIPVs to the natural enemy, despite both herbivores belonging to the same feeding guild and being targets of predation. These findings contribute to the discussion of multiple herbivory impact on induced plant defenses and its effects on the natural enemy attraction.

Role of odors in modulating host selection behavior of parasitoid wasps

Aswathi Sasidharan[1], Enakshi Ghosh[2], Radhika Venkatesan[1]
[1]Department of Biological Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, WestBengal 741246 India
[2]Graduate Degree Program in Ecology and Department of Agricultural Biology, Colorado State University, Fort Collins, CO, 80523-1177, USA

Host selection is crucial for parasitoid wasps, and odors play a significant role in this process. Parasitoid wasps utilize herbivore-induced plant volatiles as long-range cues to locate their hosts. Yet, these cues alone often do not lead to successful parasitization. Here, I will be presenting the influence of both long and short-range cues on the parasitization preferences of *Bracon brevicornis*, a generalist ectoparasitoid, and *Cotesia vestalis*, a specialist endoparasitoid. Our findings indicate that parasitoids can detect larval body odor at close range, enabling them to select the most suitable host. This larval body odor originates from plant metabolites and vary depending on the larvae's diet. In our study, *Plutella xylostella* feeding on *Brassica juncea* plants released isothiocyanates whereas sulfides were detected in the larval body odor when fed on *B. oleracea*. Additionally, these odors served as indicators of the insect's immune status, aiding parasitoids in choosing an appropriate host. This selection is critical as parasitizing an unsuitable host showed negative fitness consequences for the wasp. Further, we explored whether parasitoids can differentiate between Bt-infected and healthy hosts, confirming that they can indeed make this distinction through olfactory cues. Overall, our study underscores the essential role of various odors—from both plants and host larvae—in shaping the behavior of parasitoids. These results not only deepen our understanding of the intricate dynamics of host-parasitoid interactions but also hold potential for developing new pest control strategies that safeguard beneficial parasitoids in sustainable agriculture.

Odour hunters: Deciphering the impact of egg-emitted VOCs on host recognition *in Trissolcus basalis* and *T. japonicus* (Hymenoptera: Scelionidae)

Anaïs Sion[1,2], Ivan Hiltpold[2], François Verheggen[1], Diana la Forgia[2*]
[1]Chemical and Behavioral Ecology, Gembloux-Agro-Bio-Tech, TERRA, University of Liège, 5030 Gembloux, Belgium
[2]Entomology and Nematology, Plant Protection Strategic Research Division, Agroscope, 1276 Nyon, Switzerland

Stink bugs can be major agricultural pests causing significant economic losses. In particular, *Halyomorpha halys* and *Nezara viridula* are particularly problematic.

We evaluated the ability of two parasitoids, *Trissolcus japonicus* and *T. basalis*, to exploit short-range cues and volatile organic compounds (VOCs) emitted by stink bug eggs masses, to locate their hosts.

We hypothesized that (1) stink bugs eggs emit short-range cues that are exploited by egg parasitoids (*T. japonicus* and *T. basalis*) to locate their hosts in addition to insect chemical footprints, and that (2) the VOC blend varies according to egg age (3) parasitoids would use egg cues over indirect insect cues to locate the eggs.

We identified γ-butyrolactone and β-funebrene in the headspace of eggs of *N. viridula*. The rate of parasitism was not different between young and old eggs for *T. japonicus*, in contrast with *T. basalis* that showed preference for young eggs in contrast to *T. japonicus*. In Y-olfactometer assays, we showed that *T. japonicus* exhibited no preference between younger and older eggs, while *T. basalis* preferred younger eggs. *T. japonicus* was not attracted to footprints, whereas *T. basalis* significantly oriented toward the footprints of stink bug females. Double choice assays showed that both parasitoids oriented toward young eggs over footprints.

Our data suggest that egg parasitoid discriminate younger from older egg masses, showing that short range host search by *Trissolcus* sp. is not only depending upon footprints but also upon VOCs emitted by the eggs. These findings contribute to understanding of host-parasitoid dynamics.

Variation in the post-mating odors of *Pieris napi* butterflies is associated with variation in natural selection by eavesdropping egg parasitoids

Liana Greenberg[1], Astrid Groot[2,] Eric Schranz[1], Bas Zwaan[3], Bart Pannebakker[3], Nina Fatouros[1]
[1]Biosystematics Group, Wageningen University, Wageningen, the Netherlands
[2]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, the Netherlands
[3]Laboratory of Genetics, Wageningen University, Wageningen, the Netherlands

The odors emitted by mated female butterflies alert conspecific males of their mating status but also inform their natural enemies. *Pieris napi* females that have recently mated emit the volatile compound methyl salicylate as an antiaphrodisiac pheromone (AA), which discourages subsequent mating attempts until the female is again receptive. Tiny *Trichogramma* egg parasitoids are known to exploit the AAs of *P. brassicae* and *P. rapae* as host-finding cues. The wasps hitchhike with the odorous female butterflies to host plants where they dismount to parasitize her freshly laid eggs. This interaction likely contributes to the eco-evolutionary dynamics of these post-mating odors. To investigate, we conducted a comprehensive study of the rapidly evolving species *P. napi* across six distant sites in Westernern Europe. By assessing their odor profiles, population structure, parasitism rates, and their parasitoids' preferences, we uncovered intriguing insights. Contrary to expectations, *P. napi* post-mating odors are not species-specific. Alongside an increase in methyl salicylate, mated females emitted similarly increased amounts of benzyl cyanide (*P. brassicae* AA), indole (part of *P. rapae* AA), and guaiacol compared to unmated females. We discovered intra-specific variation in post-mating odors, with site-specific blends of these four compounds. Chemical distance is associated highly divergent parasitism rates but not with geographic nor genomic distance. Finally, we found that only mated butterflies from certain sites are attractive to *T. cacoecea*. By underscoring complexity and the influence of natural enemies, our findings challenge assumptions about the drivers and constraints of post-mating odor evolution.

Root volatiles and effects of Rhizobium-bean symbiosis on the preference and performance of a root herbivore

Camilo Rivera[1], **Tom Wakley**[1], **Carlos Bustos-Segura**[1,2], **Betty Benrey**[1]
[1]Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland
[2]Sorbonne Université, Institut National de Recherche pour L'Agriculture, L'Alimentation et L'Environnement, CNRS, Institut de Recherche pour le Développement, Université Paris-Est-Créteil-Val-de-Marne, Université Paris Cité, Institut D'Ecologie et des Sciences de L'Environnement de Paris, Versailles, 78026, France

Understanding the dynamics of belowground plant-insect interactions is essential for elucidating the factors that enhance ecosystem resilience. Nitrogen fixating bacteria (NFB) play a crucial role in this process, for nodules known as Rhizobia, facilitating the conversion of atmospheric nitrogen into soil nutrients in plant roots. This symbiotic relationship not only influences the plant's nutrient uptake but also has cascading effects on soil organisms. We investigated the impact of root volatiles in a bean-rhizobium symbiosis on the preference and performance of root-larvae of the beetle *Diabrotica balteata*. Larvae were exposed to roots of *Phaseolus vulgaris* with and without rhizobium nodules, and choice tests were conducted to assess feeding preferences. In addition, we compared the nutritional value of roots and nodules, both separately and combined, and evaluated their influence on larval growth. Further on we examined the impact of nodulated and non-nodulated root volatiles on the larval feeding preference. Our experiments demonstrated a significant preference of *D. balteata* larvae for nodulated roots, suggesting the presence of attractant cues associated with nodulation. Moreover, larvae feeding on nodulated roots exhibited faster growth and development, indicating the nutritional benefits of rhizobial symbiosis. Analysis of volatile compounds emitted by roots revealed differences in composition between nodulated and non-nodulated roots, influencing larval behavior. Our findings underscore the crucial role of NFB on belowground plant-insect interaction, involving *P. vulgaris* and *D.balteata,* highlighting the importance of volatile emission in this interaction.

Direct and indirect effects of rhizobia-bean symbiosis on higher trophic levels

Carlos Bustos-Segura[1,2], Adrienne L. Godschalx[1], Lucas Malacari[1], Fanny Deiss[1], Daniel Ballhorn[3], Sergio Rasmman[1], Betty Benrey[1]
[1]University of Neuchatel, Institute of Biology, Neuchatel, Switzerland
[2]INRAE, Versailles, France
[3]Department of Biology, Portland State University, Portland, OR, USA

Plants from the Fabaceae family establish belowground mutualistic interactions with nitrogen-fixing bacteria. Although it is known that this symbiosis benefits plant development, less is known about its consequences for plant defenses and multitrophic interactions. In this study we explored the effects of rhizobia symbiosis with bean plants on plant direct and indirect defenses, the interactions with herbivorous caterpillars and their parasitoids. We found that rhizobia had an effect on plant chemical defenses, however caterpillars performed best in plants with rhizobia, due to a more nutritious leaf tissue. Induction of plant volatiles and production of extrafloral nectar was also changed by the symbiosis. The parasitoid behaviour was not influenced by the change in plant volatiles but their performance was improved by the rhizobia interaction through the caterpillar hosts and the plant extrafloral nectar. This work unveils how the effects of a mutualistic interaction extend beyond their main interactors and cascade to other trophic levels within a complex ecological context.

Chemical communication in the plant microbiota and its impact on plant health

Sébastien Bruisson, Abhishek Anand, Alsayed Alfiky, Floriane L'Haridon, Laure Weisskopf
[1]*Department of Biology, University of Fribourg, Fribourg, Switzerland*
[2]*Food Research and Innovation Center, University of Fribourg, Switzerland*

Plants are colonized by a diverse microflora which contributes to plant health in many ways. We are interested in understanding how the plant microbiome contributes to the health of their hosts and use crops of agronomical relevance such as potato and grapevine to answer this question. We focus specifically on the role of volatile compounds emitted by plant-associated bacteria, which have strong impact on plant health through direct inhibition of pathogenic microbes or through induction of plant disease resistance. Beyond their roles in plant-microbe interactions, these volatile metabolites can have substantial impact on microbe-microbe interactions. Recent studies indicate that plant-associated bacteria and fungi can detect the presence of competing microbes via their volatile emission and react by upregulating antimicrobial volatile emission and/or siderophore production. In turn, some of the volatile chemical signals emitted by beneficial root-associated *Pseudomonas* can remotely influence the behavior of other rhizosphere inhabitants, leading to diminished siderophore production, reduced motility and increased biofilm formation. These modulated traits are of relevance for both the ability of the microbes to successfully colonize their host plant and to competitively inhibit plant pathogens. These new findings lift the veil on the complex chemical communication taking place within the plant microbiota, which can be mediated by both volatile and non-volatile signaling compounds. Understanding the basis of this communication and identifying the chemical signals leading to up- or downregulation of biocontrol-relevant traits such as siderophore production or emission of antimicrobial volatiles in plant-associated bacteria will open significant avenues for improved crop protection strategies.

POSTERS

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Host plant chemical response to oviposition by damselfly (*Lestes*)

Radana Chytilová, Aleš Dolný, Jana Koller
Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic

Plants respond to oviposition by herbivorous insects by triggering a signal cascade similar to that which is well-known following injury to plant tissue. The eggs of herbivorous insects, as immobile and seemingly inactive stages, have been intensively studied over the last three decades when considering plant-herbivore interactions. However, it is still not known how a plant responds to oviposition by an animal that does not potentially threaten it but instead helps it figuratively by consuming herbivorous insects. Dragonflies are powerful predators that consume hundreds of thousands of those insects. From an evolutionary point of view, the question arises as to whether the plant adapts its response to the oviposition of an insect predator such as a dragonfly, potentially protecting the plant from insects living in the plant tissue. Our preliminary case study was performed to verify methods, proving that monitoring hormonal responses is feasible and repeatable. The study analyzed the phytohormonal response of the plant common rush (*Juncus effusus*) to oviposition by the damselfly (*Lestes sponsa*) through quantitative analysis of stress phytohormones and cytokinins by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC). However, individual hormones and their crosstalk need more molecular and genetic data for a better understanding of the precise involvement of these pathways.

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Baculovirus manipulation modifies odour-guided behaviour in *Spodoptera exigua*

Kofi Ahadjie, Samia Mokh, Jordi Gamir
Plant Immunity and Biochemistry group, Department of Biology Biochemistry and Natural Sciences, Universitat Jaume I, Avd. Vicente Sos Baynat s/n, 12071 Castelló, Spain

A suit of parasites and viruses have been described to manipulate animal behavior, leading to their increased dispersal. Baculoviruses are dsDNA viruses that infect lepidopteran larvae, changing their behaviour to maximise viral spread. This includes enhanced locomotor activity (ELA) of infected caterpillars and/or movement to the top branches or leaves before death (called tree-top disease). However, the exact mechanisms that underlie these behavioural alterations remain elusive. Similarly, there is little information on other ways in which BVs manipulate caterpillar behaviour. Here, we hypothesize that BV might be modifying the olfactory system and chemical profile of *Spodoptera exigua* caterpillars. We will examine how the virus manipulates caterpillar behaviour in the presence of plant-derived odours and explore the possibility that infection manipulates behaviour through indirect ways by altering chemical profiles in the caterpillar. We will identify volatiles differentially emitted using TD-GC-MS, employ the two-choice assays, and track behavioural assays. We will determine which chemical compounds identified are involved in BV-induced behavioural shifts.

P-59

Beneficial microbes against arthropod pests in sustainable pepper production

G. Koutsoula, G. D. Broufas, M. L. Pappas
Department of Agricultural Development, Democritus University of Thrace, Orestiada, Greece

Plants are continuously challenged by biotic stressors. Nevertheless, plants also interact with beneficial organisms such as soil microbes which are known to improve plant growth, antagonize pathogens and prime plants against future attackers via plant defense elicitation. In our attempt to identify sustainable pest control tools to substitute chemical control, we assessed the hypothesis that soil microbes can adversely affect aboveground pests of pepper via the elicitation of plant-mediated responses. We recorded the plant-mediated effects of a series of beneficial soil fungi against key pests of pepper namely, the spider mite*Tetranychus urticae*, the aphid *Myzus persicae* and the whitefly *Trialeurodes vaporariorum*, as well as their natural enemies. Our results show that inoculating plants with different fungal strains can differentially affect herbivore populations via the plant, as well as the behavior of their natural enemies. As a next step, in the context of the ECOBOOST project we are studying the molecular and chemical mechanisms underlying the most promising identified beneficial microbe-pepper-herbivore interactions. Our results highlight the role of soil microbes in suppressing herbivore populations, possibly via the induction of plant defenses. Furthermore, it aims to identify specific molecular and chemical components of pepper direct and indirect defense that are differentially affected by pepper inoculation with the promising microbes.

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P-60

Characterization of volatiles and metabolome of domestic strawberry genotypes for the management of the red spider mite

Florencia Parpal[1], **Paulina Barrios**[1], **EstebanVicente**[2], **M. Eugenia Lorenzo**[3], **Andrés López**[1], **Andrés González**[4], **Carmen Rossini**[4]
[1]Departamento de Química del Litoral, Cenur Litoral Norte, UdelaR, Uruguay
[2]INIA Salto Grande, Uruguay
[3]Departamento de Ciencias Biológicas, Cenur Litoral Norte, UdelaR, Uruguay
[4]Laboratorio de Ecología Química, Facultad de Química, Udelar, Uruguay

Strawberries (Fragaria × ananassa Duch.) are widely recognized for their profitability, esteemed by the population, and exert significant socioeconomic influence in Uruguay. The development of resistant varieties of this fruit is crucial in our country and plays a substantial role in Integrated Pest Management (IPM) programs. The red spider mite *Tetranychus urticae* (Acari: Tetranychidae) poses a considerable threat to protected strawberry crops, underscoring the necessity for targeted control measures. This project aims to develop management resources for *T. urticae* in strawberry crops using chemical ecology tools. To achieve this, we will first characterize the profile of volatile organic compounds (VOCs) emitted by different strawberry plants and correlate this information with *T. urticae* preference. Currently, we are quantifying the relative amounts of VOCs emitted by diverse strawberry genotypes across various phytosanitary states, including those with and without *T. urticae* damage. Through multivariate analysis, we seek to identify characteristic components of different genotypes under varied health conditions. Concurrently, we will establish methodologies for identifying *T. urticae*-resistant strawberry varieties via NMR-based metabolomic analysis. This involves obtaining NMR metabolic profiles from leaf material extracts of different strawberry genotypes. The comparison of leaf metabolomes, categorized as preferred/susceptible by the genetic improvement program, will be performed using unsupervised multivariate statistical analysis such as PCA. Furthermore, supervised multivariate analysis techniques like PLS-DA and OPLS-DA will aid in developing classification models. These models will be subsequently correlated with data from genotypes undergoing *T. urticae* infestation, enabling the identification of metabolites closely linked with resistance.

P-78

Repellent from *Persicaria chinensis* against pillbug

Shin-ichi Tebayashi[1], Masashi Ishida[1], Wakana Isoda[1], Haruki Abe[1], Muhammad Agung Wardiman[1,2], Sylvia Sjam[2], Masashi Sato[3]
[1]Faculty of Agriculture and Marine Science, Kochi University, 200B Monobe, Nankoku, Kochi 783-8502, Japan
[2]Plant Pests and Diseases Department, Faculty of Agriculture, Hasanuddin University, Makassar 90245, Indonesia
[3]Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, 2393 Ikedo, Miki, Kagawa 761-0795, Japan

The development of repellents as alternatives to insecticides has expanded in recent years. However, their use in isopod pest control is limited. To develop an isopod repellent, a plant extract library from wild plants native to the Kochi Prefecture in Japan was screened for pillbug repellency. Methanol extracts of *Persicaria* (Polygonaceae) exhibited strong repellent activity. Among them, the non-pungent plant, *P. chinensis*, showed stronger repellent activity than the pungency herbal plant, *P. hydropiper*, which contained polygodial displaying deterrent activity against pest insect. Therefore *P. chinensis* was selected to isolate and identify repellent substances.

Test plants were collected in Kochi Prefecture and extracted with 90% MeOH-H_2O. And extracts was used for evaluating repellency against pillbug which was collected from the Monobe campus of Kochi University. A methanol extracts from *P. chinensis* was purified by liquid-liquid partition, and then the active substance was isolated from the aqueous layer by recrystallization. The isolate compound was evaluated a repellency and was instrumental analyzed by NMR and LC-MS.

The methanol extract of *P. chinensis* showed the strongest repellency among *Persicaria* plants tested. The active substance isolated by recrystallization from the aqueous layer on liquid-liquid fractionation was performed spectrum analyses and comparison with reference materials. Resulting, the repellent was identified as oxalic acid showed strong repellent activity of approximately 100% at 200 ppm.

No potential conflicts of interest are reported by the authors.

S17: Open symposium

Chair: *Monica Barman*

ORAL PRESENTATIONS

Caterpillars coping with chlorophyll

Vincensius Oetama, Sabine Haenniger, Riya Menezes, Wilhelm Boland, David G. Heckel
Departments of Bioorganic Chemistry and Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany

Most herbivorous lepidopteran larvae ingest large amounts of chlorophyll. Free chlorophyll and its tetrapyrrole breakdown products could be phototoxic in the presence of oxygen, due to the light-driven generation of reactive oxygen species. We examined larvae of the cotton leafworm *Spodoptera littoralis* for adaptations to cope with this potential threat. Chlorophyllide-binding proteins, previously described from *Bombyx mori*, are also present in *S. littoralis*. When two genes encoding these proteins were knocked out by CRISPR/Cas9, *S. littoralis* larvae consuming potted lima bean plants survived in the dark, but died in the light. When artificial diet was supplemented with chlorophyll or other tetrapyrroles, double-knockout larvae survived in the dark but died in the light. Double-knockout larvae consuming lima bean leaves were found to have high concentrations of some tetrapyrroles in the hemolymph. Heterologously-expressed chlorophyllide-binding protein bound to some tetrapyrroles. We hypothesize that chlorophyllide-binding proteins protect the larvae from reactive oxygen species in the aerobic environment of the hemocoel, by sequestering tetrapyrroles in the anaerobic midgut for subsequent excretion via the feces. To further test this hypothesis, we are examining the hemolymph for light-dependent generation of reactive oxygen species in the presence of tetrapyrroles.

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Buzzing after hours: Investigating fall armyworm's mating mysteries

André Richter[1,2], Astrid Groot[3], Sabine Hänniger[2,4]
[1]*Department of Medical Engineering and Biotechnology, Ernst Abbe University of Applied Sciences, Jena, Germany*
[2]*Department Insect Symbiosis, Max Planck Institute for Chemical Ecology, Jena, Germany*
[3]*Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands*
[4]*Institute of Ecology and Evolution, Friedrich Schiller University, Jena, Germany*

The fall armyworm, *Spodoptera frugiperda*, is a significant agricultural pest known for its widespread damage to key crops and its recent expansion into Africa, Asia, and Australia. In its native habitats of North and South America, this species exhibits two sympatric strains, potentially undergoing speciation despite ongoing gene flow. While the female sex pheromones remain almost consistent across strains, notable differences in the timing of sexual communication and mating behaviors have been observed. Specifically, corn-strain females initiate calling earlier in the night compared to rice-strain females, resulting in an earlier mating sequence. In contrast, pupal emergence timing does not differ between the strain, leading to questions about the precise mechanisms isolating these strains and the factors maintaining their divergent mating behaviors.

Modeling studies emphasize the role of sex-specific gene expression in perpetuating these timing differences. Therefore, this research project aims to investigate sex-specific and strain-specific variations in activity patterns, free-running period, and expression profiles of key circadian clock genes. Through this analysis, we seek to elucidate the underlying mechanisms driving the divergence in the timing of sexual communication of the fall armyworm.

Chemical and Ecological Factors Mediating Tomato Resistance to *Tuta absoluta*: Insights from Uruguayan Varieties and Wild Species

Anna Burgueño[1,2,3], Matías González[4], Federico Rodrigo[1], Andrés González[1], Carmen Rossini[1,2]
[1]Laboratorio de Ecología Química /Facultad de Química, Universidad de la República, Uruguay
[2]Laboratorio de Botánica /Facultad de Química, Universidad de la República, Uruguay
[3]Graduate Program in Chemistry, Facultad de Química, Universidad de la República, Uruguay
[4]Genética vegetal y fitomejoramiento, Instituto Nacional de Investigación Agropecuaria-Salto Grande, Uruguay

Tomato production often involves local breeding for resistance to microbial diseases, but herbivorous insects like *Tuta absoluta* (Lepidoptera: Gelechiidae) remain a significant threat. Although certain tomato trichomes and metabolites have been linked to resistance against *T. absoluta*, these associations are less understood in genotypes developed for resistance to other threats. This study examined leaf trichome density and secondary metabolites in Uruguayan tomato varieties, correlating them with *T. absoluta* oviposition preference and performance. The tested genotypes included *Solanum lycopersicum* varieties M82 (susceptible), Frontera, and HT457 (locally developed), and the resistant wild species *Solanum habrochaites* (CUPH421). The study found that egg deposition increased from CUPH421 to Frontera to HT457 and M82. Larval hatch rates were similar among the Uruguayan-developed genotypes but higher than in *S. habrochaites*. However, the life cycle duration was similar across all genotypes (GLMs). Susceptible (M82, HT457, and Frontera) and resistant (CUPH421) varieties were then classified based on *T. absoluta*-preference and performance. Susceptible varieties exhibited lower levels of nerol, tridecane, two unidentified monoterpenes, and alpha-zingiberene, and higher levels of carene-delta-2, beta-(Z)-ocimene, alpha-pinene, and alpha-phellandrene (PLS). Type IV trichomes, which exude methylketones and the lepidopteran-repelling sesquiterpene zingiberene, were present in all genotypes but in varying quantities. These findings highlight variations in susceptibility to *T. absoluta* among Uruguayan tomato varieties and identify potential chemical markers for resistance. The results suggest the need for further testing of these compounds for their potential to attract or repel *T. absoluta*, and emphasize the importance of considering pest resistance when developing new tomato varieties.

Olfactory and visual communication systems of the non-native winter crane fly, *Trichocera maculipennis*, colonizing Antarctic research stations on King George Island

Kye-Chung Park[1], **Sanghee Kim**[2], **Ji-Hee Kim**[2]
[1]*Bioprotection, New Zealand Institute for Plant and Food Research, Christchurch, New Zealand*
[2]*Division of Life Sciences, Korea Polar Research Institute, Incheon, Republic of Korea*

The non-native winter crane fly (WCF), *Trichocera maculipennis* (Diptera: Trichoderidae), has invaded several research bases across King George Island, colonizing their sewage facilities. To comprehend the visual and olfactory communication system of WCF, we conducted a series of morphological, electrophysiological, and behavioral studies. Scanning electron microscope observations revealed the presence of well-developed olfactory sensilla on both male and female WCF antennae and maxillary palps, which could be classified into distinct morphological types. Electrophysiological recordings of antennal responses to volatile compounds demonstrated strong electroantennogram responses, particularly to habitat-related compounds such as linalool. Additionally, the compound eyes of WCF adults displayed morphologically well-developed structures and exhibited robust electroretinogram responses to UV and yellow light. Laboratory bioassays using net cages and traps indicated WCF's behavioral attraction to specific visual and olfactory cues. This study furnishes valuable insights to enhance the efficacy of WCF monitoring systems and support eradication efforts in non-native Antarctic habitats.

Uncovering regional variation in sexual communication in the invasive pest species *Spodoptera frugiperda* in Africa

Renée A.H. van Schaijk[1], Emily R. Burdfield-Steel[1], Aboli Vartak[1], Idse J. Luirink[1,2], Mobolade D. Akinbuluma[1,3] Ahmed G. Hussain[4], Vera I.D. Ros[4], Frans C. Griepink[5], Georg G. Goergen[6], Fathiya M. Khamis[7], Sevgan Subramanian[7], Baldwyn Torto[7], Astrid T. Groot[1]
[1]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands
[2]Vrije University of Amsterdam, Amsterdam, the Netherlands
[3]University of Ibadan, Ibadan, Nigeria
[4]Laboratory of Virology, Wageningen University and Research, Wageningen, the Netherlands
[5]Pherobank BV, Wijk bij Duurstede, the Netherlands
[6]International Institute of Tropical Agriculture, Cotonou, Benin; [7]International Centre of Insect Physiology and Ecology, Nairobi, Kenya

Invasive species pose major threats to the natural environment, agriculture, and human health. Successful invasive species likely adapt rapidly to new environments, where they encounter, and possibly compete with, local closely related species. A particularly successful invasive pest species is the fall armyworm (*Spodoptera frugiperda*), which is native to the Americas, has invaded Africa, Eurasia and Oceania, and causes major damage and high economic losses to cultivated staple crops, especially maize. To understand the evolutionary potential of this pest in newly invaded areas, our research focuses on the question: whether, and to what extent, sexual behaviors vary between geographic populations in two regions in Africa, and whether hybridization (could) occur(s) with locally occurring *Spodoptera* species. By comparing the sex pheromone from wild-caught fall armyworm females between Benin and Kenya over two years, we found no geographic variation in the sex pheromone composition. However, we did find variation in male responses to different sex pheromone lures, which were based on the identified female pheromone variation at the two field sites. In our hybridization experiments with five *Spodoptera* species occurring in tropical Africa, we found a mating between *S. frugiperda* and *S. exigua,* but no viable offspring. We will discuss our results in light of evolution and pest management of the fall armyworm in Africa.

Adaptive innate preferences of solitary generalists and its flexibility

Aditi Mishra[1], Deepa Rajan[2], Shannon B. Olsson[1]
[1] *Naturalist- Inspired Chemical Ecology lab, National Centre for Biological Sciences, Bengaluru, India*
[2] *University of California, San Francisco, USA*

Many solitary generalist insects must locate food sources soon after emergence. This necessitates an innate search template specific enough to discriminate objects, yet general enough for the diversity of objects relevant to that species. How these tiny brains encode these cues innately is an area of intense study for ecologists and neuroscientists alike. Here we used the solitary generalist pollinator *Eristalinus aeneus* to understand how the innate food search template can arise through a small number of sensory cues spanning multiple sensory modalities. Using field and laboratory behavioral assays and electrophysiology, we found that the innate floral choices of the hoverfly *Eristalinus aeneus* are a product of contextual integration of broad plant-based olfactory cues and visual cues with high spectral intensity in the 500-700 nm range and radial symmetry.

Such a template is parsimonious yet robust for the broad ranges of food options available to a hoverfly. We also found that hoverflies can exhibit complete extinction of this innate attraction to the floral cues and retain this aversive learning for several days. Conversely, they quickly learn to associate neutral floral objects with food and loose this appetitive learning within 48-72 hours. Understanding object identification in organisms that have evolved in the natural world, such as hoverflies, highlights how mini-brains identify diverse objects like flowers and how learning finetunes their identification. Further, understanding insect search strategies can inspire efficient object classification approaches and can help us derive better strategies for pollinator conservation in the face of a rapidly changing world.

Differentiation of chemical profiles in a complex of hybrid butterfly species

Paul Doniol-Valcroze[1], Lucie Develay[1], Bruno Buatois[1], Stefan Dötterl[2], Roman Fuchs[2], Laurence Després[3], Mathieu Joron[1]*, Anne-Geneviève Bagnères[1]*
[1]CEFE, CNRS, Univ Montpellier, EPHE, IRD, Montpellier, France
[2]Department of Environment & Biodiversity, Paris-Lodron University of Salzburg, Salzburg, Austria
[3]Univ. Grenoble-Alpes, Univ. Savoie Mont Blanc, CNRS, Laboratoire d'Ecologie Alpine, Grenoble, France
** These authors contributed equally to the presented work.*

Hybridization is a pivotal driver of organismal diversification, notably through hybrid speciation, yet the full scope of factors influencing speciation and notably reproductive isolation remains only partially explored. Chemical signaling, often crucial in mate choice, can play a pre-zygotic barrier role among emerging species. In this study, we examined the cuticular chemical profiles of four alpine butterfly species of the genus *Coenonympha* (Nymphalidae: Satyrinae). Two of these species, *C. cephalidarwiniana* and *C. darwiniana*, arose through hybridization between *C. arcania* and *C. gardetta*. Employing gas chromatography coupled with time of flight mass spectrometry (GC-TOF-MS), we observed variations that were more quantitative than qualitative over numerous chemical compounds between the four species' chemical profiles, coherent with the recent evolutionary history of the species complex. Despite much closest genetic affinities with *C. arcania*, the profiles of both hybrid species exhibited striking similarity to those of *C. gardetta*, potentially impeding hybridization with *C. arcania*, consistent with observed natural hybridization rates. These findings underscore the potential critical role of chemical profile divergence in hybrid speciation and highlight the non-random sorting of parental traits during this evolutionary trajectory. By elucidating the intricate mechanisms underpinning speciation, this research contributes to our understanding of evolutionary processes and emphasizes the significance of chemical signaling in shaping reproductive isolation barriers among emerging species.

POSTERS

P-02

Conservation of indolergic olfactory receptors highlights the ecological significance of indoles in Diptera

Michal Arbel, Jonathan Bohbot
Department of Entomology, The Hebrew University of Jerusalem, Israel

Indoles are volatile organic compounds and ancient interkingdom signals used by bacteria, archaea, plants and animals. In insects, indole and its methylated analog skatole are involved in oviposition site and host selection. Indolergic odorant receptors (indolORs) were initially found and functionally characterized in mosquitoes. More recently, indolOR homologs have also been identified in *Drosophila melanogaster* and the common housefly *Musca domestica*, suggesting an origin predating the divergence between nematoceran and brachyceran flies. The latter lineage comprises species exhibiting a variety of feeding habits and occupying diverse ecological niches. How widespread indolORs is not well-documented and the ecological roles of indoles remains poorly understood. Using the two-electrode voltage clamp of oocytes expressing dipteran candidate indolORs, we have surveyed their functional evolution along the dipteran phylogenetic tree, which represents approximately 250 million years of evolution. Our functional survey includes candidate indolORs from sandflies, tephritide flies, Muscidae, Calliphoridae and other groups. Our findings support the importance of indole-sensing mediated by indolORs and raises the question of their ecological significance in this medically and agriculturally-significant group of insects.

P-06

Medicago truncatula sapogenins in plant-insect interactions: Sex-related differences in the transcriptomic responses of *Trichoplusia ni* caterpillars to the aglycone hederagenin

Yinting Chen[1], Christine Lafleur[2], Ryan J. Smith[1], Diljot Kaur[1], Brian T. Driscoll[3], Jacqueline C. Bede[1]
[1]*Department of Plant Science, McGill University, 21,111 Lakeshore, Ste-Anne-de-Bellevue, Qc, H9X 3V9, Canada*
[2]*Department of Animal Science, McGill University, 21,111 Lakeshore, Ste-Anne-de-Bellevue, Qc, H9X 3V9, Canada*
[3]*Natural Resource Sciences, McGill University, 21,111 Lakeshore, Ste-Anne-de-Bellevue, Qc, H9X 3V9, Canada*

Saponins are important defensive specialized metabolites in many legume species. Screening of *Medicago truncatula* ecotypes identified a potential correlation between foliar levels of oleanolic acid-derived saponins, such as hederagenin-derived compounds, and caterpillar deterrence. In the caterpillar gut, plant- or insect-glycosidases or the highly alkaline gut environment likely hydrolyzes the sugar group to release the sapogenin. The sapogenin hederagenin was not toxic to 4th instar caterpillars of the cabbage looper *Trichoplusia ni* nor did it act as a feeding deterrent. However, female caterpillars ate more diet spiked with hederagenin than males. RNASeq identified genes that were differentially regulated in response to the presence of hederagenin as well as distinctions between male and female caterpillars. These sex-related differences in feeding and detoxification should be taken into consideration in insecticide evaluations to minimize pesticide resistance.

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P-14

Fungus infected grain: Reaction in males and females of yellow mealworm, *Tenebrio molitor* L. (Coleoptera, Tenebrionidae)

Gabrielė Bumbulytė[1], **Ieva Lučinskaitė**[2], **Vincas Būda**[1]
[1]*Laboratory of Chemical and Behavioral Ecology, Nature Research Centre, Lithuania*
[2]*Life Sciences Center, Vilnius University, Lithuania*

Insects, considered an alternative to conventional meat in Asian cultures, offer rapid growth, minimal environmental impact, and rich protein content. These advantages position them as an excellent protein source for reducing global CO2 emissions.

The mealworm (*Tenebrio molitor*) is one of the most recognized edible insects globally, and to maintain optimal production hygiene, efforts are being made to distinguish between live and dead insects using natural repellents from their native habitats like grain. A promising method is the use of volatile compounds from the microscopic fungus *Aspergillus flavus*, which colonizes mealworm food substrates.

This study aimed to assess the impact of volatile compounds generated by *Aspergillus flavus* on adult mealworm beetles. The investigation employed a two-choice pit-fall test, utilizing vials containing control grain (no stimulus) and vials containing *Aspergillus flavus*-infected grain following infestation at different intervals (0, 5, 10, 20 days).

To evaluate the effects of *Aspergillus flavus* volatile compounds behavioral tests were carried out in Petri dishes using a two-choice assay. Behavior was tracked using the computer program EthoVision XT 12.

The tests revealed that females detected the presence of *Aspergillus flavus* from the very onset of infection (day 0), whereas male began distinguishing the infected grain only from day 5 onward. The following compounds present in grain contaminated by *Aspergillus flavus* were not behaviorally active both for females and males: 1-pentanol, 2-methyl furan, toluene, ethanol, 3-octanone and benzaldehyde. Research is in progress and data on volatiles involved in grain attractivity loss following fungus infestation will be presented.

P-42

Food plant localisation in the sugar beet weevil *Aspropartenis punctiventris* Germar

Elisabeth H. Koschier[1], Lena Dittmann[1], Bernhard Spangl[2]
[1]University of Natural Resources and Life Sciences (BOKU), Department of Crop Sciences, Institute of Plant Protection, Vienna, Austria
[2]University of Natural Resources and Life Sciences (BOKU), Department of Landscape, Spatial and Infrastructure Sciences, Institute of Statistics, Vienna, Austria

Asproparthenis punctiventris Germar (Coleoptera: Curculionidae) is widespread throughout Eurasia and is recognized as an important pest of sugar beet. Since 2017, mass outbreaks of the weevil in eastern Austria have caused significant damage to sugar beet crops. In addition to sugar beet, the weevil has also been reported to feed on other species of the Amaranthaceae family. We investigated the responses of adult weevils to the volatile leaf odours of selected food plants using a four-arm olfactometer. A bioassay procedure was developed to record the frequency of visits and the time spent by the weevils in the olfactometer quadrant with leaf volatiles, as well as their first choice of quadrant. Both females and males were equally attracted to the leaf odours of young sugar beet and chard (*B. vulgaris* subsp. *vulgaris* Cicla group) plants. Males, but not females, responded positively to the leaf odour of garden orache (*Atriplex hortensis*) and no response was observed to the leaf odour of fat hen (*Chenopodium album*) or common amaranth (*Amaranthus retroflexus*). These results suggest that *A. punctiventris* uses the odours of the leaves to locate sugar beet and other food plants, especially those with a high nutritional value. Understanding the olfactory responses of this pest may provide a basis for improved monitoring or mass trapping strategies.

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P-47

Herbivore induced resistance of hop plants against spider mites

Inka Lusebrink, Maria Obermaier, Florian Weihrauch
Bavarian State Research Center for Agriculture (LfL), Hop Research Center, Hüll 5 1/3, 85283 Wolnzach, Germany

The two-spotted spider mite (*Tetranychus urticae*) is a polyphagous pest that attacks around 90 different crops in Germany, including hops (*Humulus lupulus*). Heavily infested leaves dry out and turn gray or copper-brown and eventually fall off. The spider mite can build up very large populations in a short time during dry, hot summers and can sometimes cause enormous losses in hop quality and yield.

Observations in recent years from several field trials have shown that after surviving a severe spider mite infestation hop plants are capable to defend themselves against excessive spider mite infestation in subsequent years. The objective of this study is to investigate whether and to what extent one or two years of heavy infestation of hop plants with spider mites reduces their susceptibility to spider mites through induced resistance in subsequent years. Therefore, field and greenhouse trials are being conducted on four hop cultivars: Hallertauer Tradition, Spalter Select, Tettnanger and Herkules.

The first greenhouse trial focused on how secondary compounds of hop leaves differ between plants naturally infested by spider mites or hop aphids (*Phorodon humuli*). Therefore 18 potted plants of each cultivar were grown in a greenhouse. After four months leaves were harvested, freeze-dried, ground, and analyzed with headspace – gas chromatography / mass spectrometry.

Barely any research has been conducted on hop leaf chemistry so far, this is likely the first study comparing leaf chemistry among four different hop cultivars and how it is affected by natural infestation from spider mites and hop aphids.

P-48

The optimal choice of trap type for the recently spreading jewel beetle pests *Lamprodila festiva* and *Agrilus sinuatus* (Coleoptera, Buprestidae)

Eszter Matula[1,2], Gábor Bozsik[1], József Muskovits[3], Csenge Ruszák[4], Laura Jávorszky[4], Jochem Bonte[5], Márton Paulin[6], József Vuts[7], József Fail[2], Ágoston Tóth[4], Ádám Egri[8], Miklós Tóth[1], Zoltán Imrei[1]
[1]*Plant Protection Institute, Centre for Agricultural Research, HUN-REN, Budapest, Hungary*
[2]*Department of Entomology, Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary*
[3]*Independent Researcher, Budapest, Hungary*
[4]*Biocont Magyarország Kft., Kecskemét, Hungary*
[5]*Plant Sciences, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgium*
[6]*Department of Forest Protection, Forest Research Institute, University of Sopron, Mátrafüred, Hungary*
[7]*Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, UK*
[8]*Institute of Aquatic Ecology, Centre for Ecological Research, HUN-REN, Budapest, Hungary*

Two jewel beetle species native to Europe, the cypress jewel beetle, *Lamprodila* (Palmar, *Ovalisia*) *festiva* L. (Buprestidae, Coleoptera), and the sinuate pear tree borer, *Agrilus sinuatus* Olivier (Buprestidae, Coleoptera), are key pests of ornamental thuja and junipers and of orchard and ornamental rosaceous trees, respectively. Although chemical control measures are available, due to the beetles' small size, agility, and cryptic lifestyle at the larval stage, efficient tools for their detection and monitoring are missing. Consequently, by the time emerging jewel beetle adults are noticed, the trees are typically significantly damaged. Thus, the aim of this study was to initiate the development of monitoring traps. Transparent, light green, and purple sticky sheets and multifunnel traps were compared in field experiments in Hungary. Light green and transparent sticky traps caught more *L. festiva* and *A. sinuatus* jewel beetles than non-sticky multifunnel traps, regardless of the larger size of the colored surface of the funnel traps. Although light green sticky sheets turned out to be optimal for both species, using transparent sheets can reduce catches of non-target insects. The key to the effectiveness of sticky traps, despite their reduced suitability for quantitative comparisons, may lie in the behavioral responses of the beetles to the optical features of the traps.

P-54

Volatiles from commercial fungi act as chemical cues that influence the behavior of fungivorous pest insects

Hiromi Mukai[1], **Masahiko Tokoro**[2], **Norihisa Kusumoto**[3]
[1]*Kansai Research Center, Forestry and Forest Products Research Institute, Kyoto, Japan*
[2]*Department of Forest Entomology, Forestry and Forest Products Research Institute, Tsukuba, Japan*
[3]*Department of Forest Resource Chemistry, Forestry and Forest Products Research Institute, Tsukuba, Japan*

Most basidiomycetes form mushrooms as fruiting bodies, and are thought to have mutually beneficial relationships with various insects, among which fungivorous insects play a role in spore dispersal. Volatile C8 compounds such as 1-octen-3-ol and 3-octanone, which imbue mushrooms with flavor, have been reported to attract fungivorous insects. However, few studies have investigated the effects of these compounds on insect behavior, and the mediation of fungus–insect relationships by volatile organic compounds (VOCs) has not been studied in detail. Therefore, we conducted behavioral and neurophysiological analyses to elucidate the biological functions of fungal VOCs in fungivorous insects. We established breeding systems for two major fungivorous insect pests, *Neoempheria bifurcata* (Diptera: Mycetophilidae) and *Nemapogon granella* (Lepidoptera: Tineidae), and conducted behavioral experiments using commercial mycelial blocks of shiitake (*Lentinula edodes*: Agaricales: Omphalotaceae). Gas chromatography–electroantennographic detection analysis revealed that females of both species exhibited electrophysiological antennal responses to C8 compounds in fungal VOCs. Odor preference experiments conducted using a Y-tube olfactometer showed no significant attraction preference in either species. However, an oviposition preference experiment showed that females preferred to oviposit eggs on filter paper treated with VOCs rather than untreated filter paper. Our results indicate that fungivorous insects are receptive to C8 compounds as cues for egg-laying.

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RNAi as an emerging technology for management of *Ips typographus* (Coleoptera: Scolytinae) in the European forests

Shatarupa Sarkar[1], Sellamuthu Gothandapani[1], Kanakachari Mogilicherla[2], Amit Roy[1]
[1]*Department of Forest Genetics and Physiology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, Czech Republic*
[2]*ICAR-Indian Institute of Rice Research, Hyderabad, India*

Due to climate change, frequent and severe outbreaks of the European spruce bark beetle, *Ips typographus* have caused immense damage to the Norway spruce (*Picea abies* (L.) Karst.) population in the European forests and have drastically reduced their numbers. Traditional methods have failed to curb these infestations of tree-killing bark beetles (Coleoptera: Curculionidae, Scolytinae), thereby urgently calling for the need of an efficacious bark beetle management strategy. To check the population of coleopteran forest insect pests, RNA interference (RNAi) as a molecular tool, has piqued the interest of researchers due to its sequence-specific target gene silencing leading to insect mortality. However, use of RNAi technology against the tree-killing bark beetles has been limited until now. With the availability of *Ips typographus* genome sequence, we hypothesize that RNAi technology can be efficiently employed to control *Ips typographus* populations. We have identified RNAi core machinery genes through RNA sequencing studies and investigated their expression dynamics across different tissues and life stages of the bark beetles. Further, we have conducted bioassays to determine the efficiency of induction of mortality due to silencing of the identified target gene's and have validated the target genes through quantitative evaluation of target genes' knockdown in the beetles. Also, we elucidate the challenges of implementation of RNAi technology for *Ips typographus* management in forested landscapes and discuss the possible ways to overcome the hurdles. Our findings pave the way towards the potential application of RNAi for management of *Ips typographus* and other forest pests.

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The ecologically base enhancement of beneficial microorganisms for the control of *Meloidogyne incognita* in cucumber (*Cucumis Sativus* L.)

Win Lai Lai Swe[1], **József Fail**[1], **Ferenc Tóth**[2], **Márta Ladányi**[3]
[1]*Department of Entomology, Institute of Plant Protection, University of Agriculture and Life Sciences (MATE), Hungary*
[2]*Hungarian Research Institute of Organic Agriculture (ÖMKi), Hungary*
[3]*Department of Applied Statistics, Institute of Mathematics and Basic Science, University of Agriculture and Life Sciences (MATE), Hungary*

Climate change triggers major changes in the diversity and abundance of various microorganisms crucial for soil health, plant growth, and ecological balance and efficiency of crop protection technologies. *Meloidogyne incognita* (Kofoid and White) is a polyphagous endoparasite that causes significant yield losses across more than 2,000 plant species globally. Farmers are facing serious plant protection issues and phytosanitary risks, such as food insecurity, adverse effects of pesticide use on human health, and environmental problems. There is a need for a shift from agrochemistry to agroecology, microorganisms are a practical and cost-effective alternative to chemical control methods for root-knot nematodes (RKNs), ensuring safety. This study assesses the efficacy of four biocontrol agents (*Trichoderma asperellum, Beauveria bassiana, Fusarium proliferatum*, and *Bacillus mojavensis*) with a concentration of (1 × 10^9 cfu ml^{-1}) applied to each nursery pot. A total of 300 plants were arranged in a randomized complete block design (RCBD) over three growing seasons: spring, summer, and autumn. The nematicidal activity of each biocontrol agent was evaluated according to the physiological and morphological characteristics of cucumber plants under RKNs-infected soil. *B. mojavensis* and *F. proliferatum* showed the maximum plant height, number of leaves, flowers, and fresh plant weight compared to other treatments. The tested biocontrol agents, *T. asperellum* and *F. proliferatum* displayed the highest nematicidal activity followed by *B. bassiana, B. mojavensis*. These findings suggest that the tested beneficial microorganisms exhibit potential as biocontrol agents for suppressing root-knot nematode, *M. incognita*.

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Responses of the parasitoid *Trichogramma achaeae* to native plant volatiles

Savvina Toufexi, Eirini Anastasaki, Georgios Partsinevelos, Aikaterini Psoma, Panagiotis Milonas
Scientific Directorate of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, Kifissia, Greece

Current farming management in the Mediterranean basin is based on unsustainable practices that threaten the stability and resilience of cropping systems and cause the loss of biodiversity and abundance of natural enemies of pests. The introduction of flowering native plants in crops margin to provide additional food for natural enemies is proposed to enhance beneficial athropods in agroecosystems. In the current study, we examined the attractiveness of the parasitoid *Trichogramma achaeae* to native plants, aiming to find wild native and cultivated plants that maximize its performance. We tested the response of *T. achaeae* females in Y-tube olfactometer against *Sonchus oleraceus*, *Calendula arvensis*, *Lathyrus sativus*, *Anethum graveolens*, *Echium sp*. plants in full blossom. Additionally, volatiles from the above plants were collected using the dynamic headspace technique and analyzed by gas chromatography- mass spectrometry. The results showed a significant attraction of *T. achaeae* over the flowering plants. From the analysis, the main peaks of *S. oleraceus* were limonene, sabinene as well as sulfur compounds, while for *C. arvensis* were α-pinene, α-thujene, limonene, and 3-δ-carene. Limonene, sabinene, β-myrcene, 3-(*Z*)-hexenyl acetate were the main compounds in the headspace of flowering *L. sativus* L. plants. For *Anethum graveolens* were α-phellandrene and dill ether, and for *Echium sp*. limonene, sabinene and 3-(*Z*)-hexenyl acetate. These findings are of great importance for the planning of habitat enhancement of beneficial arthropods, to achieve the sustainable management and efficient use of natural resources.

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S18: Application of semiochemicals in sustainable insect pest management

Chairs: *Aijun Zhang, Jian Chen*

ORAL PRESENTATIONS

Enhancing ant bait specificity using semiochemicals

Jian Chen
USDA-ARS, SEA, BCPRU, Stoneville, Mississippi, USA

Bait is commonly used for managing invasive ants. Unfortunately, no ant bait products currently on the market are species-specific, indicating their non-target effect. Ants are incredibly diverse, and each species has unique ecological roles and interactions. Protecting native ants helps preserve biodiversity, which is crucial for ecosystem resilience and adaptation to environmental changes. In any invasive ant management program, preserving native ants should be a high priority. Many approaches have been proposed to improve bait specificity, such as using more selective active ingredients and phagostimulants, enhancing bait attractiveness to target ants using ant pheromones, and optimizing the timing of bait application and application rate. A new approach to enhancing fire ant bait specificity is proposed here – making bait less palatable to non-target ants without sacrificing its palatability to target ants. We discovered that fire ants feed their nestmates with their own venom, and adding certain venom alkaloids into the food does not affect the feeding of fire ants but significantly deters the feeding of other ants. Further experiments with alarm pheromone revealed the similar effect. Defensive compounds of the target ants may be useful in improving bait specificity.

Can elicitors of defenses enhance resistance in berry crops against insect pests?

Cesar Rodriguez-Saona[1], **Amanda Quadrel**[1], **Pablo Urbaneja-Bernat**[2], **James Polashock**[3]
[1]Rutgers University, Chatsworth, New Jersey, USA
[2]IRTA, Sustainable Plant Protection, Barcelona, Cabrils, Spain
[3]United States Department of Agriculture-Agricultural Research Service, Chatsworth, New Jersey, USA

Synthetic elicitors of the salicylic acid (SA) and jasmonic acid (JA) defense pathways can provide protection to crops against herbivores and pathogens. In two studies, we aimed to investigate how elicitors of plant defenses influence the resistance of berry crops against insect pests. Four commercial elicitors were tested: three SA-related (Actigard, LifeGard, Regalia) and one JA-related (Blush). In one study, we treated cranberry plants either infected by the phytoplasma causing false blossom disease or uninfected with the elicitors and evaluated the performance of the disease-vector, the blunt-nosed leafhopper (*Limotettix vaccinii*), and the non-vector spongy moth (*Lymantria dispar*). Both elicitor exposure and phytoplasma infection increased the susceptibility of cranberry plants to *L. vaccinii* and *L. dispar*. Elicitors increased nitrogen (N) and decreased carbon/nitrogen (C/N) levels; SA activators also reduced JA levels. Phytoplasma infection elevated N and SA levels and reduced C/N ratios regardless of elicitor treatment. In another study, we investigated the preference and performance of spotted-wing drosophila (*Drosophila suzukii*) on blueberries treated with elicitors and the sterilant OxiDate. Because *D. suzukii* adults use cues derived from fruits and symbiotic microbes to identify suitable feeding, mating, and oviposition sites, we expected the elicitors or sterilant to influence their behavior. In choice and no-choice experiments under laboratory, semi-field, and field settings, we showed no consistent repellent, oviposition deterrent, or larval toxicity activity for any of the products tested on *D. suzukii*. Overall, our studies found no evidence that either SA- or JA-related elicitors increase resistance to insect pests in two berry crops.

Possible use of (*E*)-2-octenal, one of the alarm pheromones of rice-ear bugs, *Leptocorisa chinensis*, for their management under field conditions

Ken-ichi Yamashita[1,4], Masayoshi Uefune[2], Rika Ozawa[3], Junji Takabayashi[3]
[1]Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, Agricultural Technology Institute, Kasai, Hyogo, 679-0198, Japan
[2]Department Agrobiological Resources, Faculty of Agriculture, Meijo University, Nagoya, Aichi, 468-8502, Japan
[3]Center for Ecological Research, Kyoto University, Otsu, Shiga, 520-2113, Japan
[4]Present address: Hyogo Plant Protection Association, Kobe, Hyogo, 676-0825, Japan

Leptocorisa chinensis is one of the most important insect pests in rice plants causing pecky rice grains. (*E*)-2-Octenal (E-2-OAL) is a major compound in the alarm pheromones of this species, and repels conspecific adults under laboratory conditions. We investigated the possible use of E-2-OAL to reduce pecky rice grains caused by *L. chinensis* adults in commercial paddy fields. We set a plot (5 m × 5 m; treated plot) in which 16 sticks (1 m high, 1.5 m intervals, 4 × 4 formations), each of which was equipped with a dispenser containing a solution of 1% E-2-OAL at its top in the fields. A control plot of the same size was established 30 m away from the treated plot in the same field. We used six different paddy fields for the experiments. The experiments were conducted at the milk-ripe stage of rice plants, a period during which *L. chinensis* adults damaged the rice grains. We found a significant decrease of pecky rice grains in the treated plots as compared to the control plots, effectively keeping the damage below the economic injury level (EIL) in the treated plots. Additional research is required to develop an efficient dispenser capable of vaporizing appropriate quantities of E-2-OAL to reduce the occurrence of pecky rice grains in paddy fields.

Control of sugar beet yellows viruses by behavioural manipulation of aphid vectors in the field via volatiles

Tarek Dardouri, Camille Delpoux, Paul-Emile Bertrand, Landry Sarles, Margot Tixeront, Ambroise Garnier, Corentin Daugan, Thomas Hecky, Fabienne Dupuy, Arcadie Fuior, Marie Rebillard, Ené Leppik
Agriodor, Rennes, France

Aphids (*Myzus persicae*) poses a global threat to numerous crops, particularly sugar beet plants, due to their damages and ability to transmit yellow virus to plants. The continuous seek for ecofriendly pest management combined with the banning of neonicotinoid substances reveals the urgency for efficient and sustainable alternatives. The management of *Myzus persicae* in sugar beet crop using volatile organic compounds (VOCs) has shown promising results. However, the use of VOCs as biocontrol method is challenged with efficacy and sustainability in sugar beet production. Consequently, the aim of this research is to validate the efficacy of blends selected in the laboratory and to validate their use as biocontrol methods in field trials.

In field studies, we used randomized block design to prove the effect of these molecules. Four replications were applied. It is revealed in the result that by utilizing a specific blend of VOCs, it is possible to regulate the behavior and biology of *Myzus persicae*. These VOCs act as repellents to winged colonizers at short distances, affecting their feeding behavior, reproduction rate, and developmental rate in wingless forms. The application of this VOC blend, which mimics the repellent properties of some plants, has been found to significantly reduce aphid populations by up to 70% in field conditions.

This approach presents a potential eco-friendly and effective solution for managing *Myzus persicae* in sugar beet crop cultivation.

Characterization of odors to predict their behavioral effects on the cabbage stem flea beetle, *Psylliodes chrysocephala*, for pest management applications

Margot Tixeront[1], **Fabienne Dupuy**[1], **Anne Marie Cortesero**[2], **Maxime R. Hervé**[2]
[1]Agriodor, 6 rue Pierre Joseph Colin, 35000 Rennes, France
[2]IGEPP, INRAE, Institut Agro, Univ Rennes, 35000, Rennes, France

Insects olfactory-guided behavior is often based on the detection of common compounds in unique relative proportions. Considering the odor as a whole unit appears promising to identify active fractions on the insect behavior and to recreate odors for integrated pest management. Different methods considering odor diversity and dissimilarity are emerging to predict odor effect on an insect behavior. A method called “compound without borders” considers the compound molecular structures and their relative proportions in the blend to characterize dissimilarity between odors. Another one, named “chemodiversity”, characterizes odor diversity based on the structural properties of the compounds, their diversity, and biosynthetic pathway. The cabbage stem flea beetle (*Psylliodes chrysocephala*, CSFB) is one of the most damaging pests of winter oilseed rape (*Brassica napus*, WOSR) in Europe, but control solutions are lacking. Using odors to disturb adult behavior and reduce crop colonization and damage is under consideration. In the present work, behavioral screenings from host plants and essential oils were conducted to identify attractive and repellent odors. These odors were then characterized using both described methods. A significant negative relationship was revealed between odor dissimilarity to WOSR and effect on the CSFB behavior. In addition, the chemodiversity between odors revealed that repellent and attractive odors are different in terms of diversity and biosynthetic pathways. These findings reveal that the more an odor is dissimilar to WOSR, the more it is likely to repel the CSFB. This study shows that these two approaches could be interesting to identify candidate odors influencing pest behavior.

Semiochemicals for monitoring the poplar bark beetle *Trypophloeus* spp. (Coleoptera: Scolytidae)

Antonio Ortiz[1], **Lucía Ruiz-Martos**[1], **Carmen Vega-Valdés**[2], **Lucía Delgado-Salán**[2], **Eva Díez-Presa**[2], **Pedro Casquero**[2], **Álvaro Rodríguez-González**[2]
[1]Department of Inorganic and Organic Chemistry. EPS Linares, University of Jaén, Spain
[2]Grupo Universitario de Investigación en Ingeniería y Agricultura Sostenible, Instituto de Medio Ambiente Recursos Naturales y Biodiversidad, Escuela de Ingeniería Agraria y Forestal, Universidad de León, Spain

Scolytine bark beetle species are capable of killing healthy trees in native populations and commercial poplar plantations. The poplar bark beetle *Trypophloeus* spp. (Coleoptera: Scolytidae), is a serious pest of poplar trees (*Salicaceae*, Genus *Populus*) across North Spain. Among the more than two hundred poplar clones available on the market, clone USA 184/411 has the highest susceptibility to *Trypophloeus* attacks. In this study, we set out to identify and evaluate the potential attractiveness of host plant volatiles released by two poplar clones with very high different susceptibility to *Trypophloeus* spp. to lay the groundwork for the development of field attractants for their populations.

The volatile chemical profiles of Populus USA 184/411 and I-214 clones were determined by GC-MS and more than 60 volatile components released by both *Populus nigra* clones were identified. GC-EAD analysis of volatiles captured by Tenax TA and SPME from bark and leaves of both clones revealed that salicyladehyde, one of the major benzenoid compounds released by *Populus nigra* trees, was the only compound that consistently elicited antennal responses from adult *Trypophloeus* beetles.

In a trapping study in three poplar plots located in León province (North Spain), shows that ethanol interacts synergistically with salicylaldehyde. *Trypophloeus* beetles were more attracted to lures combining ethanol and salicylaldehyde than lures with ethanol or salicylaldehyde alone. It will be necessary to continue with new research to continue providing new control tools for this pest.

Pheromone-based insect control in currant production, example of a small market crop dilemma

<u>Olle Anderbrant</u>[1], Glenn P. Svensson[1], Christer Löfstedt[1], Elisabeth Öberg[2], Ann-Kristin Isaksson[3], Hanh Huynh[3], Kimmo Rumpunen[4], Line Beate Lersveen Myhre[5], Sigrid Mogan[5], Gunda Thöming[6], Nina Trandem[6], Marja Rantanen[7]
[1]*Department of Biology, Lund University, Lund, Sweden*
[2]*Länsstyrelsen i Norrbottens län, Landsbygdsenheten, Luleå, Sweden*
[3]*Hushållningssällskapet Norrbotten-Västerbotten, Öjebyn Agro Park, Öjebyn, Sweden*
[4]*Swedish University of Agricultural Science, Department of Plant Breeding, Lomma, Sweden*
[5]*Norsk Landbruksrådgiving, Foss gård, Stokkeveien 4, Norway*
[6]*NIBIO, Norwegian Institute of Bioeconomy Research, Ås, Norway*
[7]*Luonnonvarakeskus (Luke), Natural Resources Institute Finland, Jyväskylä, Finland*

Commercial currant (*Ribes* spp) production covers about 3000 ha in the Nordic countries. Although currants tend to have less pest problems than strawberry and raspberry, the limited possibilities to control them, partly due to strict regulations of using pesticides, now makes research and development on pest control in currants imperative. Three lepidopteran pest species, *Lampronia capitella* (Prodoxidae), *Synanthedon tipuliformis* (Sessidae), and *Euhyponomeutoides albithoracellus* (Yponomeutidae), cause major damage in Nordic currant fields, and were subjected to mating disruption trials during 2022 and 2023 with mixed results. By applying 15-25 g of pheromone per ha from 300 dispensers a close to complete trap shutdown was achieved for *S. tipuliformis* and *E. albithoracellus*, whereas *L. capitella* was for unknown reason not affected by the treatment. For the first two species there was also a tendency of lower damage the year after treatment. Identifying other key pest challenges, as well as how to proceed with the mating disruption in a small crop like currants, will be addressed by a new network project, "Developing Integrated Pest Management in Nordic currant production". The main goal of the network is to make a foundation for the work necessary to reach a sustainable currant production in northern Europe. Networking with researchers on *Ribes* pests in other parts of the world will be an important part of the project. Thanks to Swedish farmers' foundation for agricultural research (SLF), to The Nordic Joint committee for Agricultural and Food research (NKJ), and to participating growers.

Development of effective trapping procedures for Japanese beetle to aid survey and eradication efforts in Canada

Quentin Guignard[1,2,3], Erin Campbell[4], Joel Goodwin[1,5], Troy Kimoto[6], Kenneth Haynes[7], Kiersten DeVillier[4], Jeremy D Allison[1,2,3,4]

[1]Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen Street E, Sault Ste. Marie, Ontario, P6A 2E5, Canada
[2]African Centre of Chemical Ecology, Innovation Africa @UP, University of Pretoria, South Africa
[3]Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa
[4]Canadian Food Inspection Agency, Ottawa, Canada
[5]University of Toronto, Institute of Forestry & Conservation, 33 Willcocks Street, Toronto, Ontario M5S 3B3, Canada
[6]Canadian Food Inspection Agency, 4321 Still Creek Dr, Burnaby, BC, V5C 6S7, Canada
[7]Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, KY, USA

A new population of the Japanese beetle (JB) *Popillia japonica* was detected in Vancouver (Canada). In this study, factors affecting trap captures (e.g., wind direction and speed, pheromone vs. kairomone, effect of intertrap distance and trap efficiency) were investigated in the field using semiochemical based lures. We observed that most JB flew directly upwind toward the trap, especially as wind speed increased (above 1.5 m/s). The floral lure (i.e., kairomone) and the floral lure with the pheromone captured similar numbers of JB and both captured more beetles than traps baited with the pheromone alone. Traps baited with the floral kairomone alone or with pheromone captured a 1:1 sex ratio (M:F) while traps baited with the pheromone alone captured a 3:1 (M:F) ratio. No synergism between the pheromone and the floral kairomone was observed. Within thirty seconds after approaching within 2 m of a trap baited with a lure, 43%, 35% and 22% of the beetles were captured, flew away or landed on the trap. A significant effect of intertrap distance was observed in both a high- and low-density population with stronger effects observed in the low-density population (the distance at which traps began to interfere with each other was independent of population density).To our knowledge, this study is the first to quantify in real time the flight behavior of a beetle toward semiochemical baited traps in the field and to provide evidence on the influence of insect density on trapping results for a Coleopteran insect.

Using a push and pull strategy to manage spotted wing drosophila infestation in blueberry crops

Aijun Zhang
Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, United States Department of Agriculture-Agricultural Research Service, Beltsville, MD 20705, USA

The invasive fruit fly called as spotted-wing drosophila (SWD) can infest soft-skinned fruits and cause crop losses worldwide. Application of synthetic pesticides is the only major tool for controlling this pest until now. A sustainable pest management alternative is urgently needed to reduce conventional synthetic pesticide usages. In this study, a natural product-based control method called as push-pull strategy is evaluated in blueberry field to protect crops from SWD injury. A natural occurring compound, methyl benzoate, was used as a pushing agent to push SWD away and SWD attractant was used as a pulling agent to pull SWD out of the crop fields. Our results demonstrated that methyl benzoate as a spatial repellent/oviposition deterrent can be deployed in blueberry fields to reduce the damage caused by SWD. It can help growers to apply this environmentally friendly strategy in Integrated Pest Management program to control SWD infestation and reduce the usage of synthetic pesticides for organic farming.

What does an attractive plant smell like? Investigations on the polyphagous brown marmorated stink bug *Halyomorpha halys* for developing a novel push-pull-kill strategy for organic farming

Alicia Koßmann[1], Bruna Czarnobai de Jorge[1], Tessa Strümpfler[2], Elisa Beizen-Heineke[3], Anant V. Patel[2], Astrid Eben[1], Jürgen Gross[1]
[1]Institute for Plant Protection in Fruit Crops and Viticulture, Julius Kühn-Institut (JKI) – Federal Research Centre for Cultivated Plants, Dossenheim, Germany
[2]WG Fermentation and Formulation of Biologicals and Chemicals, University of Applied Sciences and Arts (HSBI), Bielefeld, Germany; [3]Biocare GmbH, Dassel-Markoldendorf, Germany

The Brown Marmorated Stink bug (BMSB), *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae) is an invasive, polyphagous pest. Its host feeding using piercing-sucking mouthparts on fruits and vegetables causes deformations and makes the crop unmarketable. This leads to economic losses for farmers, and is further complicated due to the BMSB´s resistance to various insecticides. Currently, the only way to control the insect populations is through repeated applications of broad-spectrum insecticides, which is neither sustainable nor environmentally friendly. Thus, a different pest management strategy is necessary.

The aim of the project BIOBUG is to develop a new, biotechnical, and sustainable plant protection strategy against BMSB in organic horticulture. It consists of a push-pull-kill system with encapsulated volatile attractants, repellents, and a biological insecticide or microbial antagonist.

Understanding host plant recognition by the target insect, especially the role of plant volatiles, is important to design such a strategy. To investigate this, we conducted field monitoring of BMSB on different fruit crops during the entire growth season. When *H. halys* was found in high numbers in aggregation pheromone baited traps, volatiles of the nearby host plants and fruits were collected and analysed by gas chromatography coupled with mass spectrometry. This allowed to identify the emitted volatiles and similarities of the volatile profile among different hosts at specific times during their ripening period. Shared compounds were tested for recognition using electroantennography.

Here, I will present the first results of developing a potential attractant mixture that can be used for a push-pull strategy.

Deciphering plant-insect interactions: Volatile responses of broad bean pods to Brown Marmorated Stink bug feeding

Bruna Czarnobai de Jorge[1], Alicia Koßmann[1], Tessa Strümpfler[2], Elisa Beizen-Heineke[3], Anant V. Patel[2], Astrid Eben[1], Jürgen Gross[1]
[1]Julius Kühn-Institut (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim, Germany
[2]Bielefeld University of Applied Sciences, AG Fermentation and Formulation of Biologicals and Chemicals, Bielefeld, Germany; [3]Biocare GmbH, Dassel-Markoldendorf, Germany

The Brown Marmorated Stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a highly polyphagous pest, feeding on various plant organs but preferring fruits. In Europe, this invasive species is spreading rapidly with an expanding host range, causing damage to fruit crops and significant economic losses to agriculture.

To combat the problems caused by invasive insect herbivores, understanding the interactions between the herbivore and the plants on which it feeds is important. While several attempts to improve pest control continue to grow, it has not yet been determined if feeding by *H. halys* on different hosts induces changes in volatile chemical composition that could be exploited to disrupt its feeding behavior.

Therefore, we aimed to elucidate the interactions between BMSB and one of its host plants, the broad bean (*Vicia faba*, Fabaceae), by investigating differences in volatile compound emissions from bean pods exposed directly and systemically to BMSB feeding. To investigate this, we collected volatiles of bean pods damaged by conspecifics and undamaged (fresh or exposed to room condition). These were analyzed by gas chromatography coupled with mass spectrometry while observing the preferences of adults for these different food sources. Results indicate that BMSB feeding induced changes in specific amounts of volatile compounds emitted, as well as differences in the volatile profile that could explain differences in the food choice. Such blends of volatile compounds will be tested as attractants or repellents under field conditions, thus leading to more effective and environmentally friendly control strategies against BMSB.

Plant-Microbe-Insect Interactions: Nectar-inhabiting filamentous fungi affect the olfactory response of egg parasitoids

Jay Darryl L. Ermio[1], Antonino Cusumano[1], Patrizia Bella[1], Ezio Peri[1], Michael Rostas[2], Salvatore Guarino[3], Stefano Colazza[1]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, 90128 Palermo, Italy
[2]Agricultural Entomology, Department of Crop Science, University of Goettingen, 37077 Goettingen, Germany
[3]Institute of Biosciences and Bioresources, National Research Council of Italy, 90129 Palermo, Italy

Nectar from flowering plants used in conservation biological control (CBC) fuels parasitoids in their search and suppression of pest populations. Flowering plants used for CBC should be attractive to parasitoids to encourage high visitation rates. Although parasitoids utilize floral-based volatiles in food locations, in recent years, nectar-inhabiting microbes have gained much attention for their influence on the overall nectar scent. Nectar is an alluring environment for microbes, and it is possible to argue that their presence in nectar affects the behavior of flower visitors, including parasitoids. While specialist yeasts and bacteria are the most studied nectar microbes, how other microbes, like filamentous fungi, affect parasitoid olfactory responses is less clear. In this study, six nectar filamentous fungi were isolated from buckwheat (*Fagopyrum esculentum*), a flowering plant widely used in CBC, and used to ferment synthetic nectar. The fungus-fermented synthetic nectars were then tested on *Trissolcus basalis* and *Ooencyrtus telenomicida,* two egg parasitoids of *Nezara viridula.* In olfactometer bioassays, only nectar fermented by *Cladosporium* sp. SAAF 22.2.11 attracted *T. basalis*, while nectar fermented by *Cladosporium* spp. SAAF 22.2.11 and 22.3.29 attracted *O. telenomicida*. Volatile analyses carried out by GC-MS showed qualitative differences in compound composition among the fungus-fermented synthetic nectars, explaining the differential attraction of egg parasitoids. Our results suggest that nectar microbes affect the attraction of egg parasitoids and should be considered in CBC programs for pest management.

Oviposition of tortricid moths on substrate treated with different doses of gustatory stimuli

Carles Amat[1], Margherita Marmugi[2], Giovanni Benelli[2], Andrea Lucchi[2], Rajendra Prasad[3], Renato Ricciardi[2], César Gemeno[1]
[1]*University of Lleida-Agrotecnio-CERCA Center, Lleida, Spain*
[2]*Department of Agriculture, Food and Environment of the University of Pisa, Pisa, Italy*
[3]*ICAR-KVK Ramanagara, University of Agricultural Sciences, Bangalore, India*

Naturally occurring behaviorally modifying compounds, such as oviposition stimulants and deterrents, play a role in Integrated Pest Management programs (IPM). The effect of several gustatory stimuli on the number of eggs laid by *Cydia pomonella* (CM), *Grapholita* molesta (OFM) and *Lobesia botrana* (EGVM) was tested under laboratory conditions. Five mated females were released during the photophase in 4.5-L plastic arenas containing 4 filter paper pieces loaded with three doses (0.1 mM to 1 mM or 10-3 to 10-1) of a given tastant plus solvent control (water or ethanol), and the number of eggs laid on each filter paper was recorded 24 h later. Sugars (fructose and sucrose), salts (KCl and NaCl) and neem oil were tested on all three species, whereas tannic acid, juglone, grape seed oil and oleanolic acid were tested on EGVM alone. Neem oil was highly deterrent to all three species, although the lowest dose stimulated EGVM oviposition. Salts and sugars had mild effects, sugars being deterrent at the highest dose in all three moth species, and salts stimulating CM at the mid dose. Tannic acid, juglone, and grape seed oil strongly reduced EGVM oviposition. Oleanolic acid had no significant effect, although the trend was for increased oviposition in the EGVM. Our study pointed out stimulatory and deterrent oviposition effects triggered by several chemical compounds under laboratory conditions. Field tests are needed to determine their potential use in IPM programs.

Multitrophic interactions in agroecological-based vegetable push-pull cropping system for healthy vegetables

Daniel M. Mutyambai[1,2], **Bretor K. Mutua**[1,2], **Edward Karanja**[1], **Thomas Dubois**[1]
[1]*International Centre of Insect Physiology and Ecology, P.O Box 30772-00100 Nairobi, Kenya*
[2]*Department of Life Sciences, South Eastern Kenya University, P.O Box 170-90200, Kitui, Kenya*

Globally, vegetables especially those belonging to Brassicaceae are an essential source of micronutrients and dietary minerals, contributing to food and nutritional security of smallholder farmers. In East Africa, local vegetable production systems are highly heterogenous and characterized by a mixture of crops and cropping systems. Kale (*Brassica oleraceae* var. *acephala*) and cabbage (*Brassica oleracea var. capitata*) are the widely grown brassicas both for local consumption and export market. However, their productivity is low mainly due to several biotic and agronomic constraints including poor soils, pests and diseases which cause up to 100% yield loss if left unmanaged. Key pests of Brassicaceae include cabbage aphid (*Brevicoryne brassicae*) and diamondback (*Plutella xylostella*). To manage these pests, farmers resort to indiscriminate use of pesticides, which increase costs of production and are harmful to the growers, consumers, and environment. Borrowing from the successful maize-push-pull technology, we aimed at developing a vegetable push-pull cropping harnessing natural diversity to offer multiple ecological benefits. Through behavioural, electrophysiological and field evaluation, we developed a functional vegetable push-pull cropping system using rosemary (*Rosmarinus officinallis*) (push) and Ethiopian mustard (*Brassica carinata*) (pull) for managing key pests of brassicas. Laboratory assays showed that rosemary repelled cabbage aphids while Ethiopian mustard attracted the aphids more than the kale. Field evaluation showed reduction in pest infestation, increased parasitism and predation resulting to higher marketable and healthy kale vegetables. This system fits well within the mixed vegetable production systems in East Africa harnessing natural diversity to reduce pesticide use and increase vegetable production.

Efficacy of attract-and-kill SPLAT for management of spotted-wing drosophila in grapes and cherries in Greece

Eirini Anastasaki[1], Anastasia Kokkari[2], Stefanos Andreadis[3], Stavros Orfanos[1], Natali Kamou[2], Panagiotis Milonas[1], Teun Dekker[4], Apostolos Kapranas[2]
[1]*Scientific Directorate of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, Kifissia, Greece*
[2]*School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[3]*Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization "DEMETER", Thermi, Greece*
[4]*Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden*

The spotted-wing drosophila, *Drosophila suzukii* (SWD), is a polyphagous insect species that has been reported in Greece since 2014. SWD has become a major pest in berries and stone fruits. Current management programs for *D. suzukii* rely mainly on chemical pesticides with multiple repeated interventions during the growing season. We tested a promising Integrated Pest Management (IPM)-based practice, the attract-and-kill (A&K) method, wherein pesticides are applied to a specific point source along a lure, to different varieties of grapes and cherries in Northern Greece. The lure formulation consisted of olfactory attractants and feeding simulants specific to *D. suzukii* and pink coloring for visual attraction. The lure was formulated in a SPLAT medium (Specialized Pheromone and Lure Application Technology, ISCA Technologies, Inc.), where we added the insecticide spinosad (0.5% w/w) and applied it on the trunk of vineyards and cherry trees. The volatile profile of the lure was analyzed in weekly samples from the field by gas chromatography (GC). The lure was applied twice with a 14-d interval. The more volatile esters, ethyl lactate, and ethyl hexanoate had almost released all of their initial amounts within 2 weeks. The population of *D. suzuki* was monitored weekly with apple cider traps until harvest. Individuals of *D. suzukii* were identified morphologically and molecularly. In the experimental blocks, both *Drosophila* sp. and *D. suzu*kii catches in traps were significantly reduced either by the first or second SPLAT application, compared to control. The efficacy of the method and its adoption in IPM programs is discussed.

Possible usages of insect pheromone antagonists on pest control

Yinzhong Cui, **Yonghu Li**
Pherobio Technology Co., Ltd

Since the discovery of insect sex pheromone, great progress has been made in pest monitor and control, thanks to their advantages in safety, specificity, and non-resistance developed to the target pests. However, there are relatively fewer reports on insect pheromone antagonists, and the research on the mechanism is less clear compared to sex pheromones. Currently, the most comprehensive report is the cotton bollworm antagonist (*cis*-11-hexadecenol). In recent years, we have completed field tests of pheromone antagonists for a variety of pests, including *Spodoptera frugiperda*, *Spodoptera litura*, *Spodoptera exigua*, codling moth, etc., and found quite a few compounds which can achieve strong interference on sex-pheromone dispenser attraction. This experiment is currently only being tested in dispenser trials, and how to use the antagonist in the field still need to be explored.

Push-pull intercropping revisited: *Desmodium* does not repel ovipositing *Spodoptera frugiperda* but intercepts and decimates their offspring

Anna L. Erdei[1,2], Aneth B. David[1,3], Eleni C. Savvidou[1,4], Vaida Džemedžionaitė[1], Advaith Chakravarthy[1], Béla P. Molnár[2], Teun Dekker[1]
[1]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden
[2]Department of Chemical Ecology, Centre for Agricultural Research, Plant Protection Institute, Budapest, Hungary
[3]Department of Molecular Biology and Biotechnology, University of Dar-es-Salaam (UDSM), Dar-es-Salaam, Tanzania
[4]Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Volos Greece

The push-pull intercropping strategy has been widely praised for combining food security and ecosystem resilience in smallholder agriculture. According to the established mechanism, intercropping reduces the population of lepidopteran pests in maize fields through a stimulo-deterrent mechanism. A repellent intercrop ("push" plant), most commonly *Desmodium* spp., deters females from oviposition, while a dead-end border crop ("pull" plant) attracts them to the border where larvae cannot develop into adults.

Unexpectedly, we found that the volatile terpenoids known to deter moth oviposition were not detectable in the headspace of intact *Desmodium uncinatum* plants until after herbivory. This finding was supported by the observation that ovipositing female *Spodoptera frugiperda* were not repelled by intact *D. uncinatum* plants in behavioral bioassays. In search of an alternative mechanism, we found that neonate larvae preferred to feed on *D. uncinatum* rather than maize. However, the larvae's mobility on the plant surface was restricted and they were often impaled and immobilised by the dense network of hooked, silica-enriched trichomes. Furthermore, when arvae were fed a diet of *D. uncinatum*, no larvae survived.

Maize-*Desmodium* intercropping has inspired many efforts to apply this strategy to other pests. The additional knowledge of the underlying mechanism of this intercropping system can be used to support these efforts and rationally improve efficacy against other herbivore insects.

Halyomorpha halys fruit damage shifts but does not reduce when aggregation pheromone is used in apple orchards

Riccardo Favaro[1,2], Luca Cappellin[2], Michele Preti[3], Veronica Carnio[1], Sergio Angeli[1]
[1]Faculty of Agricultural, Environmental and Food Sciences, University of Bozen-Bolzano, Bolzano, Italy
[2]Department of Chemical Sciences, University of Padova, Padova, Italy; [3]Astra Innovazione e Sviluppo, Faenza, Italy

Halyomorpha halys (Hemiptera: Pentatomidae) is an invasive pest that inflicts significant damage to several crops. Our study examined the effects of placing "mini-sailboat" traps (MSB), a novel kind of aggregation pheromone-baited trap for *H. halys*, along the boundary of apple orchards. The spatial distribution of fruit damage was then assessed. The MSB trap has a sizable trapping surface, a visually appealing cue, and a huge sticky black sail with a water bin. 16 MSB traps were positioned along the boundaries of two apple orchard clusters extending for 1.3 kilometres. A visual evaluation of fruit damage was carried out at 107 points, progressively farther out from the orchard boundary. For every point, the incidence and severity of fruit damage were computed. According to our findings, placing MSB traps along the edge of the orchard significantly increased the fruit damage incidence for the first 45 metres from the trap; further away, the damage incidence decreased in comparison to the orchard without traps. There was a 50% cumulative damage incidence reduction from 85 m to 26 m from the traps. Our findings demonstrate the possible effectiveness of MSB traps in controlling the infestation of *H. halys* by limiting its damage in a border strip close to the traps. According to this study, using MSB traps could allow the application of insecticides more precisely, resulting in more successful pest management techniques.

Context-dependent responses of green lacewings to methyl salicylate (Neuroptera: Chrysopidae)

Sándor Koczor[1], Ferenc Szentkirályi[1], József Vuts[2], John C. Caulfield[2], David M. Withall[2], John A. Pickett[3], Michael A. Birkett[2], Miklós Tóth[1]
[1]HUN-REN Centre for Agricultural Research, Plant Protection Institute, Budapest, Hungary
[2]Protecting Crops and the Environment, Rothamsted Research, Harpenden, Hertfordshire, UK
[3]Cardiff University, School of Chemistry, Cardiff, UK

Herbivore-induced plant volatiles may provide information on the activity of phytophagous insects, thereby helping orientation of their natural enemies. Among these compounds, methyl salicylate is of special importance, several studies thus investigated its effects on predatory and parasitoid insects, including green lacewings (Chrysopidae). Although some studies found remarkable attraction of green lacewings to methyl salicylate, others reported moderate or no attraction.

To shed more light on potential mechanisms, methyl salicylate and known attractants for *Chrysopa* and *Chrysoperla* spp. lacewings were tested on their own and in combination in field experiments in Hungary.

Methyl salicylate weakly attracted the *Chrysoperla carnea* species complex, clearly outperformed by phenylacetaldehyde, a known floral attractant for these species. When presented in combination, methyl salicylate synergized attraction of both males and females. These results are in line with previous research performed in Hungary on *Chrysoperla* spp.

For *Chrysopa formosa*, methyl salicylate did not show activity, whereas nepetalactol, a known attractant for several *Chrysopa* spp., significantly attracted *C. formosa* males. When tested in combination, methyl salicylate synergized male attraction to nepetalactol. On the other hand, in combination with squalene, no synergistic effect was found.

In summary, the results suggest that the responses of green lacewings to methyl salicylate are context-dependent, and the ecological role of the compound may be more complex than previously expected.

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Extracting information from complex pheromonal profiles: Towards a better understanding of sustainable pest management

Weizhao Sun, Jan Buellesbach
University of Muenster, Institute for Evolution & Biodiversity, Muenster, Germany

The importance of parasitoids as biological control agents in integrated pest management has grown substantially in the past decades, revealing considerable advantages over pesticides for human health, agricultural quality and environmental sustainability. For optimizing their efficiency, it is of paramount importance to decipher their complex chemical communication systems which have remained largely elusive so far. The chemical ecological model system for parasitoid wasps, *Nasonia*, bears considerable potential to enhance our knowledge on the encoding mechanisms for biologically relevant chemical information. *Nasonia* females use complex cuticular hydrocarbon (CHC) profiles as sex pheromones enticing males to perform courtship and copulation behavior. We elucidated the chemical pattern conveying sexual attractiveness in these wasps as specific methyl-branching patterns essential for the functionality in sexual communication. We achieved this by functionally characterizing three fatty acid synthase genes mainly responsible for biosynthesizing the methyl-branched hydrocarbons and upholding the integrity of the CHC profiles above a detection threshold for the males. This advances our understanding of how genetic information can be translated into biologically relevant chemical information and reveals that sexual attractiveness can have a comparably simple genetic basis. Transcending these valuable insights for general chemical ecology and genetics, furthering our knowledge on the exact mechanisms behind the complex mating system of this prominent parasitoid model organism bears great potential to optimize its rearing conditions and sustainability for integrated pest management. This very viable, eco-friendly alternative to the wide application of destructive and biodiversity-reducing pesticides constitutes an important stepstone towards a more sustainable, human-health driven and eco-friendly agriculture.

Biosensor array based Odorant Binding Proteins and Odorant Receptors for detection of Red Palm Weevil infestation of palm trees

Khasim Cali[1], Binu Antony[2], Emmanuelle Jacquin-Joly[3], Corrado Di Natale[4], Arnab Pain[5], Krishna Persaud[1]
[1]The University of Manchester, Department of Chemical Engineering, Manchester, M13 9PL, UK
[2]King Saud University, Chair of Date Palm Research, Center for Chemical Ecology and Functional Genomics, College of Food and Agricultural Sciences, Riyadh 11451, Saudi Arabia
[3]INRAE, Sorbonne Université, CNRS, IRD, UPEC, Université Paris Cité, Institute of Ecology and Environmental Sciences of Paris, iEES-Paris, 78000, Versailles, France
[4]University of Rome Tor Vergata, Dept. of Electronic Engineering, 00133 Rome, Italy
[5]King Abdullah University of Science and Technology (KAUST), Bioscience Programme, BESE Division, Thuwal, Jeddah 23955-6900, Saudi Arabia

The Red Palm Weevil (RPW) (*Rhynchophorus ferrugineus*) feeds on the internal tissues of palm trees, causing damage or death to the tree. Detection of semiochemicals emitted by the palm weevil may be a powerful method for early detection if appropriate detection technologies can be developed. The elucidation of the genes involved in chemical sensing gives the basis for developing sensing devices capable of detecting semiochemicals. The RPW Odorant Binding Proteins RferOBP1768 and RferOBP23 genes as well as the aggregation pheromone receptor RferOR1, were expressed to produce synthetic proteins. In addition, *in silico* modelling identified two mutant OBPs, RferOBP23_ R49L, and RferOBP1768_ Q12V, capable of binding the aggregation pheromones, ferrugineol, and ferrugineone, with potentially better affinity than wild type proteins. After *in vitro* characterization, the proteins were immobilized onto 20MHz quartz crystal microbalance (QMB) transducers, the OR being stabilized via immobilization in lipid nanodiscs while OBPs were immobilized covalently on self-assembled monolayers of thioctic acid on the QMB. The resulting sensor array was tested against the aggregation pheromone and other environmentally relevant chemicals, then tested in the date palm field in Riyadh, Saudi Arabia, showing good differentiation between RPW-infested and non-infested palms. This may provide a tool for early detection and effective pest management of RPW.

Developing push-pull management strategies for fruit flies using oviposition deterrent as a push component

Dong H. Cha[1], Vijaya Movva[1,2], Binita Shrestha[3], Gregory M. Loeb[3], Jerry Zhu[4]
[1]Pacific Basin Agricultural Research Center, USDA-ARS, Hilo, Hawaii, USA
[2]Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee, USA
[3]Cornell AgriTech, Cornell University, Geneva, New York, USA
[4]Agroecosystem Management Research Unit, USDA-ARS, Lincoln, Nebraska, USA

A push-pull management strategy combining an attractant and a spatial repellent has been explored widely as a behaviorally-based approach for managing various insects. One of the main challenges in using a volatile spatial repellent as the ‘push’ component is the difficulty of maintaining a proper concentration of the repellent in the field. We recently identified a series of less volatile fatty acids-based oviposition deterrents for oriental fruit fly, melon fly and spotted wing drosophila. In this talk, we discuss the identification and behavioral mode of the oviposition deterrents and provide updates on on-going laboratory and field research evaluating the efficacy of a spatial repellent vs. oviposition deterrents to improve push-pull management systems for fruit flies.

POSTERS

P-03

Do options matter? Choice and no-choice assays together reveal chemical mediated resistance of cruciferous plants to the pollen beetle

Nadine Austel, **Christoph Böttcher, Ulrike Temp, Torsten Meiners**
Julius Kuehn Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection,
Königin-Luise-Straße 19, 14195 Berlin, Germany

The pollen beetle (*Brassicogethes aeneus*) is one of the major insect pests in oilseed rape (*Brassica napus* L.; Brassicaceae) causing significant yield losses. Before performing an intergeneric transfer of resistance against adult pollen beetles into oilseed rape, we have screened a wide range of *Brassica napus* accessions, *B. napus* resyntheses and wild crucifer relatives (Austel et al. 2021, PCE 44(2): 519–534). For the screening and the detection of antifeedant kairomones, we have evaluated both a no-choice bioassay on intact plants and a dual-choice assay with detached flower buds under controlled conditions. In the no-choice assay no natural resistance was observed in *B. napus* and its resyntheses, while accessions of *Sinapis alba*, *Eruca sativa* and *Barbarea vulgaris* suffered very little feeding damage. These observations were confirmed by the dual-choice assays in which a flower bud of certain accessions and/or species was offered to a male or female pollen beetle together with a standard flower bud, although not as finely-tuned as in the no-choice assays. The dual-choice assay with single flower buds and the application of crude plant extracts or standard compounds very well revealed the effect of secondary plant compounds on the feeding behaviour of the pollen beetle. While flavonoids (aglycons as well as their glucosides) did not show any deterrent effect, certain aromatic glucosinolates showed strong deterring effects on feeding pollen beetles. In conclusion, both choice and no-choice assays are needed to obtain a full view of the host plant – insect interaction and its mediation by plant compounds.

P-08

Blissus sp (Hemiptera: Lygaeidae) produce two males specific sesquiterpenoids with potential as sex pheromone

Maria Carolina Blassioli-Moraes[1], Beatriz Cardoso [2], Anariel Gomes Campos[2],
Marcus Vinícius de Sá G. Silva[2], Raúl Alberto Laumann[1], Miguel Borges M[1], Elizângela G. Fidelis[1]
[1]Semiochemicals Laboratory, Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil
[2]Students granted fellowship at Semiochemicals Laboratory, Embrapa Genetic Resources and Biotechnology

The stink bug *Blissus* sp is an important pest of pasture-grass in Brazil. The aim of this study was to identify the semiochemicals, i.e., defensive compounds from metathoracic (MTG) and dorsal abdominal (DAG) glands and sex or aggregation pheromones of this species. To collect the pheromones, volatile collections from males and females were conducted using an air-entrainment system. The insects were placed into glass chambers, and volatiles collected for 24 hours. To collect the defensive compounds the MTG from adults and DAG from nymphs were extracted using a liquid extraction with hexane. The volatile samples and glands extracts were analysed by GC-FID and GC-MS. The results showed that males and females and nymphs produce the typical defensive compounds of stink bugs, with (*E*)-2-hexenal and (*E*)-2 hexenyl acetate been the major components in adults and hexenal and 4-oxo-(*E*)-2-hexenal major components in nymphs. Additionally, males produce two specific sesquiterpenoid compounds, not present in the volatile sample of females. Further studies are being conducted to evaluate if these two male-specific components work as sex or aggregation pheromone to this stink bug species and, to elucidate the chemical structure of the compounds.

P-09

Two male specific compounds from the green belly stink bug *Diceraeus melacanthus* with potential to role as an aggregation pheromone

Maria Carolina Blassioli-Moraes, Miguel Borges, Raul Alberto Laumann R.A, Mirian Fernandes Furtado Michereff
Semiochemicals Laboratory, Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil

The stink bug *Diceraeus melacanthus* is an important pest of soybean and maize crops. Pheromones can provide useful tools for monitoring this species in crop areas. Therefore, the objective of this study was to evaluate if *D. melacanthus* produces sex-pheromone. For this, volatile collections from males and females were conducted using an air-entrainment system. The insects were placed into glass chambers, and volatiles collected for 24 hours. The volatile samples were analysed by GC-FID and GC-MS. The response of males and females of *D. melacanthus* to the odour of conspecific of both sex, volatile samples and fractions of volatile samples was evaluated *Y*-olfactometer bioassays. The results showed that males and females produce the typical defensive compounds of stink bugs, that were already reported[1]. Additionally, males produce two specific compounds, not present in the volatile sample of females. The bioassays results showed that females prefer the male odour (p=0.005), while males and female did not show a preference to females' odour when contrasted to air. Neither did males prefer the male odour when contrasted to air. In addition, females (p=0.019) and males (p=0.019), preferred the odour emitted from a volatile sample fraction containing the two male specific compounds. Further studies are being conducted to evaluate if these two male-specific components work as an aggregation pheromone to this stink bug species and, to elucidate the chemical structure of the compounds.

P-13

Function and evolution of weevil pheromone receptors

Ludvine Brajon[1], Arthur Comte[1,2], Rémi Capoduro[1], Emmanuelle Jacquin Joly[1], Nicolas Montagné[1]
[1]Institut d'écologie et des sciences de l'environnement de Paris – iEES Paris, Sorbonne Université, France
[2]Institut de Chimie de Nice – ICN, Université Côte d'Azur, Nice, France

With more than 80 000 species, Curculionidae beetles (gathering weevils and bark beetles) display an unparalleled diversity and constitute the largest family of animals on Earth. These phytophagous insects are adapted to a multitude of host plants, and some are serious pests of crops, forests or stored products. They can cause real damage to multiple crops, such as rapeseed, carrots, or date palms. They use an aggregation pheromone to colonize their host plants in order to feed and mate. To detect their aggregation pheromone, beetles have specific odorant receptors (ORs) called pheromone receptors (PRs), expressed in sensory neurons of their antennae. Currently, only pheromone receptors are known in 3 different Curculionidae species: *Rhynchophorus ferrugineus*, *Rhynchophorus palmarum*, and *Ips typographus*. These 8 PRs belong to 5 different lineages in the OR phylogeny. Surprisingly, some weevil PRs can detect VOCs from host plants in addition to pheromone compounds. The global aim of this project is to discover new PRs in Curculionidae, and characterize their function. Moreover, it will be interesting to understand how PRs have evolved and how many times they have appeared in evolution.

This could be the starting point for developing or improving integrated pest management solutions for beetle pests, based on a reverse chemical ecology approach and targeting pheromone receptors.

P-15

Responses of three entomopathogenic nematode species from the genus *Steinernema* to ethanol and 1-nonene

Rasa Čepulytė[1], **Deimantė Tiškevičiūtė**[1], **Evelina Osinska**[2], **Vincas Būda**[1]
[1]*Laboratory of Chemical and Behavioral Ecology, Institute of Ecology, Nature Research Centre, Akademijos 2, 08412, Vilnius, Lithuania*
[2]*Vilnius University, Life Sciences Centre, Saulėtekio Ave 7, LT-10257 Vilnius, Lithuania*

Entomopathogenic nematodes (EPNs) are parasites of insects and are applied as an environmentally friendly tool for controlling economically important pests. Knowledge of how infective juveniles (IJs) find their hosts could help increase their effectiveness. Chemical signals in the soil environment undoubtedly play an important role in directing IJ toward suitable insects, however, exactly which little is known.

Two novel behaviorally active compounds for EPNs' *Steinernema feltiae*, *S. carpocapsae*, and *S. kraussei* IJs were revealed. Dose responses of 1-nonene (released by EPN-infected insect cadaver) and ethanol (1-nonene solvent) revealed different behavior in the three species indicating interspecific differences in foraging. 1-Nonene was attractive to *S. carpocapsae*; ethanol to *S. feltiae* and *S. kraussei* (at high concentrations each). *Steinernema kraussei* was repelled by high concentrations of 1-nonene. Meanwhile, 1-nonene was both attractive and repellent for *S. feltiae* depending on the concentration: low were attractive, while high were repellent. Among the species analyzed, *S. carpocapsae* differed in demonstrating attraction to high concentration of 1-nonene (released by larval cadavers of late-stage nematode infection) which is more in line with the scavenger's feeding strategy. The study on chemicals involved in EPN behavior control sheds light on understanding the general biology of nematodes. EPN behavior peculiarities could be taken into consideration for EPN application in sustainable pest control.

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P-18

Behavioral response of *Plodia iterpunctella* Hübner (Lepidoptera: Pyralidae) larvae to volatile compounds from stored amaranth sticks

Mariana Cruz-Díaz[1], Victor Castrejón-Gomez[1], Humberto Reyes-Prado[2]
[1]*Departament of Chemical Ecology, Instituto Politecnico Nacional, Carretera Yautepec-Jojutla Km 8.5, calle Ceprobi No.8, San Isidro 62731, Yautepec, Morelos, Mexico*
[2]*Chemical ecology laboratory. Univerdidas Autonoma del Estado de Morelos Campus Jicarero, Carretera Galeana-Tequesquitengo s/n, Comunidad El Jicarero, Jojutla, Morelos, Mexico*

In 2020 in the state of Morelos, Mexico, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) was identified as a new pest of stored amaranth products. It was found that, although the insect can survive and reproduce in all amaranth-based products, sweet foods favor it, providing it with a shorter biological cycle and higher oviposition and life expectancy. Therefore, we developed an evaluation of the behavior of the larva to choose its food in the amaranth stores.

The bioassay was conducted in the chemical ecology laboratory, using third-stage larvae with one day of fasting, which were given a choice between volatiles of amaranth with chocolate, natural amaranth, amaranth with honey and a control, in a 4-way olfactometer. A current of pure air, chocolate, honey, and cellophane (bar wrapper) were used as controls. Finally, the volatile compounds were extracted from the amaranth bars with the dynamic aeration extraction technique, using "Super Q" as adsorbent material and were identified by gas chromatography coupled with mass spectrometry.

As a result, amaranth with chocolate and with honey are the food most chosen, showing a significant difference between them when using air and chocolate as controls (Chi-square test with Yates correction $p=<0.05$), however, there is no significant difference when changing the controls for honey and cellophane. Chocolate was the most attractive control for the insects, and the one that caused a significantly faster response (Anova test $F=16.889$, $p<0.05$), the air was the control that caused a greater dispersion of insects.

Twenty-three main compounds of the amaranth were identified.

P-20

Codling moth mate choice depends on both host plant odour and sex pheromone

Anna Laura Erdei, Maria Sousa, Francisco Gonzalez, Marie Bengtsson, Peter Witzgall
Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

The great diversity of specialist plant-feeding insects suggests that host-plant shifts can initiate speciation even in the absence of geographical barriers. Sex pheromones are thought to be able to attract insects, particularly moths, on their own. However, in the natural environment, pheromones are not perceived in isolation, but are released into a complex mixture of odours, including those emitted by host and non-host plants. We show that the sex pheromone of the codling moth (*Cydia pomonella*) is effective in attracting males only in the presence of host plant odours. In the vicinity of non-host trees, male attraction to the sex pheromone is greatly reduced. By blending the synthetic pheromone codlemone with kairomone pear ester, a strong host plant attractant, attraction to the pheromone can be completely rescued in non-host vegetation, demonstrating that this host plant-associated olfactory cue is an integral part of sexual communication.

P-21

Semiochemicals from Cattle Dung as a Potential Alternative to the Horn Fly Management

Javier Espinoza[1,2], **Joaquín Arriagada**[1], **Isabel Soto**[1,3], **Washington Aniñir**[1,3], **Marcelo Lizama**[1,4,5], **Andrés Quiroz**[1,2]
[1]Laboratorio de Química Ecológica, Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Av. Francisco Salazar 01145, Temuco, Chile
[2]Centro de Excelencia en Investigación Biotecnológica Aplicada al Medio Ambiente. Universidad de La Frontera, Temuco, Chile
[3]Doctorado en Ciencias de Recursos Naturales. Universidad de La Frontera, Temuco, Chile
[4]Doctorado en Ciencias Agroalimentarias y Medioambiente, Universidad de La Frontera, Temuco, Chile
[5]Laboratorio de Entomología Aplicada, Facultad de Ciencias Agropecuarias y Medioambiente, Universidad de La Frontera, Temuco, Chile

Hematobia irritans, commonly known as the horn fly, is an obligated hematophagous ectoparasite of cattle, considered one of the most important pests of global livestock production. Management of *H. irritans* relies heavily on broad-spectrum pesticides. Regrettably, the use of these pesticides is harmful to the environment and has led to the development of resistant fly populations. Therefore, alternative control methods are needed. The use of semiochemicals involved in horn fly intraspecific communication is one of the most promising alternatives. In this scenario, considering that four of five stage of the *H. irritans* life cycle occurs exclusively in dung, volatile compounds from cattle dung should play an important role in the horn fly behaviour. Thus, these semiochemicals could be used to management this pest. At present, volatile compounds emanating from cattle dung and their role in the *H. irritans* behaviour are not clearly known. In this study, the monoterpenes α-pinene, β-pinene, and limonene were identified by GC/MS from cattle dung volatile blends which were captured by SPME. These monoterpenes were attractants to horn flies in a Y-tube olfactometer, where β-pinene was the most attractant. All monoterpenes showed antifeedant activity and an important fly mortality in no-choice tests, but limonene was the best antifeedant and insecticidal. Moreover, α-pinene and limonene elicited the horn fly oviposition in a two-choice test, whilst β-pinene was a deterrent of the *H. irritans* oviposition. The implications of these results in relation to the horn fly management will be discussed here.

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P-25

Early detection of virus-infected seed potatoes using volatile fingerprints

Sophie Heijblom, **Silke Allmann**
Plant Physiology, department of Green Leaf Sciences, Swammerdam Institute for Life Sciences, University of Amsterdam. Amsterdam, the Netherlands

Plant viruses are a problem in the seed potato industry, due to their detrimental effects on yield, rapid spread and late detection in the field. To monitor the abundance of viruses, batches of seed potatoes are randomly screened before and after harvest. However, large-scale testing of individual tubers before planting is currently not possible.

In this project, we research an alternative method to enable large-scale early detection of virus-infected seed potatoes. We aim to achieve this by using *volatile organic compounds* (VOCs) that are released from potato tubers. Plant VOCs are chemically diverse secondary metabolites that are essential for the plant's communication with its environment. Previous research has shown that the quantitative and qualitative composition of the VOC blend from plant *shoots* changes in response to biotic and abiotic stressors, including several potato viruses. However, such a change has not been studied yet for potato tubers.

Here, we will investigate the distinctive volatile profiles of healthy potato tubers compared to those infected with either Potato virus Y (PVY) or Potato virus S (PVS). The volatiles collected from tubers grown under controlled greenhouse conditions will be analysed using gas chromatography-mass spectrometry (GC-MS) to establish a virus-specific 'volatile fingerprint.' Subsequently, we will validate the robustness of the distinctive changes by comparing the volatile profiles of healthy and infected tubers grown in the field.

These volatile fingerprints will form the basis for the subsequent development of a new, volatile-based diagnostic method for detecting potato viruses in an early stage of infection.

P-28

Monitoring of *Lobesia botrana* using attractive semiochemicals and LED-UV in vineyards of Central Chile and Catalonia, Northeastern Spain

Yasir Islam[1], Michele Preti[2], Alan L. Knight[3], Esteban Basoalto[4], Dolors Bosch[5], Ainara Peñalver-Cruz[5], Marcela A. Rodríguez[6], Eduardo Fuentes-Contreras[1]
[1]*Centro de Ecología Molecular y Funcional (CEMF), Facultad de Ciencias Agrarias, Universidad de Talca, Talca, Chile*
[2]*ASTRA Innovazione e Sviluppo Test Facility, Faenza, Italy*
[3]*Instar Biologicals, Yakima, Washington, USA*
[4]*Facultad de Ciencias Agrarias, Instituto de Producción y Sanidad Vegetal, Universidad Austral de Chile, Valdivia, Chile*
[5]*Sustainable Plant Protection (Entomology), Institute for Agrifood Research and Technology (IRTA), Lleida, Spain*
[6]*Laboratorio de Entomología Aplicada, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile*

*Lobesia botran*a (Lepidoptera: Tortricidae), is a significant pest of vineyards in Europe, Middle East, Argentina, and Chile. Monitoring of adult males of this pest is performed with sexual pheromone, but this technique is not effective in vineyards treated with mating disruption (MD). This study evaluated the effectiveness of a blend of 2-phenylethanol (2-PET) and acetic acid (AA) with light emitting diodes (LEDs) of different wavelengths: i) ultraviolet (UV), ii) green, and iii) blue, compared to 2-PET+AA without LEDs and standard sex pheromone lures, for monitoring *L. botrana* populations. The trials were performed in vineyards of Catalonia (NE Spain) and Maule (Central Chile). The research was conducted in Spain for second and third flights from the start of June (early summer) to end of August (late summer) in 2023, and in Chile from late September (early spring) to the end of March (late summer) in 2023/2024. Pheromone traps captured only male *L. botrana* adults, while 2-PET and AA, with or without LED-UV, green, and blue LEDs, attracted both male and female adults. During late summer, the blend of 2-PET and AA with LED-UV significantly captured more males and females compared to other treatments. The blue and green LEDs also attracted *L. botrana*, but with lower capture rates than LED-UV. Non target capture of other Lepidoptera, Diptera, Trichoptera, and Neuroptera were also captured. Our findings suggest that 2-PET and AA, especially with LED-UV light, improved monitoring of *L. botrana* in vineyards, offering valuable insights for pest management in viticultural regions.

P-39

Mating disruption of *Conogethes punctiferalis* (Lepidoptera: Crambidae) in chestnut orchards

Junheon Kim[1], Seongchae Jung[2], Young Un Kim[2]
[1]*Forest Entomology and Pathology Division, National Institute of Forest Science, Seoul, 02455, Republic of Korea*
[2]*AD Corp., Andong, Gyeongsangbuk-do, 36729, Republic of Korea*

Chestnut (*Castanea crenata* Siebold & Zucc.) is one of the major agroforest products in Korea, and *Conogethes punctiferalis* is a major pest of chestnut fruit. Pheromone-based mating disruption (MD) has emerged as a promising eco-friendly approach to reduce population levels and finally to reduce the damage of fruits. Field trials were conducted over two years (2022-2023) in two commercial chestnut orchards in central Korea, Orchard A and Orchard B, both infested with *C. punctiferalis*. The MD treatments effectively reduced male captures of *C. punctiferalis* in MD treatment plots compared to control plots. Mating inhibition rates ranged from 70.5% to 95.1% in 2022 and from 87.8% to 94.9% in 2023. MD efficacy (%) was calculated based on the total number of chestnut fruits collected and the number of damaged chestnut fruits by *C. punctiferalis*. In 2022, MD efficacy by 50 mg/ha amount (TS) in Orchard A was 73.4%, and in Orchard B was 79.1%. In 2023, MD efficacy by double amount treatment (TD, 100 mg/ha) and twice treatment (TT, 50 mg/ha at June and August) in Orchard A was 59.8% and 78.2%, respectively, and in that in Orchard B was 50.9% and 64.9%.The study confirms the efficacy of pheromone-based MD in reducing *C. punctiferalis* populations in chestnut orchards and damage to chestnut fruits.

P-44

Semiochemicals from byproducts of sugarcane mills attractive to *Stomoxys calcitrans*

Raúl Alberto Laumann[1], **Beatriz Guedes**[1,2], **Miguel Borges**[1], **Maria Carolina Blassioli-Moraes**[1], **Paulo Henrique Duarte Cançado**[3], **Antonio Thadeu Medeiros de Barros**[3]
[1]*Semiochemicals Laboratorys, Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil*
[2]*Zoology Postgraduate Program, Universidade de Brasília – UnB, Brasília, DF, Brazil*
[3]*Embrapa Gado de Corte, Campo Grande, MS, Brazil*

The stable fly, *Stomoxys calcitrans* (Diptera: Muscidae), is considered a pest to livestock in several countries due to its blood-feeding habits. In some regions of Brazil, the stable fly outbreaks have been associated with organic byproducts, generated in sugarcane and alcohol mills, which favors the oviposition and development of immature stages of this fly. The massive proliferation of the stable fly in mills and subsequent feeding on livestock farms demands efficient methods for outbreak prevention. Insecticide-impregnated traps are a valuable option in integrated management strategies, potentially becoming more efficient with adding chemical attractants. The present study aimed to select volatiles from sugarcane and alcohol mill byproducts attractive to adults of *S. calcitrans*. In olfactometry bioassays three byproducts: vinasse, filter cake, and vinasse sediment sludge, were attractive to stable flies. Preference bioassays, conducted in a four-choice olfactometer, showed that filter cake was the most attractive substrate for these flies. To identify the most attractive compounds, aeration of the substrates and electroantennography studies were conducted. Chemical analysis of volatile samples of each substrate showed differences in compound composition. Olfactometry bioassays confirmed that filter cake aeration volatile samples were more attractive than vinasse and sediment sludge samples. Electroantennography analysis revealed that twelve volatile compounds from the filter cake volatile samples elicited electrophysiological responses. A blend of eight electrophysiological active compounds showed significant attraction to stable flies in bioassays and is under investigation as a potential synthetic attractant.

P-50

Enhancing soybean field monitoring: Evaluating the efficacy of *Euschistus heros* pheromone nanoemulsion for smart pest control

Mirian F. F. Michereff[1], João Vitor Machado[1], Miguel Borges[1], Raúl A Laumann[1], Luciano P Silva[2], Maria Carolina Blassioli-Moraes[1]
[1]Semiochemicals Laboratory, Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil
[2]Nanotecnology Laboratory, Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil

The use of the *Euschistus heros* sex pheromone can be a valuable tool to monitoring its presence in soybean field, providing information for a more efficient and smart control application. The objective of this study was to evaluate the efficacy of a nanoemulsion containing the *E. heros* pheromone, the compound methyl 2,6,10 trimethyltridecanoate. For this, bioassays in Y-olfactometer were conducted with female's *E. heros* responding to the odour released by the nanoemulsions and to evaluate the effect of these nanoemulsions on soybean plants and in the egg parasitoid *Telenomus podisi* foraging behaviour, 1 ml of the nano emulsion containing 1mg of the pheromone was sprayed in the leaves of soybean plants. The release rate of the pheromone from the soybean plants was monitored along 29 consecutive days, and the attractiveness of these plants to *T. podisi* was evaluated along the same period. The results showed that *E. heros* females were attracted to nanoemulsions containing the pheromone along the first 15 days and *T. podisi* females were attracted to plants treated with the nanoemulsion at days 5, 9, 19 and 23 after spraying. The response of the egg parasitoid appear to be related also with the level of (*E*,*E*)-⊠-farnesene and methyl salicylate; that had their emission enhanced on sprayed plants compared to untreated plants. The nanoemulsion is a promising tool for use in soybean fields as it is biodegradable, easy to apply and induce the emission of soybean volatiles that are involved in the attraction of natural enemies.

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P-55

Electrophysiological and behavioral responses of cabbage aphid (*Brevicoryne brassicae*) to rosemary (*Rosmarinus officinalis*) volatiles, a potential push plant for vegetable push-pull cropping system

Bretor Katuku Mutua[1,2], Thomas Dubois[1], Komivi Senyo Akutse[1,3], Benjamin Muli[2], Edward Nderitu Karanja[1], Daniel Munyao Mutyambai[1,4]
[1]International Centre of Insect Physiology and Ecology, P. O. Box 30772-00100, Nairobi, Kenya
[2]Department of Agricultural Sciences, South Eastern Kenya University, P.O Box 170-90200, Kitui, Kenya
[3]Unit for Environmental Sciences and Management, North- West University, Potchefstroom 2520, South Africa
[4]Department of Life Sciences, South Eastern, Kenya University, P.O Box 170-90200, Kitui, Kenya

The cabbage aphid (*Brevicoryne brassicae*) is a major pest of kale (*Brassica oleraceae* var. *acephala*), an important vegetable that is grown worldwide due to its high nutritional and economic value. *Brevicoryne brassicae* poses a great challenge to *B. oleraceae* var. *acephala* production, causing significant direct and indirect yield losses. Farmers overly rely on synthetic insecticides to manage the pest, with limited success owing to its high reproductive behavior and development of resistance. This necessitates a search for sustainable alternatives to mitigate these challenges. This study assessed behavioral responses of *B. brassicae* to odors from rosemary (*Rosmarinus officinalis*) and *B. oleraceae* var. *acephala* headspace volatiles in a Perspex four-arm olfactometer. We identified and quantified volatiles emitted by each of the two plants and those eliciting antennal responses using coupled gas chromatography-mass spectrometry (GC-MS) and GC-electroantennograhic detection (GC-EAD), respectively. Our findings revealed that *B. brassicae* spent more time in the arms of the olfactometer that contained *B. oleraceae var. acephala* volatiles compared to the arm that held *R. officinalis* volatiles. GC-MS analysis revealed diverse and higher quantities of volatile compounds in *R. officinalis* compared to *B. oleraceae* var. *acephala.* GC-EAD analysis showed that antennae of *B. brassicae* detected linalool, α-terpineol, verbenone, geraniol, camphor, and borneol from the volatiles of *R. officinalis*, and sabinene, γ-terpinene, and β-caryophyllene from *B. oleraceae* var. *acephala* volatiles. Our findings demonstrate the potential of *R. officinalis* as a repellent plant against *B. brassicae* and could be utilized as a 'push' plant in an intercropping strategy against this pest.

P-68

Eurasian spruce bark beetle cytochrome P450 and glutathione S-transferases play a crucial role against conifer monoterpene defence

Sellamuthu Gothadapani*, Zubair Ashraf, Aisha Naseer, Roman Modlinger, Jaromir Hradecky, Amit Roy
Faculty of Forestry and Wood Science, Czech University of Life Sciences Prague, Czech Republic

Ecosystem functioning is influenced by the complex interactions between plants and herbivores, especially in forest ecosystems where bark beetles constantly attack trees like Norway spruce (*Picea abies*). Conifers have formidable defensive mechanisms triggered by foreign elicitors, which result in the synthesis of allelochemicals (i.e., monoterpenes). Alternatively, bark beetles (*Ips typographus*) have developed detoxifying strategies to overcome or withstand conifer defences. Here, we investigated the gene expression plasticity in bark beetles after exposure to the conifer host with different defence vigour to identify bark beetle genes or gene families that responded to higher amounts of monoterpene challenge. We used an RNA-seq-based approach followed by independent monoterpene exposure and enzymatic assays to accomplish the study goals. Our results revealed the ecological relevance of having expanded cytochrome P450 and glutathione S-transferases in these beetles. We gathered additional evidence that these gene families in bark beetles were actively involved in defence against conifer monoterpenes. Further downstream studies on selected detoxifying enzymes will reveal the functional relevance of those genes and may provide the key targets for future bark beetle management using molecular tools like RNA interference (RNAi).

P-81

Efficacy of EPNs against invasive pest *Otiorhynchus salicicola* adults: Behaviorally active compounds and lethality

Deimantė Tiškevičiūtė, Rasa Čepulytė, Sandra Radžiutė, Vincas Būda
Laboratory of Chemical and Behavioral Ecology, Institute of Ecology, Nature Research Centre, Vilnius, Lithuania

Entomopathogenic nematodes (EPNs) are obligate parasites of insects and are applied as environmentally friendly means for controlling economically important pests. Although EPN biopesticides are widely available, little is known about EPN-insect chemoecological interactions. Recently a broad-spectrum polyphage weevil *Otiorhynchus salicicola* had spread massively in Europe causing significant damage to plants, particularly to raspberries and strawberries. Despite EPNs are suggested for biocontrol of some weevils, no evidence on the efficacy of these products on *O. salicicola* is available. In this study, we tested the response of three species of EPNs from the genus *Steinernema* to exudates and frass of *O. salicicola* adults. The two-choice behavioral test showed that *O. salicicola*-related compounds were attractive to EPNs and some behaviorally active ones were revealed. We also evaluated the effectiveness of each EPN species on adult weevil mortality and the best performing EPN species in terms of concentration and timing was determined. Therefore, our results contribute to a better understanding of EPN and *S. salicicola* chemical interactions.

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P-88

Early monitoring and detection of insect pests in agriculture using plant volatiles

Sergio E. Ramos[1], **Christian Geckeler**[2], **Stefano Mintchev**[2], **Meredith C. Schuman**[1]
[1] *Spatial Genetics Lab, Department of Geography, and Chemistry, University of Zurich, Zurich, Switzerland*
[2] *Environmental Robotics Lab, Department of Environmental Systems Science, ETH, Zurich, Switzerland*

Arthropod pests are among the main causes of crop yield and economic losses, accounting for around 20% of global annual losses. Early detection of pests in crop fields is key for targeted management, allowing farmers to reduce pesticide costs and environmental impact and minimize crop losses. Herbivore-induced plant volatiles (HIPVs) are emitted immediately upon herbivore damage to plants and with increasing complexity over time following the initial attack, and as herbivores continue to feed. HIPVs hold a great potential to be used as early-stress signals to detect the attack of arthropod pests at their earliest possible stages. Nevertheless, collecting and detecting HIPVs in open headspace under realistic crop field conditions is not trivial, as volatiles are not preconcentrated and can be lost due to oxidation reactions under open air. Here, we present preliminary results from experiments conducted under semiopen conditions in maize where self-made and low-cost volatile vacuum robots can be deployed to collect open headspace samples. Additionally, we highlight the challenges associated with volatile collection and identification when dealing with realistic open-field conditions, and suggest how HIPVs can be effectively used for the early detection of insect pests.

S19: Marine chemical ecology

Chairs: *Jörg Hardege, Christina Roggatz*

ORAL PRESENTATIONS

Chemical ecology in the Antarctic marine benthos: The challenges of human pressures and natural hazards

Conxita Avila
BEECA Department (Invertebrates), Faculty of Biology, Universitat de Barcelona, Spain

In recent years, our research group at the Universitat de Barcelona has been experimentally analyzing and describing the human pressures and natural hazards that challenge the organisms living in the Antarctic marine benthos. We aim to ascertain how factors such as increasing sea water temperatures and ocean acidification affect these benthic organisms and thus how changes in their ecological interactions and chemical ecology are also modified. We also analyzed the effects of all these changes on symbiosis, diseases, and the appearance of invasive species. Some species were artificially exposed to higher than environmental temperatures in the laboratory to determine potential changes in their natural compounds and also in their microbiomes. In other experiments, crude extracts from selected species were tested against potential allopatric predators in order to understand the potential impact of the arrival of invasive species. Examples in sponges, cnidarians, molluscs, tunicates and others will be presented as a summary of these research topics. Overall, our results indicate that marine benthic communities in Antarctica are exposed to potentially dramatic changes in the next years as global change unfolds.

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How the carpet shell clam (*Ruditapes decussatus*) responds to amino acids, predators, and conspecific and heterospecific bivalve cues: Electrophysiological responses of the osphradium

Ana Rato[1,2*], Sandra Joaquim[2,3], Domitília Matias[2,3], Peter C. Hubbard[1]
[1]Centre of Marine Sciences (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, Faro, Portugal
[2]Department of Sea and Marine Resources, Portuguese Institute for Sea and Atmosphere (IPMA, I.P.), Olhão, Portugal
[3]Interdisciplinary Centre of Marine Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal

Sensing chemical cues is essential for several aspects of bivalve biology, such as detection of food and predator avoidance. However, little is known about chemosensory systems in bivalves and how they perceive the surrounding environment. Considering this, electrophysiological responses were recorded from the osphradium of the carpet shell clam, *Ruditapes decussatus*. Stimuli from different odorant groups [amino acids, bile acids; predator-released cues and signals from conspecific and heterospecific bivalves (mussels and oysters)] were used. As in oysters, the osphradium in clams proved to be sensitive to most proteinogenic L-amino acids, evoking negative, tonic, and concentration-dependent responses. However, it was insensitive to acidic amino acids (L-glutamic and L- aspartic acid), L-arginine and to bile acids (cholic, taurocholic and taurolithocholic acid). Interestingly, crab-released cues failed to evoke any response, whether fed or unfed. Nevertheless, cues from injured bivalves (conspecific and heterospecific) evoked strong responses. Similar to other bivalves, the osphradium in clams proved to be a chemosensory organ, detecting putative food-related odorants and alarm cues. That clams cannot detect predator-released cues, even those from unfed crabs, may indicate that they use cues released by injured prey to avoid predators. Further research is needed to understand how clams make use of such sensory input by correlating the current results with behavioural analysis.

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Conspecific chemical and visual cues/signals that attract coastal barnacle larvae

Takefumi Yorisue[1,2], **Shinnosuke Mori**[3], **Kiyotaka Matsumura**[4]
[1]*Institute of Natural and Environmental Sciences, University of Hyogo, Hyogo, Japan*
[2]*Museum of Nature and Human Activities, Hyogo, Japan*
[3]*Department of Biology, Keio University, Yokohama, Japan*
[4]*School of Marine Science, Kitasato University, Sagamihara, Japan*

Larval settlement is an important process that drives population and community dynamics of marine invertebrates. Barnacles are the one of the suitable animals for the study of larval settlement because of the ease of conducting laboratory assays on larval settlement. While it is well known that barnacle cyprid larvae sense chemicals secreted from adult barnacles, there is limited information on how they to specific cues in order to locate settlement sites. In a previous study, a proteinaceous settlement pheromone, Waterborne Settlement Pheromone (WSP), was purified from adult barnacles, *Amphibalanus amphitrite*, but it is unclear how WSP guides barnacle cyprids to settlement sites. Here, a series of settlement assays were conducted to investigate the concentration dependence and species specificity to recombinant WSPs. We report that low concentrations of barnacle WSP decrease the probability of settlement of conspecific larvae, whereas high concentrations have the opposite effect. We suggest that this is because weak WSP informs cyprids that suitable settlement sites with adult barnacles are distant, thereby inducing them to extend the larval phase. We also found that both *A. amphitrite* and *A. improvisus* cyprids responded similarly to allo-specific WSPs as they did to con-specific WSP stimuli, suggesting that WSPs are not species-specific. Additionally, a visual signal, possibly an autofluorescence emitted from adult wall plates, attract barnacle larvae. Our study opens the door to future research to determine how the harmony of these signals guide larvae to settlement sites.

Exploring the metabolomic response of marine sponge *Aplysina aerophoba* Nardo 1843 to global change: Insights from PARAFAC HPLC-DAD analysis

Gemma Llàcer[1], Julio Díaz[1,2], Juancho Movilla[2], Guillem Mateu-Vicens[1], Antoni Sureda[1,3], Carlos Pagán-Galbarro[4], Manuel Miró[4], Pere Ferriol[1]
[1]Interdisciplinary Ecology Group, Biology Department, University of Balearic Islands, Palma de Mallorca, Spain
[2]Instituto Español de Oceanografía, Centre Oceanogràfic de Balears, Palma de Mallorca, Spain
[3]Research Group in Community Nutrition and Oxidative Stress, Department of Fundamental Biology and Health Sciences, University of Balearic Islands, Palma de Mallorca, Spain
[4]FI-TRACE Group, Department of Chemistry, University of Balearic Islands, Palma de Mallorca, Spain

Many benthic invertebrates, such as sponges, develop chemical-mediated defensive strategies against potential predators and fouling. However, the accelerated global change could disrupt these vital functions, thereby altering the intricate relationships within organisms. This study aims to assess the potential impact of rising temperatures and ocean acidification, both consequences of climate change, on the Mediterranean demosponge *Aplysina aerophoba* Nardo, 1843. Specifically, we delve into its metabolomic response to these stressors. Over a three-month period, specimens of this marine sponge were subjected to experimental conditions, including different temperatures (20 ºC, 25 ºC and 30 ºC) and two levels of acidification (pH= 7.80 and pH= 7.65). Throughout the experimental period, incubations were carried out to study respiration and nutrient uptake/release. Organic solvents were used for extracting the bromotyrosine derived compounds; additionally, HPLC and liquid chromatography–mass spectrometry (LC-MS) were performed for further analysis and quantification. Furthermore, statistical analysis using PARAFAC HPLC-DAD was conducted to provide additional insights into the metabolomic profile of the samples. The current study has revealed a change in *A. aerophoba* chemical composition, and therefore in the amount of the different metabolites produced, against temperature and pH variations. These changes may affect their ability to survive in the field and, since Porifera are significant components of benthic biodiversity, the entire ecosystem could be compromised. Responses to stressors are very specific to each group of invertebrates, thus, further research about abiotic global change influencing them is required.

Keywords: marine chemical ecology, metabolomics, global climate change, temperature stress, ocean acidification, PARAFAC HPLC-DAD

Deciphering the molecular functions of brown algal CYP enzymes in defense signaling through metabolomics and functional genomics

Maëlle Zonnequin[1], Ludovic Delage[1], Cédric Leroux[2], Karine Cahier[2], Marine Vallet[3], Georg Pohnert[3], Gabriel Markov[1], Catherine Leblanc[1]
[1]Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, France
[2]Sorbonne Université, CNRS, FR2424, Metabomer-Corsaire, Station Biologique de Roscoff (SBR), 29680 Roscoff, France
[3]Max Planck Institute for Chemical Ecology and Friedrich Schiller University Jena, Germany

The oxylipin pathways are known to be involved in plant and animal defense signaling. In brown algae, an evolutionary independent eukaryotic lineage, oxylipins deriving from both C18- and C20- Polyunsaturated Free Fatty Acids are produced during stress defense responses, but the biosynthetic pathways and their exact roles as signals during biotic interactions are still unknown. Genomic approaches have identified several CYP5164 genes, homologous to the plant CYP74 gene family, that play an important role in biotic interactions between plants, insects and endophytes. To decipher the biological functions of CYP genes in the brown algal model, *Ectocarpus sp.7,* we have performed both un-targeted and targeted metabolomic analyses to compare the global metabolome of control and stressed algal cultures and to investigate the occurrence or absence of specific oxylipins of CRISPR knock-out mutants for the CYP5164B1 and wild-type strains. Liquid chromatography high-resolution mass spectrometry analysis is used to mine for differences in the overall metabolic profiles. In parallel, production of recombinant CYP5164B1 proteins is carried out to characterize and validate their biochemical activities *in vitro* to identify brown algal-specific substrates. These approaches will indicate whether the mutant oxylipin profiles are consistent with de previously determined catalytic activity of the recombinant enzyme.

The combination of *in vivo* metabolomic approaches and targeted biochemical characterization will allow the integration of CYP5164 activity in a more global metabolic context in a brown algal model. Moreover, these results will contribute to a better understanding of CYP-based defense and brown algal chemical signaling during biotic interactions.

Metabolomics and functional genomics of halogenation mechanisms in brown algae

Peti-Jean Eurydice[1,2], Delage Ludovic[1], Leroux Cédric[3], Cahier Karine[3], Puppo Rémy[2], Marie Arul[2], Prado Soizic[2], Leblanc Catherine[1]
[1]Laboratoire de Biologie Intégrative des Modèles Marins, UMR 8227, Sorbonne Université – CNRS, Station Biologique de Roscoff, Roscoff, France
[2]Molécules de Communication et Adaptation des Micro-organismes, UMR 7245, CNRS-MNHN, Paris, France
[3]Corsaire-MetaboMer platform, FR 2424, Sorbonne Université – CNRS, Station Biologique de Roscoff, Roscoff, France

Some brown algae are known to concentrate halogens such as bromine and iodine and to produce halogenated metabolites. Volatile or not, they are still poorly described. In addition the enzymatic processes and the function of halogenation remain uncertain in these marine organisms. Those compounds may have important roles in signalling and/or defense during biotic interactions and physiological responses to environmental changes. My thesis work aims to explore the production of halogenated metabolites and the role of halogen metabolism in brown macroalgae through functional genomics and metabolomics analyses. One of the putative halogenating key enzymes, vanadium-dependent bromoperoxydases (vBPO), was inactivated using the CRISPR-Cas9 method in the brown algal model *Ectocarpus siliculosus*. The extinction of vBPO activity has been validated for three independent knock-out mutants. Chemical extractions using different polar and non-polar solvents for metabolomic analysis were carried out. Several analytical optimisations led to the detection of nearly 1,000 features in the wild-type strains under standard culture conditions. Workflows, based on liquid chromatography-high-resolution mass spectrometry or gas chromatography-mass spectrometry data and utilizing analytical tools will enable the comparison and description of whole metabolomes between strains, as well as the specific characterization of the halometabolome. The current study will provide new knowledge about the chemical diversity of halogenated metabolites and their biosynthesis in brown algae.

POSTER

P-64

Dynamic chemical interactions in marine diatom-bacteria biofilms

Anja Ruhl[1,2], **Joel Bose**[1,2], **Lena Keller**[1,2], **Christina C Roggatz**[1,2]
[1] *Dynamic Ecological Chemistry, Faculty of Biology & Chemistry, University of Bremen, Bremen, Germany*
[2] *UFT – Center for Environmental Research and Sustainable Technology, University of Bremen, Bremen, Germany*

Communities of photosynthetic and heterotrophic microorganisms are fundamental to life and play essential roles in maintaining carbon, oxygen and other key element cycles on Earth. Research over the past decade has revealed a growing number of signals, cues and metabolites involved in the chemical crosstalk of these communities to mediate their interactions and functions. In aquatic biofilms on hard substrates, diatoms and bacteria share micro-habitats in which the interplay of photosynthesis and respiration can cause steep gradients and substantial diurnal fluctuations of pH and oxygen that could affect their interactions. Here, we assess the temporal and spatial scale of these abiotic dynamics in marine diatom-bacteria biofilm systems and present first insights into the potential dynamic effects on the molecules involved in community crosstalk based on chemical modelling.

S20: Synthetic lures for insect pest management

Chairs: *J. Paul Cunningham, R. Andrew Hayes*

ORAL PRESENTATIONS

Comparing the effect of host and lure on capture of spotted lanternfly (*Lycorma delicatula*) in circle traps

Everett Booth[1], Emily K. L. Franzen[1,2], Sarah Devine[1,2], Joseph A. Francese[1], Ann M. Ray[2]
[1]United States Department of Agriculture, Animal & Plant Health Inspection Service, Forest Pest Methods Laboratory, Buzzards Bay, USA
[2]Department of Biology, Xavier University, Cincinnati, USA

Since its introduction to North America the spotted lanternfly (SLF), *Lycorma delicatula*, has spread relatively unchecked. The preferred host of SLF in the native range is tree of heaven (TOH), *Ailanthus altissima*, although they are highly polyphagous throughout their development. Survey programs have relied primarily on circle traps placed on TOH and baited with methyl salicylate lures. However, recent work has suggested methyl salicylate may not significantly increase attraction of SLF to traps under some conditions. Additional studies indicate that maple (*Acer* spp.) and black walnut (*Juglans nigra*) are comparable hosts for trapping SLF when TOH is absent. We conducted assays to compare SLF trap catch between baited and unbaited trees of three taxa. Paired replicates of traps were placed on TOH (n=24), maple (n=14), and black walnut (n=18), and monitored during all life stages of SLF. A total of 64,310 SLF were collected across all traps and treatments. We observed no significant differences across any SLF life stages between baited or unbaited trees for any of the tree taxa tested. Our work suggests that methyl salicylate lures do not substantially increase trap catch or detection, and therefore, unbaited and baited circle traps are equally effective for monitoring. Our results are consistent with observations of other authors demonstrating that preference for TOH generally increases over the life of SLF. Nevertheless, alternate hosts can be used for trapping when TOH is not present. The results of this work may reduce costs and simplify trapping protocols for SLF survey programs.

Investigating the release rates of different controlled release devices containing blends of 10 pheromones for non-specific monitoring of invasive and destructive beetles

Michael Fraser, **Bob Setter**
Synergy Semiochemicals Corp.

When releasing semiochemicals in the field for use against insect populations individual release devices allow the best control of release rate over time. The compound being released can be paired with plastics appropriate for controlled release over longer periods extending from 60-120 days, depending upon the moiety and temperature. But when 5 or 10 compounds are required using a single release device for each compound can quickly become unaffordable. In these circumstances, researchers commonly make a cocktail of compounds and release it from a single device.

While a pooled approach can be cost effective, control over release rates of each compound is compromised. The relative ratio between compounds in the pool may also change over time if the compounds have different chemical properties, dependant upon how they react with each other in the mix and the interface of the controlled release membrane or substrate. Depending upon the device used releasing a mixture of compounds could result in a constantly changing ratio in the blend of compounds being released over time.

This study compares the performance of a 10-compound blend of cerambycid pheromones in different controlled release devices. We compared the effectiveness of cotton wicks in small plastic bags to two different types of polyethene bubble caps, one bubble cap with all 10 pheromones vs 3 bubbles with the compounds grouped by the chemical and releasing characteristics. We also investigate the release rates via gravimetric analysis and the relative releasing ratios of the compounds through gas-chromatography.

Biological flowable beneficials attractant to fight aphids

O. Guerret, J. Fournil, F. Briand
M2i Group, ITB, Tereos, CNRS

As part of a project committed to finding organic and sustainable alternatives to neonicotinoids (NNI) in beets, M2i conducted a 3-year research program involving the sector, the French Technical Institute for Beetroot and the CNRS (french scientific public institute).

The objective of the project was to develop a formulated biocontrol product using semiochemicals (of aphids and predators) to carry out population control on beet aphids. The aim was to spray a liquid formulation of green aphid predator attractants that could be used as a substitute or in addition to organic or conventional insecticide treatments in order to limit the impact of *Myzus persicae*, and therefore the risks of viruses, and to guarantee producers a better yield.

We carried out experiments in olfactometry, then proofs of concept in the field, first in trapping and then in micro-plot experiments before in 2022 & 23 to conduct trials in large areas according to different modalities and technical itineraries. The results are convincing in terms of reducing green aphid populations, virus damage leading to increased yields for farmers, both within reasoned organic and conventional technical itineraries.

The product is now ready to enter the market for sugar beets and soon in orchards.

Live fast, die young: Zingerone (ZN) ingestion in *Bactrocera jarvisi* males

Most Mottakina Akter[1], Md Forhad Hossain[1], Md Hafiz All Amin[1,2], Suk Ling Wee[3], Soo Jean Park[1], Vivian Mendez Alvarez[1]
[1]Applied BioSciences, Macquarie University NSW 2109, Australia
[2]Hajee Mohammad Danesh Science and Technology University Dinajpur 5200, Bangladesh
[3]Biological Science and Biotechnology, Universiti Kebangsaan Malaysia 43600 Bangi, Selangor, Malaysia

Bactrocera jarvisi is a native Australian fruit fly species (Diptera: Tephritidae), which is strongly attracted to and feeds on zingerone (ZN) from flowers and fruits. Young males of *B. jarvisi* are attracted to ZN while they are immature, and after ZN ingestion, males show higher mating success. We studied the effect of ZN ingestion on male sexual maturation, competitive mating behaviour, and female attraction of lab-reared flies. Dissections of male reproductive organs at day 8 after emergence showed that males attracted to ZN were more sexually developed than males unattracted to ZN. ZN-fed males achieved sexual maturation by 10 days after emergence while unfed males became sexually mature by 15 days after emergence. Also, ZN-fed males had the highest mating rates the day after being fed with ZN and the shortest mating latency. ZN-fed males had higher mating success in competitive mating assays and attracted more females in Y-maze olfactometer bioassays. More sexually developed males were attracted to ZN but not allowed to feed on it also showed higher mating rates suggesting that aromatherapy effect. Feeding on ZN allows males to develop faster and have a mating advantage but it also seems to have a physiological cost as shown in the longevity assays with ZN-fed males dying younger than unfed males. *B. jarvisi* wild male flies were attracted to ZN at 30 days after emergence and were more sexually developed than the unattracted ones. As with the lab-reared male, feeding on ZN accelerated the sexual development of wild males.

Age-dependent epicuticular chemistry and its chemoreception in the melon fly, *Zeugodacus cucurbitae*, revealed a synthetic attractant for females

Vincent Jacob[1], **Antonio Pompeiano**[2,3], **Lucie Vaníčková**[2]
[1]UMR PVBMT, CIRAD, Saint-Pierre, La Réunion, France
[2]Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno, Czech Republic
[3]Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

The melon fly *Zeugodacus cucurbitae* is a major pest of Cucurbitaceae crops with a large geographical spread over Oceania, Asia and Africa. The study of pheromonal communication in this species has so far been limited to the chemical identification of epicuticular and glandular compounds, which have not induced strong behavioral responses. We investigated further the age-dependent production and chemoreception of epicuticular components in both sexes of *Z. cucurbitae*, and the behavioral response they induce. Using two-dimensional gas chromatography coupled with mass spectrometry, we studied the epicuticular composition of virgin males and females aged 5, 15, 25 or 30 days after emergence. Sex-specific chemical profiles were observed in mature flies, but not in young individuals. The olfactory sensitivity of both sexes to natural and synthetic epicuticular compounds was explored using gas chromatography coupled with chopped triple electroantennographic and electropalpographic detectors. We found no olfactory responses to the predominant epicuticular compound of males, N-(3-methylbutyl) acetamide (dose-response dependence in females: $F_{3,24}=0.61$, $p=0.61$). Instead, both sexes were sensitive to a derived compound, 2-methoxy-N-(3-methylbutyl) acetamide (females $F_{3,20} = 13.1$, $p < 10^{-4}$; males $F_{3,20} = 25.9$, $p < 10^{-6}$), and to four other male compounds. When blended in a natural ratio, they induced a robust behavioral attraction of unmated females in a six-choice olfactometer (glmm Poisson family, $p < 0.01$). The synthetic attractant for female melon flies we identified could play a role in pest management, with further testing needed to confirm its effectiveness in field conditions.

Change of the chemical profile of the semisynthetic "bisexual" lures by time

Júlia K. Jósvai [1], Erdei Anna L. [1,2], Katalin Hári [3], Magdolna O. Szelényi, Sándor Kecskeméti[1]
[1]Department of Chemical Ecology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary
[2]Department of Plant Protection Biology, Swedish University of Agricultural Science, Alnarp, Sweden
[3]Institute of Plant Protection, MATE, Budapest, Hungary

Numerous volatiles originating from host plants or feeding sources have been identified and successfully used in order to attract various groups of insects. However, the attractiveness of these lures is subpar compared to sex pheromone traps, so researches focus to discover more effective combinations.

Adding red wine to well-known feeding attractants of different insect groups (noctuid moths – Noctuidae, pest moths – Tortricidae, pest wasps – Vespidae) significantly increased the attractiveness in Hungarian field experiments, suggesting wine emits behaviourally active compounds. However, these semisynthetic bisexual lures (SBLs) while increases the number of the target species, they also raise the number of species caught. Our study aims to find compounds emitted from wine to enhance effectiveness of known synthetic baits.

In our field experiments, the attractiveness of the SBLs changed over time: some species were more likely to be caught with fresh baits, while others were not affected by lure aging. To identify compounds, we collected volatiles from field-aged baits at different periods (SPME, GC-MS).

The amount of most detected volatiles in the headspace samples decreased as aging progressed: there were some components – e.g. benzyl alcohol – which could be found only in traces after a week, while other compounds were present in the aged baits only. Our results help to select volatiles which could be implemented in future behavioural or field tests.

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Development of pheromone-based tools for wireworm management in the United States

Jacqueline M. Serrano[1], **Livy Williams III**[2], **Wim van Herk**[3], **Gerhard Gries**[4], **Jocelyn G. Millar**[5]
[1]USDA-ARS Temperate Tree Fruit and Vegetable Research Unit, Wapato, WA 98951, USA
[2]USDA-ARS Vegetable Research Unit, Charleston, SC 29414, USA
[3]Agriculture and Agri-Food Canada Agassiz Research and Development Centre Agassiz, BC V0M 1A2 Canada
[4]Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada
[5]Depts. of Entomology and Chemistry, University of California, Riverside CA 92521, USA

Wireworms (Coleoptera: Elateridae), the larvae of click beetles, are insect pests that inhabit the soil and feed on many staple crops such as cereals and root vegetables. These pests have become increasingly problematic in North America in recent years, in part due to the lack of effective management strategies. Several species are prominent pests of several crops throughout North America, and the below ground life of wireworms makes them difficult to detect and manage. Until recently, there were no pheromone-based tools available for use in managing wireworms in the U.S. and Canada, excluding the invasive *Agriotes* species. The need for such tools resulted in collaborative research that has led to sex pheromone identifications of many click beetle speciesm including some major pest species. We investigated the utility of a pheromone and attractant lure developed for major pest species in the genera *Limonius* and *Melanotus*. Field studies compared the capture rate of male beetles using different lure types and doses of pheromone. In a separate field study, trap type and placement were compared with the different lure types to determine the best options for trapping male beetles. Through these field experiments, we discovered that the capture rates of male beetles for different species. This will aid with future work to developed into effective Integrated Pest Management strategies that will be useful for the management of wireworms.

Response of a parasitoid wasp, *Ascogaster reticulata* to host sex pheromones

Yooichi Kainoh[1], Makoto Hattori[1], Jun Tabata[2], Shigeru Matsuyama[1]
[1]*Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan*
[2]*National Agriculture and Food Research Organization, Tsukuba, Japan*

The parasitoid wasp, *Ascogaster reticulata*, parasitizes the smaller tea tortrix *Adoxophyes honmai*. The wasp reportedly uses wing scales from the host moth as a cue to locate host egg masses. The moth's abdominal tip scales surrounding the sex pheromone gland adhere densely to its egg masses, evidently being deposited during oviposition. Therefore, assuming that the abdominal tip scales carry host moth sex pheromone, we examined responses of female wasps to host pheromones. Three synthetic pheromone blends were prepared: blend A (with a reported ratio, (*Z*)-9-TDA, (*Z*)-11-TDA, (*E*)-11-TDA, 10-Me-DA=63:31:4:2), blend B (monitoring dispenser volatile ratio, 10:4:1:85), and blend C (only the 4th component, 0:0:0:100). Each blend was spotted on a piece of filter paper (1x1cm) and choice tests were conducted to observe antennal responses of female wasps. These results showed a relatively strong response to component B and almost no response to A and C. This suggests that female wasps use host sex pheromone as a cue to find host egg masses, and that they are responsive to the mixture of the dispenser volatile ratio rather than that of the reported ratio.

Virgin males of *Tuta absoluta* prefer synthetic pheromones, while virgin females attract more mated males: Implications for mass trapping efficacy

Eraldo Lima
Department of Entomology, Federal University of Viçosa, Viçosa, MG, Brazil

Mass trapping holds significant potential for controlling the tomato leafminer, *Tuta absoluta*, by using a high density of traps with synthetic sex pheromones to capture males and keep the population low. However, the technique's success has been inconsistent in tomato fields. Failures are mainly attributed to multiple matings by females and competition between females and traps. In Lepidoptera, males transfer sperm and nutrients to females via a spermatophore during copulation. Virgin and mated males of *T. absoluta* cannot be visually distinguished, but mated males produce smaller spermatophores with successive matings. This enables the estimation of males' reproductive status, thus assessing the mating status of field-collected males. A method was developed to determine the mating status of males by measuring spermatophore size. The average spermatophore size from the first and second copulations was used for males attracted by virgin females in the field. Males producing spermatophores below 0.1082 ± 0.0158 mm^2 were considered mated, while those above this threshold were considered virgin. Field-collected males were trapped using three attractants: (i) virgin females, (ii) synthetic pheromone (*E,Z,Z*-3,8,11-14:Ac), and (iii) a combination of synthetic pheromones (*E,Z,Z*-3,8,11-14:Ac + *E,Z*-3,8-14:Ac). The mating status of these males was determined by measuring spermatophores in the lab. Results showed males trapped with *E,Z,Z*-3,8,11-14:Ac were virgin primarily; those with the pheromone combination included equal proportions of virgin and mated males, and those with virgin females included more mated males. These findings suggest that using the main compound alone could improve mass trapping success by reducing competition with females.

Refinement of a lure for cucumber fly, *Zeugodacus cucumis*

Jodie Cheesman[1], Stefano de Faveri[1], Mai Nantawan[1], Matthew Siderhurst[2], R Andrew Hayes[3]
[1]Horticulture and Forestry Science, Queensland Department of Agriculture and Fisheries, Mareeba, Qld, Australia
[2]ARS, USDA, Hilo, HI, USA; [3]Forest Research Institute, University of the Sunshine Coast, Dutton Park, Qld, Australia

A nine-component synthetic lure based on cucumbers was developed as an attractant for the melon fly *Zeugodacus curcubitae.* The commercially available form of this lure has only seven components. The commercial lure was found to attract the cucumber fruit fly, *Z. cucumis*, a horticulturally important pest in Australia for which there is no known male lure. This study investigated optimisation of this lure by subtraction bioassay and/or by addition of new compounds from other attractive fruits. *Zeugodacus cucumis* is electrophysiologically active to all the original nine components when tested individually, and to both the nine- and seven-component blends.

Subtraction bioassays show different preferred lures based on fly species. In the USA, bioassays showed the nine-component lure is better in attracting *Z. cucurbitae.* In Australia, *Z. cucumis* attraction was not different between seven- and nine-component lures.

Volatiles were analysed from several additional cucurbits know to attract *Z. cucumis.* Two *Luffa* spp. were largely similar in compounds identified and overall relative abundances, but new compounds identified are potentially metabolites from the lipoxygenase (LOX) pathway. Further evidence for adding *Luffa* volatiles to the existing cucumber lure was found when these volatiles elicited strong EAG responses from *Z. cucumis*.

Field testing of multiple lures to date has been constrained by an unexpected drop in the size of field populations of *Z. cucumis*. Given the promising EAG results with *Z. cucumis*, we suggest that LOX pathway volatiles identified from *Luffa* species may increase the attraction of *Z. cucumis* if added to the seven-component lure.

POSTERS

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Plant-origin attractants for economically important pest *Otiorhynchus salicicola* (Coleoptera, Curculionidae) differ in males and females

Sandra Radžiutė, Violeta Apšegaitė, Laima Blažytė-Čereškienė, Vincas Būda
Nature Research Centre, Vilnius, Lithuania

Otiorhynchus salicicola (Coleoptera, Curculionidae) is a new alien species in Lithuania, rapidly spreading in Europe during the last three decades. These polyphagous weevils have pest status because they damage many species of shrubs and trees, ornamental and horticultural plants, and can cause significant harm to red raspberries and strawberries. The pests damage both the underground and the above-ground parts of the plant: larvae feed on roots meanwhile adults on leaves. Since the use of conventional pesticides and the increase in their use are harmful both to the environment and to humans, monitoring and environmentally friendly control measures, which are still not developed today, are necessary. To develop them, chemo-ecological studies on the insect pests were carried out. The headspace volatiles of undamaged and adult-damaged host plant *Syringa vulgaris* (Oleaceae) were collected and seven electrophysiologically active compounds for males and females were determined. Six compounds were identified (hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexenal and (*E*)-2-hexenal) and behavioural reactions of *O. salicicola* males and females to the volatiles were registered. In Y-olfactometer hexanol and (*E*)-2-hexen-1-ol were significantly attractive for males, however, those were not attractive for females. Females showed directional movement toward (*E*)-3-hexen-1-ol. These three attractive compounds will be tested under field conditions in future studies to evaluate their effectiveness both for pest population monitoring and as a potential pest insect control tool.

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P-80

Seasonal abundance, host preferences, and economic importance of Horse Flies (Insecta: Diptera: Tabanidae) in Sub Saharan Africa: A case study in Ghana, West Africa

James A. Timbilla, **Scott C. Sherman**
Department of Biological Sciences and Geology, Queensborough Community College – City University of New York, Bayside, NY, USA

Horse Flies (Insecta: Diptera: Tabanidae) are distributed in many biogeographical regions of the world including Sub Saharan Africa. The host-seeking and hematophagous habits of the Tabanidae and their ability to transmit pathogens make them of considerable biological and economic importance. A case study was conducted in Ghana, West Africa between 2014 and 2017 on the perceived importance of three genera (*Atylotus* Osten Sacken, 1876; *Haematopota* Meigen, 1803; and *Tabanus* Linnaeus, 1758) to humans, and to livestock care using a reconnaissance survey questionnaire developed by the authors. The questionnaire included colored photographs to help agricultural, veterinary extension officers and farmers identify the genera. The results obtained from the Sahel, Semi-Deciduous and Forest Savannah Transition ecological zones of Ghana indicate that all three species occur in the study zones with *Haematopota* sp. and *Tabanus* sp. being the most abundant. The insects tend to proliferate during the wet season, which correlate with the rainfall distribution. The insects are known to suck blood from and cause harm to humans, cattle, sheep, goats, donkeys, horses and pigs, with humans and cattle being the most impacted. Possible management strategies and or control of the Tabanidae in Sub Saharan Africa from chemo-ecological perspectives are discussed.

We are indebted to Mr. Henry Yamgah (Ministry of Agriculture, Ghana) and Mr. Samuel Adom (Crops Research Institute, Ghana). We also thank Ms. Keiko Fujitani (Brooklyn, NY); Ms. Christiana Timbilla, Mr. John Timbilla and Miss Victoria Timbilla (Wharton, NJ); Ms. Teresa Salas and Terrence Rohan (Queensborough Community College, Bayside, NY).

S21: E-niche Young Researcher Award

Chair: *Anne-Geneviève Bagnères*

ORAL PRESENTATIONS

Bioactive potential of *Ailanthus altissima* bark extract: A promising ecofriendly approach for pest control in stored grains

Radenka Kolarov[1], Dejan Prvulović[1], Sonja Gvozdenac[2]
[1]*Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia*
[2]*Institute of Field and Vegetable Crops, Novi Sad, Serbia*

Conventional pesticides are synthetic chemicals that are toxic, could have negative effects on non-target organisms and may be carcinogenic to humans. Pests can be controlled in an alternative way using less hazardous and eco-friendlier methods, based on chemical ecology principles, such as using biopesticides Translating basic research into practical products and technologies compatible with the Green Deal agenda and Integrated Pest Management (IPM) programs that can facilitate environmental health, ecosystem services, and sustainable agriculture is the main challenge. Plants synthetize secondary metabolites which could be used as alternatives to synthetic pesticides for pest control. Invasive plant species *Ailanthus altissima* possesses effective defence mechanism against other plants and insects, due to the presence of bioactive substances in its tissues. This work aimed to investigate the effect of 5% extract of *A. altissima* two-month-old bark on two economically harmful storage insects, *Sitophilus oryzae* and *Tribolium confusum*. The repellency of the bark extracts of *A. altissima*, to which they were exposed during a certain period, was tested. After an initial period, *S. oryizae* showed a preference for the treatment, with a majority opting for it. However, a significant shift occurred later, with the majority of insects moving towards the control group. A similar trend was observed with *T. confusum*, where initially a substantial portion favored the control, and this preference increased over time. These findings highlight the strong repellent effect of *A. altissima* against storage insects, affecting both *S. oryizae* and *T. confusum*.

Combined acute ozone and water stress alters the quantitative relationships between O_3 uptake, photosynthetic characteristics and volatile emissions in *Brassica nigra*

Kaia Kask, Eve Kaurilind, Eero Talts, Astrid Kännaste, Ülo Niinemets
Chair of Crop Science and Plant Biology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51006 Tartu, Estonia

O_3 entry into plant leaves depends on atmospheric O_3 concentration, exposure time and openness of stomata. O_3 negatively impacts photosynthesis rate (A) and might induce the release of reactive volatile organic compounds (VOCs). Water stress reduces stomatal conductance (g_s) and O_3 uptake and can affect VOC release and O_3 quenching by VOC, but the interactive effects of O_3 exposure and water stress, as possibly mediated by VOC, are poorly understood. Well-watered (WW) and water-stressed (WS) *B. nigra* plants were exposed to 250 and 550 ppb O_3 for 1 h, and O_3 uptake rates, photosynthetic characteristics and VOC emissions were measured through 22 h recovery. The highest O_3 uptake was observed in WW plants exposed to 550 ppb O_3 with the greatest reduction and poorest recovery of g_s and A, and elicitation of lipoxygenase (LOX) pathway volatiles 10 min–1.5 h after exposure indicating cellular damage. Ozone uptake was similar in 250 ppb WW and 550 ppb WS plants, and O_3-dependent reduction in photosynthetic characteristics was moderate and VOC emissions were little affected. Water stress alone did not affect the total amount and composition of VOC emissions. The results indicate that drought ameliorated O_3 stress by reducing O_3 uptake through stomatal closure.

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