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Chemical Ecology Down Under: From Molecules to Ecosystems

Abstracts

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Plenary speakers

In alphabetical order (by first name of presenter)

Chemical communication between the Eucalyptus snout beetle, its hosts and the egg parasitoid *Anaphes nitens*

Prof Almuth Hammerbacher[1]

[1]Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa

Gonipterus weevils are commercially important defoliators of *Eucalyptus* trees in their invasive ranges in Europe, South America and Africa. In most of these areas, the weevil was brought under economic control by introducing an egg-parasitic wasp, *Anaphes nitens*. However, recently a resurgence of weevil outbreaks has been observed globally and the causes for this are unknown. To gain a mechanistic understanding of the factors driving the resurgence of the weevil, a series of laboratory and field studies were conducted to better understand the invasiveness and chemical ecology of *Gonipterus* sp. n. 2 in South Africa. Our results showed that the weevil's invasiveness is closely linked to its persistent core microbiome, its ability to manipulate nutrient and secondary metabolite levels in hosts and its effective detoxification of a wide range of *Eucalyptus* defence metabolites. Attacked trees emit volatiles that are attractive to *A. nitens*, but this is off-set by the weevil's increased survival rate on modern commercially planted *Eucalyptus* varieties. Taken together, this research shows that the weevil's adaptation to its introduced habitats as well as anthropogenic factors may be involved in driving the weevil's resurgence.

Neuroecology of locust olfaction

Prof Dr Bill S Hansson[1]

[1]Max Planck Institute for Chemical Ecology, Jena, Germany

Over the past five years, we have dedicated significant time and effort to understanding the olfactory foundation of behavior in migratory locusts, *Locusta migratoria*. Through two extensive studies, we examined both the peripheral and central olfactory systems, uncovering highly unusual traits such as superspecific olfactory receptors and a previously unknown, ring-shaped coding principle in the brain. A third study explored a specific, odor-mediated interaction. In the animal kingdom, cannibalism is a dietary strategy, and among dense populations of migratory locusts it is common. Our research delved into the olfactory interactions associated with this behavior. We discovered that in dense swarms, locusts emit an anti-cannibalistic pheromone called phenylacetonitrile (PAN). The level of cannibalism changes in correlation with PAN production in a density-dependent manner. After identifying the anti-cannibalistic role of PAN, we investigated the olfactory basis of its impact. Utilizing the *Drosophila* empty neuron system, we pinpointed the olfactory receptor that recognizes PAN. With this knowledge, we applied genome editing to deactivate this receptor, completely eliminating the negative reaction to PAN. Additionally, we removed the gene responsible for PAN production. Locusts that could not produce PAN lost their protective advantage and were more frequently targeted by their own species. Ultimately, we unveiled an anti-cannibalistic mechanism based on a specialized pheromone within dense locust swarms. Overall, I will share new research into the olfactory system of migratory locusts and provide examples of its critical role in influencing behavior.

Benefits of being chemically divers

Prof Caroline Müller[1]

[1]Bielefeld University, Bielefeld, Germany

Chemical diversity, or chemodiversity, is a fascinating trait that is attracting more and more attention due to its diverse ecological functions and intriguing evolution. Variation in chemical phenotypes of organisms plays a fundamental role in shaping species interactions, and can be studied using different metabolomics approaches combined with laboratory and field bioassays. From the plant perspective, inter- and intraspecific plant chemodiversity influence the specific outcome of plant-herbivore, plant-pollinator and also plant-microbe interactions. Moreover, not only the individual plant chemodiversity matters, but plant neighbourhood chemodiversity is likewise very important for driving antagonists and mutualists and thus impacting plant fitness, as I will demonstrate with our data from the highly chemo-diverse plant species *Tanacetum vulgare*. From the herbivore perspective, plant metabolites are used for nutritive and non-nutritive purposes. The uptake and detoxification of plant metabolites, but also sequestration of plant metabolites into body tissues modulates the individual phenotypic variation in herbivores, especially insect species. This chemodiversity at various levels thus influences niche realisation processes, such as niche choice, conformance and construction of individuals. I will highlight several examples of these intriguing functions of chemodiversity.

Plant–plant communication mediated by leaf volatiles

Prof Junji Takabayashi

[1]Kyoto University (Center for Ecological Research), Otsu, Japan

For over 30 years, I have been studying the chemically mediated interactions between insects and plants. In particular, I have studied on the ecological functions of volatiles emitted from plants infested by herbivorous arthropods (herbivory-induced plant volatiles: HIPVs) and volatiles emitted from artificially damaged plants (primarily green leaf volatiles: GLVs). Our research group has demonstrated that HIPVs and GLVs attract the carnivorous natural enemies of herbivores (induced indirect defense), and activate defense responses in neighboring undamaged plants (plant-plant communication). In this presentation, I will discuss our research findings related to plant-plant communication under laboratory and field conditions. First, I will present some mechanistic studies of plant-plant communication. Then, I will present data on the application of volatiles emitted from artificially damaged weeds on crop seedlings, such as soybeans, rice, and corn, to improve their performance. Compared to unexposed seedlings, those exposed to volatiles from artificially damaged weeds for two to three weeks exhibited superior defense against subsequent herbivory. At harvest, the yield of exposed crops was significantly higher than that of unexposed crops. These results suggest that exposing crop seedlings to volatiles from artificially damaged weeds could artificially induce volatile-mediated plant-plant communication, which could contribute to crop production.

From chemical ecology to biotechnical crop protection - sustainable agriculture in the face of globalization, biodiversity crisis and climate change

Prof Dr Jürgen Gross[1]

[1]Julius Kühn-Institut, Dossenheim, Germany

Modern agriculture is being challenged by a variety of new developments: Global warming, for example, is causing the immigration of alien pests into previously cooler regions. Global trade is also displacing other species, which can then become invasive in their new habitat and threaten crop harvests. At the same time, the biodiversity crisis is ensuring that fewer and fewer conventional chemical pesticides are available. By studying chemical-mediated insect-plant interactions, new active substances can be found that are suitable as attractants or repellents that can be used for monitoring, mass trapping or in push-and-pull or attract-and-kill strategies. The effects of semiochemicals can be further enhanced by the inclusion of physical signals like colour or vibration. The role of applied chemical ecology in interaction with modern plant breeding, nature conservation and climate protection will be presented and discussed.

Molecular mechanisms of mating-mediated olfactory behavioral plasticity in the oriental fruit fly

Li Xu[1], Hong bo Jiang[2,3], Jin jun Wang[2,3]

[1]Anhui Agricultural University, Hefei City, Anhui Province, China, [2]College of Plant Protection, Southwest University, Chongqing, China, [3]Academy of Agricultural Sciences, Southwest University, China

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is one of the most destructive and invasive pests, causing huge economic losses to the world fruit and vegetable industries. Currently, the most economic and effective way to control *B. dorsalis* is olfaction-based trapping, highlighting the critical importance of studying the olfactory system for its prevention and management. Insects rely heavily on their olfactory system for various behaviors, including foraging, mating, and oviposition. Numerous studies have demonstrated that insects can adjust their olfactory behaviors in response to different physiological states and environmental conditions. In this study, we discovered that female flies exhibit significant shifts in olfactory preferences for sex pheromones and host plants/volatiles after mating. The virgin females showed a markedly higher preference for sex pheromones compared to mated females. However, once mating, this preference drastically reversed. The mated females displayed significantly reduced attraction to sex pheromones and a pronounced increase in preference for host plants and their volatiles. Subsequently, based on transcriptomic analysis, along with HPLC, ELISA and CRISPR/Cas9 assays, we elucidated the critical role of biogenic amines in mediating mating-induced olfactory behavior plasticity in female *B. dorsalis*. Additionally, we further elucidated the molecular mechanisms of specific host volatiles induced oviposition behavior in *B. dorsalis*. These findings not only elucidate the mechanisms underlying mating-mediated olfactory behavioral plasticity in *B. dorsalis*, but also provide a theoretical foundation for developing high-efficiency attractants targeting females flies.

Integrative perspectives on social insect chemical signaling

Nathan Derstine[1,2]

[1]Pennsylvania State University, Pennsylvania, United States, [2]USDA-NIFA - University of Richmond, Virginia, United States

Integrative perspectives on social insect chemical signaling

While chemical communication is a fundamental part of all insect life, social insects produce semiochemicals which mediate interactions beyond those of their solitary ancestors. In addition to “typical” sex pheromones produced by the reproductive castes, social insects produce fertility signals which facilitate reproductive division of labour among females. The chemical identification and diversity of those signals, their biosynthetic origins, and the characteristics that impact their evolution are intertwined and active questions. Once identified, it is possible not only to better understand how chemical signals regulate social behaviours, but also how they might be dis-regulated by environmental stressors such as pesticides, or used to manage species which are invasive or problematic. My research foci have included identifying novel sex pheromones in social wasps, which could be a foothold to identify unknown pheromones in related invasive *Vespula* species, understanding the molecular underpinnings of caste specific reproductive signals in social bees, and attempting to test the sender-precursor hypothesis for signal evolution by comparing the Dufour’s gland secretions of bees from solitary and social lineages. Overall, this talk will span multiple taxa in Hymenoptera, integrating classical chemical ecology with molecular, behavioural, and physiological approaches to evaluate the role and evolution of insect pheromones.

Harnessing chemical ecology: From cross species disruption to sniffer bees

Prof David M. Suckling[1]

[1]The Royal Society of New Zealand Te Apārangi, Nelson, New Zealand

The 1983 publication of the light brown moth (LBAM) sex pheromone enabled field collection and mapping insecticide-resistant populations, but a commercial supplier enabled us to completely disrupt mating by 1987. In the export orchards of New Zealand, insecticides were soon replaced by residue-free moth pheromones. Four export pest species were removed with a single application of pheromone release device, a major success for chemical ecology with global parallels in other species.

But new incursions of invasive agricultural and forestry insects into peri-urban areas present a rapidly rising and more complex challenge. The need for innovation in socially-acceptable tactics for pest eradication became especially obvious after public opposition to large scale government aerial eradication responses, in Auckland, Hamilton and San Francisco, for example. Aerial pheromones have been applied in responses such as spongy moth in Eastern USA, but an unpopular pheromone programme in California against LBAM was terminated after the trapped population was widespread.

Our team took on to develop and demonstrate new tactics such cross species mating disruption at urban field scale. Micro-encapsulated sprayable moth sex pheromone was applied to mass-reared sterile male medflies then released in urban Perth, in order to disseminate pheromone to disrupt the LBAM. Male moths were found in medfly-specific traps, and catches in moth traps in the medfly release areas were briefly reduced.

In the rationale of seeking novel solutions to invasive species, we have investigated the potential of trained honeybees to detect diverse biosecurity targets in the lab and open field. Such high risk ideas can serve as stepping stones towards more practical but entirely novel solutions in biosecurity, which are sorely needed against high risk pests. The potential for novel relatively-benign chemical ecology-led solutions against invasive pests remains high, but mounting evidence suggests that invasive insects costs are outstripping innovation and adaptation rates.

Keynote speakers

In alphabetical order (by first name of presenter)

Effects of CO_2 and heatwaves on trophic interactions mediating biological control systems

Dr Adriana Jeanette Najar Rodriguez[1]

[1]Plant And Food Research, Christchurch, New Zealand

Climate change is widely regarded as one of the greatest challenges currently facing natural and productive systems. Consequently, a significant amount of research on climate change mitigation and adaptation has been conducted internationally. Although our understanding of how productive systems will respond to climate change, with a focus on plants and herbivores, has advanced significantly in the last two decades, the effects of climate change on tritrophic systems involving plant-insect herbivores and natural enemies have rarely been investigated.

Elevated CO2 concentrations have increased since the preindustrial era and are predicted to almost double by the end of this century, leading to an increase in the average global temperature of over 2.2°C. Heat waves, on the other hand, defined as periods when maximum temperatures are above seasonal norms by 5°C for at least five consecutive days, are increasing in frequency and intensity with climate change. Higher trophic levels, such as predators and parasitoids, are predicted to be more strongly affected by climate change than herbivores because they may have smaller thermal windows and because of altered ecological interactions with lower trophic levels.

Here, we will present the results of our past and ongoing research testing the effects of (a) elevated versus ambient CO2 on chemically-mediated interactions in a model system comprised of Brassica plants, the specialist aphid *Brevicoryne brassicae* and its endoparasitoid *Diaeretiella rapae*; and (b) heatwaves of varying intensity and duration on the fitness and reproductive success of invasive herbivores and their natural enemies. The implications of our results on the stability and functionality of trophic interactions mediating biological control systems as impacted by climate change will be discussed.

Harnessing chemical ecology for sustainable biological control

Dr Adriana Jeanette Najar Rodriguez[1], Dr Gonzalo Avila[2], Dr Andrew Chan[2], MSc Karina Santos[2], Dr Jinping Zhang[3], BSc Zi-Jian Song[3], Dr Xin Lü[4]

[1]Plant And Food Research, Lincoln, New Zealand, [2]Plant and Food Research, Auckland, New Zealand, [3] MARA-CABI Joint Laboratory for Bio-safety, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [4]Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou, China

Addressing biosecurity threats—such as invasive pests (both vertebrate and invertebrate), weeds, and diseases—requires multidisciplinary approaches due to the complex challenges posed by climate change, global trade and travel, changing land use patterns, and the pressing need to reduce dependence on chemical inputs. Among existing strategies, biological control remains one of the most sustainable tools available for managing these risks.

Chemical ecology, which explores the chemical mechanisms driving both bi-trophic (e.g., plant–insect herbivore, plant–pathogen, insect herbivore–natural enemy relationships) and more complex multitrophic interactions, provides powerful insights for manipulating insect behaviour in targeted and environmentally sustainable ways. Integrating chemical ecology into biological control programs can strengthen all stages—from target and agent selection to conducting risk assessment, post-release evaluation, and integrated management strategies.

In this presentation, we will highlight ongoing research at the intersection of biological control and chemical ecology. Through selected case studies, we will demonstrate the advantages of this synergy and explore how chemical ecology can be further integrated into biological control to maximize its potential as a sustainable pest management tool.

Evolutionary origins of terpene synthesis in butterflies

Kathy Darragh[1]

[1]Indiana University, Bloomington, United States

Terpenes are one of the most structurally diverse compound classes and have key functions in interactions between organisms. Butterflies use terpenes for multiple functions including courtship signals, anti-aphrodisiac pheromones, and defense compounds. 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. Previously, we identified a novel terpene synthase in *Heliconius* butterflies, unrelated to plant enzymes and other previously described insect terpene synthases. We hypothesized that this was a novel *Heliconius* trait, resulting from lineage-specific duplication. Building on this work, we are currently trying to trace the origins of terpene synthesis in butterflies. We have found evidence of widespread terpene production in butterflies. We have identified at least two independent origins, with shared origins between groups that diverged during the Cretaceous. These new findings suggest that the evolution of terpene synthesis in butterflies is ancient, occurring around the time of the rise of the angiosperms independently in multiple lineages.

The critical need for new insect attractants to enhance biosecurity measures in Antarctica

Dr Sanghee Kim[1], Dr Ji Hee Kim[1], **Dr Kye Chung Park**[2]

[1]Korea Polar Research Institute, Incheon, South Korea, [2] The New Zealand Institute for Bioeconomy Science, Lincoln, New Zealand

As global temperatures rise, the introduction of non-native insect species into the Antarctic region has become increasingly frequent. Two such species, the winter crane fly (*Trichocera maculipennis*) and the moth fly (*Psychoda alternata*), have now been persistently detected over several years in the maritime Antarctic, particularly on King George Island. This ongoing presence has led to international recognition of the urgent need for a comprehensive, region-wide monitoring system for invasive insects. However, based on over a decade of field experience, current methods, primarily UV light traps and manual inspections, have proven inadequate for reliably detecting these species due to lack of effective attractants. To address this gap, we have begun introducing new tools to Antarctic research stations that enable the on-site development of novel insect attractants. Despite logistical and resource constraints, preliminary results indicate that developing viable attractants in situ is both feasible and promising, offering a critical advancement for invasive species surveillance across Antarctica.

Semiochemistry+: Opportunities for cross discipline collaboration for primary production industries

Lloyd Stringer

[1]The New Zealand Institute for Bioeconomy Science, Lincoln, New Zealand

There is an increasing drive to develop alternative, non-pesticide-based solutions to protect human and environment health in primary production. To date, advances in semiochemistry have enabled industries to produce quality products with low-nil residues. Because of this growing demand for alternative tools in production and the role chemical ecology has played in demonstrating positive effects, complementary alternative nature-based solutions are poised to see great uptake in primary production.

Here I will talk about the great opportunities for applied chemical ecologists when they are able to collaborate with researchers from other disciplines, such as those investigating other modes of communication like acoustic and vibratory signals, through to plant breeding, recognising that plants can sense and respond to future biological threats.

There are ongoing biotic, abiotic and regulatory challenges for food producers globally. By harnessing our collective knowledge of the conversations occurring in the environment, we can support the ongoing sustainability of food production.

Effects of acute and chronic exposure to ozone pollution, in the context of climate change, on the olfaction and behavior of a specialized pollinator

Emilie Balthazar[1], Benoit Lapeyre[1], Solène Liegois[1,3], Alice Kloop[1], Joana Sauze[2], Clément Piel[2], Alexander Milcu, Emmanuel Gritti[2], **Magali Proffit**[1]

[1]CEFE-CNRS, Montpellier, France, [2]Ecotron-CNRS, France, [3]IRBI, France

Anthropogenic environmental changes are a real threat to pollination and associated ecosystem services. In plant-pollinator interactions, host recognition by pollinators relies on the emission of floral odors, a complex mixture of volatile organic compounds (VOCs), by plants and their perception by pollinators. While several recent studies have demonstrated that acute (short-term) exposure to high concentrations of air pollutants such as ozone (O_3) or high temperatures can impact plant-pollinator chemical communication, studies assessing the effects of chronic (long-term) exposure to these stressors are critically lacking. In the present study, we investigated the effects of acute and chronic exposure to increased ozone concentrations, alone or in combination with high temperatures, on the olfaction and behavior of the specialized pollinator of the Mediterranean fig tree (*Ficus carica*), *Blastophaga psenes*. In order to reproduce acute and chronic exposure to O_3, short-term (3 h) and long-term (throughout the insect development period) experiments were conducted under controlled conditions in mesocosms. During these exposures, daily fluctuations in environmental conditions were reproduced. We exposed pollinators to two cross-treatments (O_3 and temperature), with three O_3 concentrations (maximum value of 20 ppb as control, 80 ppb as average concentration expected in 2100, 120 ppb as maximum annual concentration) and two temperature regimes (control temperature and control + 3°C as expected in 2100). After exposure, we assessed certain ecological traits involved in chemical communication between plants and pollinators: VOC detection by antennae (using electrophysiology recording), attraction to VOCs (using an olfactometry test), and pollinator activity. Acute or chronic exposure to these two environmental stressors can affect pollinator behavior and their perception of VOCs. The different effects of these two types of exposure of pollinators to environmental stress factors will be compared and discussed.

Identification and knockout of a herbivore susceptibility gene enhances planthopper resistance and increases rice yield

Dr Peng Kuai[1], Yonggen Lou
[1]Zhejiang University, Hangzhou, Zhejiang, China

Brown planthopper (BPH, *Nilapavata lugens*) and white backed planthopper (WBPH, *Sogatella furcifera*), are amongst the most destructive pests on rice. However, plant susceptibility (*S*) genes have not yet been exploited for crop protection. Here, we identified a leucine-rich repeat protein, OsLRR2, from susceptible rice varieties that facilitate infestation by BPH. OsLRR2 interacted with OsSERK1 and OsSERK2 and thereby impaired their binding to two defence-related receptors, OsPEPR1, OsFLS2, and a growth-related receptor OsBRI1. Knockout of *OsLRR2* increased induction of mitogen-activated protein kinases, defence phytohormone signalling, and secondary metabolite production. This increased BPH resistance and improved plant growth. Field trials showed that knockout of *OsLRR2* significantly reduced BPH infestation and enhanced natural biological control by attracting natural enemies. Yield of a susceptible variety was increased by 18% in insecticide-treated plots that eliminated planthoppers, and by 25% in untreated plots. These results underscore the pivotal role of *OsLRR2*, offering a promising pathway for pest population suppression and rice yield increase. Importantly, our findings show how this mechanism can be exploited to produce high yielding elite rice varieties that are resistant to devastating pest insects, using highly efficient breeding methods such as gene editing and marker-assisted breeding.

Whispering sweet (N)othings: are floral aldoximes index signals for nectar amino acids?

Dr Robert Raguso[1]

[1]Cornell University, Ithaca, United States

Night blooming flowers have long attracted human attention through their powerful, perfume-worthy fragrances. Previous research has confirmed the key role played by such fragrances in attracting hawkmoths as pollinators. Interestingly, the fragrances of jasmines, tobaccos, evening primroses, sacred datura and Darwin's star orchid contain pungent nitrogenous volatiles (indole, nitriles, aldoximes) first identified by Roman Kaiser for their "animalic" organoleptic qualities. Indeed, indole was recently shown to be a key hawkmoth attractant leading to reproductive isolation between species of *Ipomopsis plants*. The nitrogenous aldoximes are directly derived from essential amino acids (LEU, ILE, VAL and PHE) via a single CYP79 enzyme: might hawkmoths use these volatiles as index cues for the presence of nectar amino acids, the only source of N for adult moths? Using the yellow evening primrose (*Oenothera flava*) as a model system, we asked 1) whether nectar AAs and volatile aldoximes are statistically correlated, 2) whether moths preferentially visit flowers scented with aldoximes, 3) whether moths show innate or learned preferences for nectar AAs, 4) whether nectar AAs increase moth fitness, and 5) how flowers of *O. flava* make aldoximes. We found that volatile aldoximes are strong predictors of total nectar AAs (total nectar N), but not specifically their biosynthetic source AAs (LEU, ILE). *Hyles lineata* moths show strong, dose-dependent attraction to the full aldoxime blend, to 3-methylbutyraldoxime but not to 2-methylbutyraldoxime. Manduca sexta moths prefer artificial nectar spiked with AAs over sugar alone, but only when [sucrose] is high (25%). Female *H. lineata* that drink nectar with AAs live marginally longer (than on sugar-only meals) but lay significantly more viable eggs. Finally, petals of *O. flava* have a highly expressed cyp79 gene that is sufficient to reproduce the full complement of aldoximes and nitriles emitted by *O. flava* when heterologously expressed in leaves of *Nicotiana benthamiana*.

Exploiting herbivore-induced plant volatiles for crop protection

Ted Turlings[1,2,3]

[1]Henan University, Kaifeng, China, [2]University of Neuchatel, Neuchatel, Switzerland, [3]Penn State University, State College, USA

Natural enemies of herbivorous pests often use plant-provided signals to locate plants that carry potential prey. Particularly intriguing are so-called herbivore-induced plant volatiles (HIPVs), which various plants release in large quantities only when they are attacked by insects. I will discuss ways in which HIPVs can be exploited for crop protection, with a focus on our efforts to utilize these highly specific plant-produced signals for the real-time detection of agricultural pests. With specific odour sensors we have been able to accurately distinguish healthy plants from plants that are under attack by insects or are infected by pathogens. We envision that these odour sensors can be placed on robotic rovers and allow farmers to determine the presence of specific pests on their crops before these pests do serious harm. The same rovers could also be used to apply biocontrol agents to control these pests, only when and where it is necessary. This should eventually result in a novel, cost-effective and sustainable crop protection strategy.

New GPCR family responding to volatile pheromones in the marine worm *Platynereis dumerilii*

Dr Victoria C. Moris[1], Dr Daniel Thiel[2], Junior Prof Lauren Saunders[3], Prof Isabel Beets[4], Dr Jörg Hardege[5], Prof Gáspár Jékely[3]

[1]Université Libre de Bruxelles, Brussels, Belgique, [2]Living Systems Institute, University of Exeter, Exeter, United Kingdom, [3]Centre for Organismal Studies (COS), University of Heidelberg, Heidelberg, Germany, [4]Department of Biology, KU Leuven, Leuven, Belgium, [5]Department of Biological and Marine Sciences, University of Hull, Hull, United Kingdom

Chemical detection is crucial to various behaviors such as mating in both marine and terrestrial animals. So far, chemosensory systems of marine species remain poorly-studied compared to those of terrestrial species. The marine worm *Platynereis dumerilii* is a promising species to study chemosensation. As broadcast spawner, *P. dumerilii* males and females use pheromones to induce rapid swim speed or to trigger the release of their gametes synchronously. We aim to identify the receptors of these pheromones to better understand chemical detection systems in marine animals. Combining differential transcriptomics, molecular phylogeny, GPCR deorphanization and HCR in situ hybridization, we discovered a new family of GPCR receptors responding to volatile pheromones and carried out its initial characterization. Based on HCR and SEM imaging, receptors of this new family are expressed in the dorsal part of the modified parapods, which are specific to mature males and females. We showed that two promising receptors from this GPCR family are activated by volatile pheromones released by the worm (5-methyl-3-heptanone, 3,5-octadien-2-one). This new family cluster indicates a major duplication event which is likely *Platynereis* or *Nereis* specific. We hypothesize that this radiation event might be linked to the numerous ketones and other similar volatile compounds that are released by the worms, possibly acting as a pheromone bouquet, similar to what is observed in insects. The characterization of this new receptor family will improve our understanding of chemosensation in marine annelids and also contribute establishing *Platynereis* into a new molecular genetic model for chemical ecology.

From olfactome to attractome: Cross-species conservation of peripheral olfactory tuning predicts behavioural sensitivity in *Bactrocera dorsalis*

Vincent Jacob[1], Gaëlle Ramiaranjatovo, Nicolas Slomiany
[1]Cirad UMR PVBMT, Saint-pierre, Réunion

Identifying which host-emitted volatiles mediate insect attraction remains a major challenge, particularly in polyphagous species exposed to complex and variable odour environments. Although insects can detect numerous volatile compounds, the individual contribution of each to host-driven attraction varies widely. Some volatiles enhance attraction across diverse chemical backgrounds, even at low concentrations, whereas others have minimal or context-dependent effects. We hypothesised that this behavioural variability reflects differential evolutionary selection pressures shaped by species-specific ecology. To test this in polyphagous Tephritidae fruit flies, we explored, as a proxy for host-driven selection, whether sensory tuning to fruit volatiles correlates with host range, and assessed the behavioural responses of *Bactrocera dorsalis* females to those compounds. More precisely, we screened antennal responses to mango and strawberry guava volatiles in eight species (tribe Dacini and Ceratitidini), five of which are polyphagous (n=8-9 individuals per fruit and Tephritidae species), using gas-chromatography coupled with triple electroantennographic detection via chopping modulation. The correlation between antennal response intensity and host-range was assessed across species while controlling for phylogenetic relatedness. We then tested the behavioural responses of *B. dorsalis* females to 45 fruit compounds, at four air concentrations (n=32 individuals per compound and concentration), using a high-throughput behavioural assay (Flywalk). Compounds preferentially detected by polyphagous species elicited behavioural responses with significantly higher sensitivity, defined as a lower concentration required to achieve maximal attraction (bootstrap test based on inter-individual variability in sensitivity estimates, 1000 replicates, p=0.05). Furthermore, a blend of the most attractive compounds showed no saturation effect, maintaining strong attraction across a broad concentration range. These findings reveal a predictive link between conserved olfactory tuning in polyphagous Tephritids and behavioural valence in *B. dorsalis*, supporting the rational design of semiochemical blends for pest control.

Understanding mosquito smell system: A new frontier in mosquito control

Dr Wei Xu[1], Dr Aniruddha Agnihotri[1]
[1]Murdoch University, Murdoch, Australia

Mosquito-borne diseases such as malaria, dengue, Zika, and Japanese encephalitis continue to pose a major threat to global health. Current mosquito control strategies rely heavily on insecticides; however, the emergence of insecticide resistance and climate-driven expansion of disease vectors highlight the urgent need for innovative, sustainable, and environmentally friendly alternatives. This project aims to explore malaria mosquito olfactory receptor systems as a novel frontier for vector control, targeting the molecular mechanisms that govern host-seeking behaviour. Chemical attractants represent a promising, eco-friendly strategy for mosquito management, yet identifying effective attractants remains challenging due to the complexity of host-emitted volatiles. Recent research has identified several human breath compounds that elicit strong physiological and behavioural responses in malaria vector mosquitoes, *Anopheles stephensi* and *Anopheles farauti*. However, the mosquito receptors responsible for detecting these volatiles remain largely unknown. This study aims to bridge this knowledge gap by identifying and characterising mosquito olfactory receptors involved in detecting key host-derived compounds. Specifically, we will: (1) identify candidate receptors responsive to target volatiles; (2) functionally characterise these receptors using in vitro electrophysiological assays; and (3) investigate their behavioural roles using RNA interference (RNAi) and behavioural testing. These insights will enable the rational design of novel attractants, repellents, or receptor-targeted interventions. Ultimately, this project will contribute to the development of next-generation, olfaction-based vector control tools that disrupt mosquito host-seeking behaviour, reduce disease transmission, and support long-term biosecurity and public health outcomes. By targeting the molecular interface between mosquitoes and their hosts, we seek to establish a new paradigm in mosquito control grounded in receptor biology and chemical ecology.

Oral Presentations

In alphabetical order (by first name of presenter)

Developing a novel multisensory push-pull strategy for ultrasound sensitive insect pests

Mr Advaith Chakravarthy[1,3], Ms Sophie Hunt[1], Mr Tomas Sullivan[1], Dr Ryo Nakano[2], Dr Paul Szyszka[3], Dr Christian Mille[4], Dr Adriana Najar-Rodriguez[1], Dr Flore Mas[1]

[1]The New Zealand Institute for Plant and Food Research, Lincoln, New Zealand, [2]National Agriculture and Food Research Organization (NARO), Tsukuba, Japan, [3]Department of Zoology, University of Otago, Dunedin, New Zealand, [4]Institut Agronomique néo-Calédonien, New Caledonia

Despite rigorous biosecurity measures, invasive moth species continue to threaten primary industries. Current management strategies rely heavily on conventional chemical interventions, highlighting the urgent need for sustainable and ecologically sound alternatives. In this project, we aim to develop an innovative pest management strategy based on the well-established push–pull system, enhanced through the integration of multiple sensory modalities—a first-of-its-kind. This approach will combine the repellent effect of bat-like ultrasound ("push") with attractive insect odours such as sex pheromones or host-plant kairomones ("pull"), simultaneously targeting the auditory and olfactory systems of ultrasound-sensitive moths. Notably, major invasive moth pests in (e.g., *Spodoptera* spp., *Helicoverpa* spp.,) along with a biosecurity risk species (*Eudocima* spp.,) have evolved in regions where echolocating bats are common predators. These moths have adapted to evade predation by bats by developing ultrasonic hearing - an evolutionary arms race we seek to exploit for pest control. By synthetically producing danger-signalling ultrasounds, replicating bat hunting calls, and using attractive insect odours, we aim to trigger both the innate predator-avoidance behaviours and mate/host-finding mechanisms of our target pests. This new multisensory push-pull approach will drive moths away from crop interiors while simultaneously luring them toward odour traps at the field margins. We will combine neuro- and electro-physiological techniques to examine both the peripheral detection mechanisms and, for-the-first time, the central integration of ultrasound and odour cues at the insect brain level. These studies will be complemented with behavioural assays conducted both in controlled laboratory conditions and field trials. Our goal is to generate foundational knowledge on how invasive moth pests perceive and respond to a multisensory environment while navigating through the landscape, contributing to the development of a novel sustainable and ecologically grounded pest control system.

Effective semiochemical strategies for vector management

Dr Agenor Mafra-neto[1], Dr Teun Dekker[2], Mr Jesse Saroli[3], Dr Carmem Bernardi[1], Ms Vanessa Soria[1], Dr Kim Spencer[3]

[1]Isca Technologies, Inc., Riverside, United States, [2]BioInnovate Global AB, Sodra Sandby, Sweden, [3]ArcGen Pro Technologies, Golden Valley, United States

Insect semiochemicals offer abundant opportunities to develop alternative strategies to complement existing vector control products. This has been demonstrated in a similar manner by their use in agricultural pest management, wherein semiochemical-based techniques have effectively controlled harmful insects even in situations where conventional pesticides have failed or where regulation has phased out older strategies. To achieve effective, sustainable control of mosquito vectors of disease, the control efforts made for vector control must be diversified. Rather than continuing to rely on a limited number of insecticidal chemistries and intervention modes, the next generation of control strategies must diversify by manipulating different behaviors and sensitivities, and targeting mosquitoes at multiple life stages. This discussion will cover four technologies demonstrating the potential of semiochemicals to broaden and improve vector control programs. ***SPLAT BAC*** utilizes semiochemicals attractive to both adult and immature mosquitoes to enhance the larvicidal effect of the application on target breeding sites. ***Vectrax*** targets adult mosquitoes of both sexes of a variety of vector species in a flowable matrix that utilizes floral attractants and phagostimulants to act as an easily applicable targeted toxic bait. ***Trojan Cow*** utilizes a mammalian host cue to divert mosquitoes from preferred human hosts and onto livestock treated with an insecticidal anti-worming medication, reducing transmission of vectored disease to humans. Finally, ***Halo Shield*** uses a blend of botanical volatiles to create a spatial repellent that can protect groups of people in treated areas from host-seeking mosquitoes.

Influence of pollinator-associated exterior surface bacterial communities on blueberry floral volatile organic compounds

Aiko Lignon[1], Dr Manpreet Dhami[3,1], Prof Eirian Jones[1], Assoc Prof Clive Kaiser[1], Dr Flore Mas[2]
[1]Lincoln University, Lincoln, New Zealand, [2]The New Zealand Institute for Plant and Food Research Limited, Lincoln, New Zealand, [3]Manaaki Whenua Landcare Research, Lincoln, New Zealand

When insect pollinators visit flowers, microorganisms may be transferred from insects to flowers or vice versa. It has been shown that yeast can change the volatile profile of flowers, impacting insect visitor attraction. Therefore, we were interested in investigating whether different pollinators and their associated bacterial communities could also influence floral volatile organic compounds (FVOCs). We compared whether the different species of insect pollinators visiting flowers of northern highbush blueberry (*Vaccinium corymbosum*) 'Duke' influenced the FVOC profiles of both pre- and post-pollination. We hypothesised that there would be changes in post-pollination FVOC profiles of 'Duke' blueberries, depending on the exposure to different pollinator species. Different insects carry different microorganisms, which could also influence pollination success and visits from other pollinators. Through caged experiments, we exposed individual 'Duke' blueberry plants to one of four different types of insect pollinators (bumble bee (*Bombus terrestris*), honey bee (*Apis mellifera*), drone fly (*Eristalis tenax*), and a native New Zealand bee (*Lasioglossum sordidum*)). We sampled both pre- and post-pollination stages for each pollinator group to analyse FVOCs using dynamic headspace volatile sampling and to characterise the bacterial community through 16S rRNA Illumina sequencing of epiphytic washes from the external surfaces of pollinators. We found that distinct bacterial communities were associated with each pollinator species after pollination, but we did not detect any significant effect on FVOCs post-pollination profiles. The only changes observed in FVOCs were between pre- and post-pollination profiles (which was significant with a *P*-value of 0.002). Therefore, the bacteria associated with the exterior surface of each pollinator do not seem to influence FVOCs production.

Characterising the secondary metabolite and genetic mechanisms of aphid-wheat interactions in aphid resistant ancestral wheat.

Dr Alexander Borg[1], Dr John Caulfield[1], Dr Jozsef Vuts[1], Dr Mike Birkett[1]
[1]Rothamsted Research, Harpenden, United Kingdom

The barley yellow dwarf virus (BYDV), a vector-borne wheat pathogen of major importance, is spread by aphid vectors *Sitobion avenae* (English grain aphid) and *Rhopalosiphum padi* (bird cherry-oat aphid). Effective and sustainable BYDV management is currently lacking, with insecticides being the only effective option for BYDV control via vector management. Host-plant vector resistance holds promise as a sustainable solution for BYDV management but is lacking in modern wheat. The ancestor wheat *Triticum monococcum* MDR049 shows promising signs of resistance against *S. avenae* and *R. padi* in both laboratory and field settings. Here, we aimed to characterise the secondary metabolites involved in aphid-wheat interactions responsible for resistance in MDR049.

Through dynamic headspace collection, olfactometry, GC-electroantennography (EAG), GC-MS and authentic standards, 21 volatile organic compounds (VOCs) were identified and confirmed to be involved in the aphid repellent activity of MDR049, with the ratio of this VOC blend important in inducing either attraction or repellence. Bioassay-guided fractionation of leaf extracts via preparative-HPLC and aphid artificial feeding assays isolated a leaf secondary metabolite (SM) involved in the aphid antibiotic activity of MDR049. This SM was purified, and identified through LC-MS/MS, NMR and authentic standards, with activity confirmed using aphid artificial feeding assays. Transcriptomic analysis revealed a high level of differential gene expression in MDR049 upon aphid herbivory, lacking in the aphid-susceptible *T. monococcum* MDR037, with top differentially expressed genes putatively identified as being involved in defence, such as an (E)-β-farnesene synthase and recognition receptors.

Overall, we have identified the VOCs, leaf SMs and putative genes involved in the aphid resistance mechanisms of MDR049.Ongoing work is aiming to confirm/identify the genes and biosynthetic pathways involved in the SM-based aphid resistance mechanisms of MDR049 and investigate hexaploid wheat introgression lines with incorporated MDR049 genetic material for the aphid resistance observed in MDR049.

Attractancy of beta-caryophyllene to male Oriental fruit fly

Alvin Kah Wei Hee, Pradeepa Hewa Ranaweera
[1]Universiti Putra Malaysia, Serdang, Malaysia

The Oriental fruit fly, *Bactrocera dorsalis* is amongst the world's most destructive and invasive pests of fruits in the tropical and subtropical regions. The availability of a potent male lure, methyl eugenol has contributed significantly to success in the surveillance, monitoring and control of those flies. The recent discovery of new lures offers the advantage of capturing other pest species of fruit flies. Thus, we propose that probit regression analysis be used to evaluate the quantal response of male flies that were attracted to those lures. As such, the attractancy of those lures can then be compared. This is crucial in determining for their use in fruit fly control programs involving Male Annihilation Technique (MAT) and Sterile Insect Technique (SIT). Here, we report on the attraction of *B. dorsalis* males to a sesquiterpene, β-caryophyllene (CP). Probit regression analysis was undertaken to determine the quantal response of sexually mature male flies that were attracted to CP in cage bioassays. We discovered that, as a measure of male *B. dorsalis*' sensitivity of CP, the median dose of CP required to elicit a positive response in 50% of the tested fly population (ED_{50}) was 3.7 mg. This value was over 10,000x higher than known ED_{50} of *B. dorsalis*' male attraction to ME. Higher ED_{50} values imply lower lure attractancy. Thus, the much weaker attraction of *B. dorsalis* male to CP than to ME implies that CP cannot be considered for use in fruit fly control programme. Our findings imply that in any fruit fly surveillance and monitoring programme, selection of lures must consider the specificity and potency of each compound to target pest fruit fly species. The probit regression analysis of male fly quantal response to lure offers such information.

Are yeasts helping a tree-killer thrive?

Ms Ana Patricia Baños Quintana[1,2], Leandro Manuel Santiago Padilla[1,3,4], Martin Kaltenpoth[2], Jonathan Gershenzon[1], Maximilian Lehenberger[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany, [2]Department of Insect Symbiosis, Max Planck Institute for Chemical Ecology, Jena, Germany, [3]Berlin Institute of Health at Charité–Universitätsmedizin Berlin, Berlin, Germany, [4]Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin Institute for Medical Systems Biology (BIMSB), Berlin, Germany

The Eurasian spruce bark beetle (*Ips typographus*) is Europe's most destructive forest pest, causing annual timber losses of up to 70 million m^3 over the past decade. Rising global temperatures have shortened its life cycle and increased stress on its primary host, the Norway spruce (*Picea abies*), leading to mass outbreaks in which beetles shift from attacking weakened or fallen trees to infesting and killing healthy ones. In addition to increased temperature, drought and extreme weather also contribute to these outbreaks. While abiotic factors driving this shift in population dynamics are well recognized, the contribution of biotic factors remains less understood.

Throughout its life, *I. typographus* interacts with intra- and interspecific competitors, natural enemies, and symbiotic microorganisms, including fungi, bacteria, and yeasts. Although the beetle's fungal exosymbionts have been extensively studied in recent years, the ecological roles of its gut microbiome are less explored. It has been hypothesized that the gut microbes may contribute to the beetle's adaptive success by supplying nutrients, producing defensive compounds, or detoxifying the defence secondary metabolites of the host tree.

Our research combines classical microbiology, molecular biology, analytical chemistry, behavioural assays, and in-beetlo microbial community manipulation assays to better understand the ecology of *I. typographus*-associated yeasts and their involvement in the success of their host. Our main findings demonstrate that the yeast community can transform the Norway spruce's constitutive chemical defences into bioactive compounds that protect the beetle galleries against fungal pathogens. Additionally, we show that these microorganisms may also be an important source of sterols and B-vitamins for their host. We conclude that these lesser-studied fungi have metabolic capabilities that enable them to be the early colonizers of the galleries and to provide protection to the beetles, which in turn vector them to newly colonized trees during oviposition.

Plant invasion down under

Dr Andrea Clavijo McCormick[1,2], Evans Effah[2], Benjamin Pearson[2], Maria Minor[2], Alastair Robertson[2]

[1]Ministry for Primary Industries, Wellington, New Zealand, [2]Massey University, Palmerston North, New Zealand

Invasive plants are generally considered detrimental to ecosystems due to their ability to outcompete native species, disrupt natural habitats, and negatively impact local biodiversity. However, our growing understanding of invasive plant ecology suggests that their ecological impacts are far from uniform. This presentation shares results from two separate studies exploring below-ground interactions in the Central Plateau of New Zealand's North Island, home to Tongariro National Park—a dual UNESCO World Heritage site recognised for its cultural and natural value. The first study examines the effects of root extracts from two invasive species—heather (*Calluna vulgaris*) and Scotch broom (*Cytisus scoparius*)—on the germination and seedling growth of the native mānuka tree (*Leptospermum scoparium*), compared to conspecific extracts and those from another native species, red tussock (*Chionochloa rubra*). Results show that invasive root extracts exhibit stronger allelopathic activity on mānuka than those from conspecifics or another native. However, the effects of the two invasive species differ markedly from one another. The second study investigates soil properties and arthropod communities (micro- and mesofauna) associated with each of these four plants under field conditions. Interestingly, soil characteristics and fauna assemblages did not cluster by invasive status. Instead, mānuka and broom showed greater similarity, as did red tussock and heather. These findings challenge simplistic narratives in invasion ecology, revealing complex, species-specific interactions shaped by context.

We can’t know what we can’t catch: considering trap treatments post-PFAS

Dr Ann Ray[1], Emily Franzen[1]
[1]Xavier University, Cincinnati, United States

Traps for wood-boring insects are typically treated with aqueous suspensions of polytetrafluoroethylene (PTFE, a.k.a. Fluon®) to improve capture and retention of specimens during field assays. Formulations of PTFE vary in their cost, ease of application, initial effectiveness, and persistence over multiple field seasons. Disruption to global supply chains, changes to formulations of commercially available products, increasing costs, and concerns about environmental impacts of PTFE and other per- and polyfluoroalkyl substances (PFAS) have highlighted the need to consider alternative trap conditioners for wood-borer traps. We present here results of field assays testing different formulations and application methods for PTFE vs. non-PFAS trap conditioner and discuss considerations for the future of wood-borer trapping as access to PFAS becomes more difficult.

Semiochemical-based alternative concepts for the management of wireworms

Miss Anusha Mohan-Kumar[1,2], Dr Gareth Thomas[1], Dr John C. Caulfield[1], Dr Ben de Lacy Costello[2], Dr Benedikt Kurtz[3], Dr Pete Maxfield[2], Dr Jozsef Vuts[1]

[1]Rothamsted Research, United Kingdom, [2]University of the West of England, United Kingdom, [3]Syngenta Crop Protection AG, Switzerland

Wireworms (*Agriotes* spp.), the larvae of click beetles (Coleoptera: Elateridae), are polyphagous soil pests of vegetable and cereal crops across Europe and North America. They cause 10-37% seedling mortality in cereals by feeding on the crown and roots. Stringent pesticide regulations and a shift towards sustainable practices, including minimal tillage, have inadvertently increased wireworm infestations. Therefore, sustainable pest management strategies, including using semiochemicals, are necessary.

Semiochemicals are development- or behaviour-modifying compounds that are involved in intra- or inter-species interactions. Like aboveground pests, soil herbivores are attracted to or repelled by volatile organic compounds (VOCs) that are abundant in the gas phase and diffuse through soil pores. Hence, the aim is to identify and characterize novel root-emitted VOCs that serve as host-finding cues for wireworms.

In agricultural systems, plants encounter biotic stresses, that can alter their VOC profiles. To understand how these changes influence wireworm host-finding behaviour, different wheat varieties were tested under conditions of wireworms (belowground), aphids (aboveground), and combined herbivory.

Soil olfactometer assays revealed that wireworms exhibited behavioural preferences for certain wheat varieties, whereas others were unattractive. This suggests that wireworms can differentiate between varieties of the same species. Additionally, wireworms showed a preference for plants damaged by congeners over healthy plants but exhibited no such preference for plants under aboveground or dual herbivory. VOC extracts collected through air entrainment showed treatment-dependent VOC profiles across varieties, although emission levels varied. Root damage by wireworms induced VOCs that were absent in healthy and aboveground herbivore-damaged plants, indicating qualitative changes specific to belowground herbivory.

Future research will focus on identifying active VOCs that trigger behavioural preferences in wireworms. These compounds could be used in slow-release formulations for wireworm population monitoring in farmlands, attract and kill strategies for crop protection and may further support breeding programs that select for pest resistance traits.

TRSYMB1 is a novel transcription factor which regulates secondary metabolism and symbiosis in *Trichoderma*

Dr Artemio Mendoza-Mendoza[1], Dr Maria Fernanda Nieto-Jacobo[1], Prof Michael Rostas[2], Dr Michal Kuchar[1], Prof John Hampton[1]

[1]Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, New Zealand, [2]Department for Crop Sciences, Georg-August-Universität Göttingen, Germany

Endophytic microorganisms protect plants against biotic and abiotic stresses, enhancing growth, productivity, biodiversity, and ecosystem function. Surprisingly, fungi that can be devastating and widespread plant pathogens, including *Fusarium* spp., have also been identified as asymptomatic endophytes in healthy host and non-host plants. Moreover, strains of *Trichoderma*, a facultative fungal symbiont and the fungal species applied most successfully in agriculture, have shown positive, neutral, and sometimes detrimental effects in plants. In this presentation, we will describe the role of a membrane-bound transcription factor, TRSYMB1, which regulates the *Trichoderma*-plant root symbiosis. A deletion mutant lacking the *trsymb1* gene (Δtrsymb1) causes inhibition of seedling emergence and produces severe maceration in tomato and *Arabidopsis* plants. Interestingly, the *trsymb1* gene is conserved in some fungal pathogens. The lack of the trsymb1 gene affects nitrate assimilation by regulating the nitrate gene cluster in *T. virens*. Remarkably, different secondary metabolites are differentially expressed in response to ammonium or nitrate through TRSYMB1.

This finding presents a unique opportunity to understand the molecular mechanisms that control the endophyte-pathogen equilibrium in both host and non-host plants, not only with *Trichoderma* but also with pathogens such as *Fusarium oxysporum*.

Bioactivity of *Trichoderma* secondary metabolites against forest tree pathogens in New Zealand

Ms Nicky Hambrook[2], Prof Rosie Bradshaw[2], Dr Nicole Xu[1], Prof Michael Rostas[3], Prof Ulises Esquivel-Naranjo[1], Ms Helen Whelan[1], Prof John Hampton[1], **Dr Artemio Mendoza-Mendoza**[1]

[1]Lincoln University, Lincoln, New Zealand, [2]School of Food Technology and Natural Sciences, Massey University, New Zealand, [3] Georg-August-Universität Göttingen, Göttingen, Germany

We investigated the impact of secondary metabolites (SMs), secreted by *Trichoderma atroviride*, on the growth of major disease pathogens affecting New Zealand's forest trees. These included foliar pathogens affecting *Pinus radiata* and root pathogens affecting *Agathis australis* (kauri). We identified these metabolites using Gas Chromatography coupled to Mass spectrometry. By using commercially available SMs, diverse tree pathogens were directly or indirectly exposed in vitro. We found that several SMs exhibited fungicidal or fungistatic effects when directly exposed to *Dothistroma septosporum*. We also observed growth being limited by indirect exposure to some SMs for the pathogens *Phytophthora pluvialis, Phytophthora cinnamomi*, and *Cyclaneusma minus*. We will explore the potential to expand this promising proof-of-concept study through further pathogen screening, with the ultimate goal of advancing to in planta trials—bringing us one step closer to sustainable, biology-driven solutions for disease management.

Glucosinolate profiles linked to reduced flea beetle (*Phyllotreta cruciferae*) damage in *Brassica napus* X *Sinapis alba* introgressed lines

Dr Aziz Ullah[1], Dr Altaf Hussain[1], Dr Sally Vail[2], Dr Boyd Mori[1]
[1]University of Alberta, Edmonton, Canada, [2]Agriculture and Agri-Food Canada, Saskatoon, Canada

Flea beetles (*Phyllotreta* spp., Coleoptera: Chrysomelidae) are major, economically damaging canola (*Brassica napus*) pests in Canada. Feeding damage to canola seedlings negatively affects plant establishment and growth. Insecticides are the primary means of management. The potential for resistance to insecticides underscores the significance of investigating alternative management strategies, such as host plant resistance. To date, little insect resistance has been found within *B. napus*, whereas *Sinapis alba* has been shown to be resistant or tolerant to several insect species. Introgressed lines of *B. napus* and *S. alba* offer promise to identify host plant resistant traits and develop canola quality lines. In this study, we investigated host plant resistance in introgressed lines of *B. napus* and *S. alba* and explore the underlying mechanisms of resistance. We conducted feeding bioassays with *P. cruciferae* collected across the Canadian Prairies and quantified glucosinolates, chlorophyll content, trichrome density, linear electron flow (LEF), and dry biomass from introgressed, *B. napus* and *S. alba* seedlings. Additionally, six lines representing resistant, moderately susceptible, and highly susceptible categories were selected based on preliminary screening and used for repeated assays.

Our analyses identified 15 distinct glucosinolate profiles among the introgressed *B. napus* and *S. alba* seedlings, with significant variation observed across different lines. The higher glucosinolates were correlated with higher dry biomass, chlorophyll, and LEF content. Trichomes were observed in only the *S. alba* parental line, suggesting they played a limited role in resistance. Notably, the introgressed lines with elevated glucosinolate contents experienced significantly less feeding damage compared to lines with lower concentrations of glucosinolates. Furthermore, Random Forest analysis identified glucosinolate content as a key predictor of resistance. This study underscores the potential of introgressed resistant lines in enhancing defense against flea beetle damage.

Piezo mediates oviposition in shielding gaps to protect moth eggs from parasitoid wasp

Dr Baiwei Ma[1]

[1]Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

Where to lay eggs is critical for insect females to avoid risks and ensure offspring survival. Females of the oriental armyworm, *Mythimna separata*, deposit their eggs inside narrow slits to evade predators. However, the sensory mechanism by which they choose the optimal slit for egg-laying remains unclear. Here, we demonstrate the requirement of mechanosensation for selecting oviposition slit. Females preferentially deposit eggs in leaf sheath or in folded paper gaps, thereby shielding their eggs from parasitism by *Trichogramma dendrolimi*. Specifically, females prefer the opening of 0.5 mm in rigid gaps. Surgical ablation of ovipositor bristles leads to disrupted gap selection and increased parasitism. Mechanosensitive receptor Piezo is found within these bristles and responds to the mechanical deflection of a single bristle. Piezo deletion phenotypes bristle ablation in random egg-laying and vulnerability to parasitoids. Collectively, our findings provide insight into the involvement of mechanosensation in insect egg-laying site choice.

Production of pheromones in *Camelina* for sustainable pest control

Dr Baojian Ding[1], Dr Honglei Wang[1,2], Dr Per Hofvander[3], Dr Edgar Cahoon[4], Dr Christer Löfstedt[1]

[1]Xianghu Lab, Hangzhou, China, [2]Department of Biology, Lund University, Lund, Sweden, [3]Department of Plant Breeding, Swedish University of Agricultural Sciences (SLU), Alnarp, Sweden, [4]Center for Plant Science Innovation & Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, USA

Pheromones have become an environmentally friendly alternative to conventional insecticides for pest control. Most current pheromone-based pest control products target lepidopteran pests of high-value crops, as today's manufacturing processes cannot yet produce pheromones at low enough costs to enable their use for lower-value crops, especially commodity crops. To lower the cost of pheromone synthesis and promote their wider use in agricultural pest management, use of transgenic plants to, in essence, 'grow' pheromone precursors or components represents a green chemistry alternative to synthetic pheromone production. This not only reduces the cost of these production processes but also eliminates the need for the petroleum-based and other chemical feedstocks used in conventional pheromone synthesis. We genetically engineered *Camelina sativa* to produce sex pheromone precursor of several moth species. Seed oil were extracted and pheromone precursors were transformed to the final pheromones. Furthermore, we demonstrated the biological activities and economic feasibility of pheromone production in plant factories by metabolic engineering of an oilseed crop.

Hot breath, quick exit: Aphids flee mammalian heat via TRPA1

Prof Jonathan Bohbot[2], **Prof Bing Wang**[1], Prof Guirong Wang[1], Dr Lulu Yang[1]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Department of Entomology, The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel

Animals have developed scent-triggered avoidance for threat evasion and survival. Here, we found that aphids exhibit a unique heat-guided herbivore-avoidance mechanism. We identified the TRP channel ApisTRPA1 in *Acyrthosiphon pisum* as a key receptor for sensing rapid temperature increases. Two isoforms of ApisTRPA1, ApisTRPA1-A and ApisTRPA1-B, are both expressed in neurons located in the primary rhinaria, which are essential for acute heat detection. Among them, ApisTRPA1-B exhibits a stronger response to temperature gradients. Furthermore, we found that ApisTRPA1 is a multimodal receptor and acts as a chemosensor activated by natural plant compounds. These findings shed light on aphid physiological adaptations to thermal cues and may inform the development of eco-friendly aphid management strategies.

Pollination of by sexual deception via a proposed pro-pheromone mimicry mechanism

Ryan Phillips[4], Seeger van Kints[5], Ben Ong[2], Alyssa Weinstein[3], Rod Peakall[3], Gavin Flematti[2], **Björn Bohman**[1,2,3]

[1]Swedish University of Agricultural Sciences, Sweden, [2]University of Western Australia, Australia, [3]Australian National University, Australia, [4]La Trobe University, Australia, [5]University of Tasmania, Australia

The slipper orchids, *Cryptostylis,* represent an extreme pollination strategy amongst the sexually deceptive orchids, as it is one of very few plants that induces ejaculation by some of the male insect pollinators. Despite being recognised almost 100 years ago as sexually deceptive, their chemical pollinator attractants inducing this sexual behaviour have remained unknown. In addition, *Cryptostylis* is the only sexually deceptive genus so far known to exploit ichneumonid wasps for pollination, and therefore may provide new insights into the chemistry of sexual deception. In a previous study using preparative chromatography, synthesis and field bioassays, the pollinator of *Cryptostylis ovata*, *Lissopimpla excelsa*, was attracted to (*S*)-2-(tetrahydrofuran-2-yl)acetic acid, although no pseudocopulation to this compound was observed in the bioassays.

Sex pheromones are typically volatile compounds underpinning the chemical sexual communication among conspecifics. In insects, however, there are cases where females of some species do not produce the active pheromones, but rather precursors (pro-pheromones) that are subsequently modified by external processes to the bioactive compounds. Here, we identify three long chain alkenes, which are predominantly present in the pollinator-attracting labellum of *C. ovata*, and show that these compounds, when present in large amounts, induce pseudocopulation in *L. excelsa*. Importantly, we discovered that the predicted oxidation products from these compounds, a mixture of volatile aldehydes, are also strongly attractive to the pollinator, at much lower amounts. In combination with the previously identified long-range attractant 2-(tetrahydrofuran-2-yl)acetic acid, the blend of aldehydes frequently elicited pseudocopulation in bioassays. As the same most abundant of the three alkenes, (*Z*)-8-tridecene, was found in extracts of female *L. excelsa*, this compound is proposed as a putative "pro-pheromone mimic" in *C. ovata* and pro-pheromone for *L. excelsa*.ody text goes here.

Integrative analysis of allelopathic activity and metabolite profiling in *Moringa oleifera* root extracts on native, pasture and weed species

Blair Moses Kamanga[1], Dr Donita Cartmill[1], Mr Paul Berrett[1], Mr Craig McGill[1], Dr Andrea Clavijo-McCormick[1,2]

[1]Massey University, Palmerston North, New Zealand, [2]Ministry for Primary Industries, Wellington, New Zealand

Integrative analysis of allelopathic activity and metabolite profiling in *Moringa oleifera* root extracts on native, pasture and weed species

Abstract

Moringa oleifera is a fast-growing tree native to northern India, valued for its nutritional and medicinal properties. As global demand increases, moringa is being introduced into new regions. While the species is not yet cultivated in New Zealand, there is growing in integrating it into dairy systems. However, there are concerns regarding its potential ecological impact, particularly its allelopathic traits that could impact native and introduced plant species. This study investigated the allelopathic effects of moringa root extracts on native, pasture, and weed species in New Zealand, and conducted a targeted metabolomics analysis of the potential compounds responsible for the observed allelopathic effects. Root extracts from two moringa provenances were assessed across concentration gradients (1–100%) using seed germination and seedling growth bioassays. LC-MS and principal component analysis (PCA) identified potential allelochemicals, including caffeic acid, indole-3-acetyl-L-tryptophan, Isoquercetin, 8-hydroxyquinoline, fumaric acid, and kaempferol-3-glucoside. No significant chemical differences were found between the provenances. Statistical analysis using generalized mixed linear models and Type III fixed effects revealed a strong species-specific response to moringa root extracts. Significant inhibitory effects were observed on mean germination time ($F = 6808.03$, $p < .0001$) and root length ($F = 29434$, $p < .0001$), with higher extract concentrations causing a progressive decline in seedling performance across all test species ($p < .0001$). These findings highlight moringa's strong allelopathic potential with White clover - a key pasture species being the most negatively affected, suggesting that moringa may not be suitable in combination with this species. Planting nearby native species should also be avoided; however, ryegrass was not affected. Conversely, its suppressive effects on common weeds such as crabgrass and fathen indicate possible benefits for weed management. This research highlights the importance of ecological assessments before introducing new species and offers valuable insights into the chemical ecology of moringa in novel environments.

Improved Queensland fruitfly lures – Longevity versus high efficiency

Prof C. Rikard Unelius[1], Dr Suresh Ganji[1], Jodie Cheesman[2], Dr Andrew Bloomfield[1], Dr Stefano de Faveri[2], Dr Ashraf M. El-Sayed[3]

[1]Department of Chemistry and Biomedical Sciences, Linnaeus University, Kalmar, Sweden, [2]Department of Agriculture and Fisheries, Horticulture and Forestry Science, Mareeba, Australia, [3]The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand

Background. The Queensland fruitfly [QFF, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae] is one of the most severe insect pests on horticulture in Australia. Originating from tropical coastal Queensland it is now established in Eastern Australia and some Pacific Islands. Already ten years ago, there were >30 reports of undertaken responses to Queensland fruitfly incursions in Australia, French Polynesia, the Cook Islands, and New Zealand. The economic losses arise not only by direct fruit yield losses, but also by direct and indirect management costs and by restrictions to sell fruit in international and domestic markets in QFF-free parts of Australia. Cuelure is known to attract more than 280 species of Tephritid fruit flies. Cuelure is attractive but has not found to be released by the QFF flies. This suggests that there exists a natural compound that has not been detected yet.

The aim of this project was to discover more effective attractant(s) for the Queensland fruitfly based on syntheses and testing of rationally selected analogues of cuelure.

Science outcome. In this investigation a chemical bait with superior attractivity compared to Cuelure has been discovered. It attracts more flies and performs better over time. The availability of an improved attractive lure should have a significant positive impact on monitoring and control of the Queensland fruitfly, the most important fruit fly pest in Australia.

Divergent evolutionary pressures shape olfactory sensitivity of the maxillary palps in Tephritidae fruit flies

Chaymae Fennine[1,4], Sebastian Larsson Herrera[1,2], Tibebe Dejene Biasazin[1], Wittko Francke[3], Sergio Angeli[4], Teun Dekker[1,2]

[1]Swedish University of Agricultural Sciences, Alnarp, Sweden, [2]National Species Management, Wageningen, Netherlands, [3]Institute of Organic Chemistry, University of Hamburg, Hamburg, Germany, [4]Free University of Bozen-Bolzano, Bozen, Italy

Olfaction is a rapidly evolving sense. Given its diverse functions, from finding ecological niches to selection of mates, we hypothesized that olfaction is subjected to divergent evolutionary pressures. We compared the olfactory sensitivity of five species of Tephritidae fruit flies to two broad classes of volatiles: general niche-related volatiles (food and fruit odors) and volatiles used in sexual communication (pheromones and 'parapheromones'). We then analysed whether the differential sensitivities across species harbor 'signals' of such contrasting evolutionary pressures. As recent studies highlight the maxillary palps as key auxiliary olfactory organs for detecting both classes of volatiles, we focused our sensory analysis on this auxiliary olfactory organ. Using gas chromatography coupled to electropalpographic detection (GC-EPD), we recorded sensory responses from five species with a diverse phylogenetic and ecological separation. Besides, considerable overlap in detection was observed across taxa, the maxillary palp exhibited sex and clade-specific divergence in sensitivity to pheromones and parapheromones. Cluster analysis of sensitivities to (para)pheromones aligned strongly with the species' phylogeny. In contrast, cluster analysis of sensitivities to general food and fruit odors strongly followed ecology rather than phylogeny. Clearly, the selection pressures that shape the evolutionary direction of olfactory sensitivity to (para)pheromones and niche-related odors are diametrical opposites, reminiscent of stabilizing versus directional selection. Understanding the detection and evolution of distinct volatile classes provides valuable insights into evolutionary-ecology of olfaction, studies on olfactory receptors, and sensory and preference coding, and supports the rational development of novel lures to manage these pest insects.

Chemical ecology in the biological control of weeds: the role of sex pheromones in the species delimitation and monitoring of moth biocontrol agents

Dr Cody-Ellen Murray[1], Dr Michelle Rafter[2], Dr Adriana Najar-Rodriguez[3], Dr Andrew Twidle[2], Mrs Lee-Anne Manning[2], Mr Andrew White[1], Mrs Kelli Pukallus[1], Dr Dean Brookes[1], Dr Gimme Walter[1]

[1]The University of Queensland, Brisbane, Australia, [2]CSIRO, Australia, [3]Plant and Food Research, Lincoln, New Zealand

Accurate species delimitation of target pests and prospective agents are integral for the success of biological control programs. Inaccurate identifications can not only result in poor management outcomes but can also have catastrophic off-target impacts. In moths, female sex pheromones are often the first step in species-specific mate recognition systems, however shared compounds and cross-attraction is not unheard of in closely related species, meaning taxonomy determined on this basis alone is often not sufficient for applied outcomes. Such is the case in *Eueupithecia cisplatensis* and *E. vollonoides*, cryptic species of geometrid moths introduced to Australia as biocontrol agents of the pastural weed *Parkinsonia aculeata*. Reciprocal pheromone trapping tests in the field revealed that males are attracted to the sex pheromones of females of both species and laboratory tests indicated significant overlap in diel mating patterns and behaviour, resulting in viable hybridisation in crosses between *E. vollonoides* females and *E. cisplatensis* males. Despite this, molecular assessment of their population genetics found that these moths exist as highly discrete genetic entities both in the native range of South America and the introduced range of Australia, despite areas of sympatry at both locations. This example highlights the need for an integrated chemical, behavioural and molecular approach to be used iteratively throughout weed biocontrol programs to tease apart ecological nuances and clarify species boundaries in prospective agents. Further, in the example of *Eueupithecia* species, pheromone trapping with live unmated females proved to be an effective tool for monitoring the establishment and spread of these moths across their host plant's patchy and remote distribution. Preliminary GCMS and EAD analyses also identified a shared chemical compound in the pheromone profile of females from both species ((11Z,13Z)-hexadeca-11,13-dienal), which may be investigated further to develop a synthetic lure for further evaluation of this biological control program.

An iOR-based biosensor for the detection of fall army worm (*Spodoptera frugiperda*)

Dr Colm Carraher[1,2], Dr Brady Owen[1], WenJuan Huo[1], Alaigne Mare[1], Leroy Bird[1], Dr Sarah Wilson[1], Jung Shin[1], Jonathan Good[1], Dr Andrew Kralicek[1]

[1]Scentian Bio Ltd, Auckland, New Zealand, [2]Plant and Food Research Ltd, Auckland, New Zealand

Globally the food and fibre sectors are under increasing threat from insect pests, meanwhile consumers are demanding more sustainable production with less chemical treatments. There is a wealth of work and emerging solutions around biologics and IPM, but this relies on early detection which is infeasible today. To address this problem, we are creating novel sensors that mimick insect olfaction to detect the volatile organic compounds (VOCs) insects use to communicate. To do this we use synthetic biology to produce odorant receptors, which are ligand-gated ion channels, and integrate them into membranes where we use electrophysiology approaches to track current passing through the channel over time.

Insect olfactory receptors are membrane bound proteins that are expressed in the dendrites of olfactory sensory neurons in the antennae. Membrane proteins are inherently difficult to work with *in vitro*, however, at Scentian Bio we have successfully expressed and purified more than 70 receptors from 5 different insect species. We now have a pipeline in place to predict the mostly likely receptor to respond to a given ligand and allow the expression and purification of novel receptors within weeks of receiving the gene. We can then insert these receptors into our biosensor platforms and achieve PPB levels of detection.

Here we will present our work on the detection of fall army worm (*Spodoptera frugiperda*), one of the most damaging horticultural pests globally, that has recently been established in NZ. The economic risk and high level of interest in developing management strategies makes it the perfect target for our biosensor technology. The key component of the FAW pheromone is (Z)-9-tetradecyl acetate. We have purified the pheromone receptor from *S. frugiperda* and demonstrated its functional response to the key pheromone compound. We will describe these data and our plans to develop a field-ready device for FAW detection.

Development of a carob moth (*Ectomyelois ceratoniae*) semiochemical lure targeting gravid females

Dr Daniel Geiberras[1], Dr Umar Lubanga[1], Mr David Madge[2], Ms Cathy Taylor[2], Dr Daniel Anderson[2], Dr Samantha Edley[2], Assoc Prof J Paul Cunningham[1,3]

[1]Agriculture Victoria Research, Bundoora, Australia, [2]Agriculture Victoria Research, Irymple, Australia, [3]La Trobe University, Bundoora, Australia

Carob moth (*Ectomyelois ceratoniae*) is a polyphagous pest that causes significant economic damage to high-value nut and fruit commodities, including almonds, pistachios, macadamias, pomegranates, stone fruits and pome fruits. In Australia, this insect causes around 1-4 % damage to almond crops, in an industry that has a farmgate value exceeding $1 billion. Current control measures for carob moth include orchard hygiene, biological control with parasitoids or generalist predators, and insecticidal sprays. Male moth activity can be monitored using traps baited with sex pheromone mimic lures. However, female moths are of greater concern, as each can lay over 100 eggs which develop into crop-damaging larvae. Unfortunately, there are no established methods to monitor female moth activity.

We have developed a lure to specifically target gravid female carob moth, using host-derived odours that act as oviposition cues. Cage experiments revealed that used diet from a laboratory-reared carob moth culture was the preferred oviposition site for gravid female moths over fresh diet, almonds and fruits. The volatile profile of the used diet was examined by DHS-GC-EAD-MS to identify volatiles that elicit a response in gravid female carob moth. Results were used to develop an 11-component synthetic lure. After showing initial promise in the 2021/2022 field season, the lure has undergone an optimisation process through field testing over each subsequent growing season. The addition of further attractive volatiles from other sources has been explored and six of the original 11 compounds have been eliminated from the blend. Our 2024/2025 season trails yielded two six-component blends which each trapped 50% more female moths than our best lure blend from the previous season. These promising results mean that the feasibility of using the lure in an attract and kill solution is now under consideration.

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Pyrethroids sensory detection in the malaria vector *Anopheles gambiae*

Dr Simplice Kambou[2], MSc Adeline Valente[1], Dr Philip Agnew[1], Dr Anna Cohuet[1], **Dr David Carrasco**[1]

[1]French National Research Institute for Sustainable Development (IRD), Montpellier, France, [2]Institut de Recherche en Sciences de la Santé, Bobo Dioulasso, Burkina Faso

Insecticide-based vector control has been recognised as the principal contributor of the malaria-incidence reduction since 2000. Recently, this trend has worryingly stalled or even reversed in some countries. One identified reason of such stall is the evolution of insecticide resistances to the widely used pyrethroid insecticides in malaria vectors (*Anopeheles sp.*). Among them, behavioural resistance, i.e. behavioural modifications allowing to surpass the negative effects of insecticides, has been acknowledged but the mechanisms involved are relatively unknown in malaria vectors. The sensory detection of pyrethroid molecules by malaria mosquitoes has been proposed as one of the potential mechanisms that could explain some forms of behavioural resistance. Indeed, several studies have identified a sensory-guided effect, such as repellency or even in some cases attraction, of malaria mosquitoes when confronted with pyrethroid-based vector control tools. Yet, the functional mechanisms of pyrethroid sensory detection in *Anopheles* sp. have not been yet described.

In this work, in a series of behavioral experiments, we determined that laboratory-reared *An. gambiae* (Gilles; Diptera, Culicidae) and wild *Anopheles* sp. mosquitoes are able to detect pyrethroid molecules from a distance and also, we identified that antennae and tarsi are the organs involved in such detection. Secondly, we confirmed using electrophysiological techniques (EAG and SSR) the presence of sensory receptors having pyrethroid molecules as agonists in *An. gambiae*.

All these findings shed light into the functional mechanisms of pyrethroid sensory detection in malaria mosquitoes, and they open the door to further study their role in the still vastly unexplored evolution of behavioral resistances in disease vectors.

Linking volatile chemical landscape to parasitic activity of *Fopius arisanus* in mango orchards

David Emmanuel Arzac[1]

[1]Cirad, La Réunion, France

Insects locate suitable oviposition sites by detecting odour cues emitted by their hosts, while navigating a broader field of volatile compounds, an odour landscape that structures their environment. Does host exploitation efficiency depend on the surrounding chemical landscape? To address this, we studied the spatio-temporal variability of both the background parasitism pressure of *Fopius arisanus*, a biological control agent targeting the oriental fruit fly *Bactrocera dorsalis*, and the chemical composition of ambient air in mango orchards. Our study was conducted across four distinct phenological stages of mango trees and four orchards, sampled simultaneously at each stage, with three replicates per stage and orchard. For each replicate, we deployed simultaneously five sentinel hosts, consisting of banana artificially infested with *B. dorsalis* eggs, alongside eight Tenax cartridges connected to air pumps, later analysed via gas chromatography-mass spectrometry. We showed that both the parasitic activity of *F. arisanus* and the chemical composition of ambient air varied significantly across time and space. Parasitism activity (percentage of parasitized eggs) differed significantly among phenological stages ($F(3,218) = 16.5$, $p < 10^{-8}$), with high levels during ripe mango stage (Tukey, $p < 0.01$). Each phenological stage also exhibited a specific volatile profile (PERMANOVA $F(3,363) = 15.21$, $R^2 = 0.10$, $p < 0.001$), superimposed upon a common background odour. During the ripe mango stage, variability in parasitic activity ($F(3,56) = 330.96$, $p < 10^{-9}$) and in chemical composition (PERMANOVA $F(3,84) = 1.99$, $R^2 = 0.06$, $p < 0.001$) was observed between orchards. One orchard stood out with high levels of parasitic activity, and its distinct chemical profile was characterised by high levels of hexanal and 3-carene, and low levels of 4-penten-1-ol and methyl salicylate. This study highlights volatile compounds that may modulate *F. arisanus* behaviour, offering leads for agroecological strategies in crop protection.

Olfactory learning in *Pieris brassicae* butterflies is dependent on the intensity of a plant-derived oviposition cue

Msc Dimitri Peftuloglu[1]

[1]Wageningen University, Wageningen, Netherlands

Specialist herbivores rely on specific cues to identify their host plants; however, these innate stimuli are often difficult to detect from a distance and herbivores might learn to associate additional olfactory or visual information with these cues. For many butterflies, oviposition occurs only when specific gustatory cues are detected by the tarsi, allowing a mated female to identify ideal sites for offspring development. However, upon substrate location, simultaneously occurring stimuli might be integrated in memory and used in a later moment as additional cues to locate oviposition sites. In the present study, we asked whether mated *Pieris brassicae* butterflies are able to associate a novel olfactory stimulus, vanilla scent, with an innately meaningful oviposition stimulus, the glucosinolate sinigrin. In addition to this, we asked whether memory recall was dependent on the intensity of the neuronal response generated by sinigrin tarsal detection. In a first moment, mated butterflies were conditioned by exposure to a paper disc sprayed with different sinigrin concentrations and coupled with a vanilla scent source. To assess memory formation, animals were later exposed to the same paper discs used during conditioning, either bearing vanilla scent or a control solvent, but not sprayed with sinigrin. Memory recall was assessed by measuring 1) time spent on each disc type, 2) number of visits to each disc type, 3) number of oviposition events on each disc type. Tests were performed indoors (greenhouse) and outdoors (flight tent) and in both cases butterflies were able to form memory after conditioning. Moreover, electrophysiological recordings on the tarsal taste sensilla used to detect sinigrin allowed to observe that memory recall occurred only for animals conditioned with sinigrin concentrations giving the highest neuronal responses. This study presents novel insights on how butterflies can integrate different sensorial information to successfully complete crucial tasks such as oviposition, showing how cognitive plasticity might contribute to adaptation in a rapidly changing environment.

Chemical ecology of honey bee responses to brood injuries

Dr Elisa Pal[1], Louise Jeandroz[1], Charlène Dumas[1], Dr Maxime Hervé[2], Benjamin Basso[1], Dr Fanny Mondet

[1]INRAE, UR 406 Abeilles et Environnement, Avignon, France, [2]INRAE, IGEPP, University of Rennes, Rennes, France

In honeybees (*Apis mellifera*), hygienic behaviour is a social immunity trait which can improve disease and parasite resistance in the colony. This behaviour has been bred for economic purposes for decades with conventional selection methods such as the pinned-killed brood (PKB) and the freeze-killed brood (FKB) assays. However, the underlying mechanism behind the PKB assay is not well understood. Thus, in this study, the odour profiles of injured brood (PKB, FKB) and non-injured brood were investigated to identify potential compounds that could trigger hygienic behaviour. The removal behaviour response of injured brood from sealed cells was also measured in the field at different times, as well as the development of the nymphs after being injured. Volatile organic compounds (VOCs) and cuticular hydrocarbons (CHCs) from injured and non-injured brood were collected via headspace solid-phase microextraction (HS-SPME) and solvent extraction, respectively. Results showed that different VOCs and CHCs are emitted between injured and non-injured brood, with variations depending on the type of injury and the age of brood. In the field bioassays, the cleaned cell proportion after injury increased over time and the hygienic responses vary depending on the age of the brood. This effect may be attributed to the varying levels of necrosis at different developmental stages caused by the wound inflicted by the pin. Overall, these findings expand our knowledge on the mechanism behind the hygienic behaviour, and how the brood signals its status (injured, dead). We also highlighted candidate compounds potentially involved in triggering this social immunity response.

Conspecific chemical cues in *Ciona intestinalis*: Linking larval behaviour, brain activity, and cue composition

Dr Emily Claereboudt[1], Dr Marios Chatzigeorgiou[1]
[1]Michael Sars Centre, University of Bergen, Bergen, Norway

Chemical communication plays a key role in the lives of many marine invertebrates, including tunicates. We are investigating how larvae of the solitary ascidian *Ciona intestinalis* respond to waterborne cues released by conspecifics, using a multi-level approach that combines behavioural tracking, calcium imaging, and non-targeted metabolomics. To assess the chemical nature of these cues, we analysed seawater conditioned by either one adult or 200 larvae using untargeted metabolomics. Larval-conditioned water contained a greater abundance of metabolites compared to adult-conditioned water, suggesting that larvae release a rich mixture of compounds into their environment—possibly as signals to other larvae or even adults. Ongoing work focuses on whether and how these cues influence behaviour and neural activity. We are applying machine learning-based tracking to quantify larval movement patterns in response to conditioned water. In parallel, we are conducting in vivo calcium imaging to identify brain regions involved in detecting and processing conspecific cues. Together, these approaches aim to reveal how *Ciona intestinalis* larvae produce, detect, and respond to conspecific chemical cues. This work contributes to a broader understanding of chemical communication and neural processing in early-diverging chordates, and highlights *Ciona* as a powerful system for studying the evolution of sensory processing and behaviour.

The potential of synthetic sex pheromone among other technologies to suppress *Vespula* species wasps

Eric Edwards[1], Robert Brown[2], Ashraf El Sayed[3]

[1]Department Of Conservation, Wellington, New Zealand, [2]Manaaki Whenua Landcare Research Institute, Lincoln, New Zealand, [3]Plant & Food Research Institute, Lincoln, New Zealand

Common and German wasps (*Vespula vulgaris, V. germanica*) have invaded New Zealand and other southern and northern temperate countries. Their impacts are significant for people, livelihoods and nature. Honeydew or carbohydrate from a range of sources fuels wasps. New Zealand has among the highest *Vespula* wasp densities recorded anywhere, across a million hectares of beech forest containing native honeydew insects. Wasps are calculated to cost the New Zealand economy ~$80m US dollars per year and cause ~5% of honey-bee hive losses.

While pest insect control is typically done at the farm scale or in urban areas, we seek potential technologies to suppress the impact of wasps at the scale of >200 square kilometres. Disruption of wasp populations via synthetic sex pheromone may be relatively feasible and additive in contrast to use of toxins, pathogens, natural enemies or molecular genetic approaches.

A new approach for detection of Tasmanian devil facial tumor disease: VOC analysis and canine scent detection

Dr Flora Gouzerh[1], Dr Frédéric Thomas[1], Rodrigo Hamede[2], Beata Ujvari[3], Lionel Brazier[1], Jordan Meliani[1], Bruno Buatois[4], Mélanie Bonet[1], Anne Xuereb[5]

[1]CREEC/ MIVEGEC, Centre de Recherches Ecologiques et Evolutives sur le Cancer/ Maladies infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle UMR IRD 224-CNRS 5290-Université de Montpellier,, Montpellier, France, [2]School of Natural Sciences, University of Tasmania, Hobart, Australia, [3]School of Life and Environmental Sciences, Deakin University,Waurn Ponds, Victoria, Australia, [4]Centre d'écologie Fonctionnelle et Evolutive,CEFE, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, MONTPELLIER, France, [5]Centre de Biologie pour la Gestion des Populations , CBGP , INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, MONTPELLIER, France

The use of volatile organic compounds (VOCs) as biomarkers for disease detection is rapidly expanding across species, including wildlife. Non-invasive sampling of odors—via feces, urine, or exhaled air—offers a promising approach to assess health status without the need to capture or handle animals. Among diseases that alter metabolic processes and VOC profiles, cancer remains largely unexplored in wild species. The Tasmanian devil (*Sarcophilus harrisii*), an endangered marsupial, presents a unique case as it suffers from two naturally occurring transmissible cancer: Devil Facial Tumor Disease (DFTD). For a better understanding of the disease and better monitoring of cancer-infected areas, it would be interesting to develop non-invasive and non-capture detection tools. For this, in this study, we investigated whether VOCs emitted from feces differ between visibly infected and healthy devils. We combined gas chromatography–mass spectrometry (GC-MS) analysis with SPME technique, and canine olfactory detection to test the feasibility of VOC-based diagnosis. To control for potential dietary confounders, we also performed a metabarcoding analysis of fecal contents. We identified 25 volatile compounds in devil feces, several of which differed in abundance between sexes—10 being more abundant in males, potentially reflecting sex-specific functions such as reproductive signaling. Cancer appeared to affect VOC emission in a sex-specific manner, with three compounds differing in females (including benzaldehyde, which increased in cancerous individuals) and one in males. Despite these limited changes, the detection dog was able to generalize and discriminate cancerous from healthy individuals of both sexes with an accuracy exceeding 90%. This integrated, non-invasive approach demonstrates the potential of odor-based diagnostics for wildlife disease surveillance and conservation management.

Yeast-derived volatiles orchestrate an insect-yeast mutualism with oriental armyworm moths

Dr Baiwei Ma[1,2,4], Prof Hetan Chang[1], Dr Mengbo Guo[3], Dr Dong Ai[2], Jiayu Wang[2], Run Chen[2], Dr Xiaolan Liu[1], Prof Bingzhong Ren[4], Prof Bill S Hansson[5], **Prof Guirong Wang**[1,2]

[1]Agricultural Genomics Institute At Shenzhen, Chinese Academy Of Agricultural Sciences, Shenzhen, China, [2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [3] Zhejiang A & F University, Hangzhou, China, [4]School of Life Sciences, Northeast Normal University, Changchun, China, [5]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans Knoell Strasse 8, Jena, Germany

Interactions among insects, plants, and microorganisms are fundamental to ecosystem dynamics, with floral nectar and pollen serving as key resources for various organisms. Yeasts, such as *Metschnikowia reukaufii*, commonly found in nectar, influence nectarial attraction through volatile compounds (VOCs), yet the underlying biological mechanisms remain elusive. Here, we show that isoamyl alcohol, a prominent yeast VOC, attracts oriental armyworm moths (*Mythimna separata*) to pollen-rich, yeast-fermented nectar. In a series of electrophysiological and behavioral assays, we show that isoamyl alcohol activates a single class of highly specific olfactory sensory neurons expressing the olfactory receptor MsepOR8. In the moth antennal lobe, these neurons target the AM2 glomerulus, which responds to isoamyl alcohol. Genetic disruption of MsepOR8 leads to complete abolition of both physiological and behavioral responses to isoamyl alcohol, resulting in an impaired ability to locate nectar sources. Moreover, we show that isoamyl alcohol-induced foraging behavior fosters a mutualistic relationship between yeast and moths to some extent, enhancing yeast dispersal and increasing moth reproductive success. Our results unveil a highly specific mechanism by which a yeast-derived VOC facilitates insect-yeast mutualism, providing insights into insect-microbe interactions within pollination ecosystems.

Three odorant-binding proteins (OBPs) involved in the perception of kairomone 3-carene in *Dendroctonus valens*

Honglei He[1]

[1]AnHui agricultural university, Hefei, China

The red turpentine beetle (RTB), *Dendroctonus valens* LeConte (Coleoptera: Curculionidae, Scolytinae), is a destructive invasive pest of conifers which has evolved a sensitive olfactory system for locating host plants. Its odorant-binding proteins (OBPs) not only play crucial roles in olfactory processes but also represent promising molecular targets for novel pest control strategies. We identified significantly upregulated expression of three OBPs (OBP5, OBP6, OBP18) in transcriptome data following 3-carene exposure. Employing quantitative real-time PCR (qPCR), we found the three OBPs all show high expression levels in the antennae of both male and female adults, while OBP6 and OBP18 are also highly expressed in the legs. By the fluorescence competition binding assays, we revealed the binding affinity of three OBPs to 3-carene, while molecular docking simulations further validated this specific interaction. Our results help elucidate the molecular mechanism of the olfactory perception in *D. valens* and facilitates the development of host volatile-based attractants.

Exploring the combined use of biocontrol agents *Trichoderma atroviride* and *Engytatus nicotianae* against the tomato psyllid (*Bactericera cockerelli*)

Dr Emiliano Veronesi[1], Sarah Cairns[1], **Dr Hossein Alizadeh**[1], Prof John Hampton[1], Robbie Maris[2], Dr William Godsoe[1], Prof Stephen Goldson[3], Dr Andrea Clavijo McCormick[4]

[1]Faculty of Agricultural Sciences, Lincoln University, Lincoln, New Zealand, [2]School of Accounting, Finance and Economics, University of Waikato, Hamilton, New Zealand, [3]AgResearch, Lincoln, New Zealand, [4]School of Agriculture and Environment, Massey University, Palmerston North, New Zealand

Control of tomato potato psyllid (TPP), *Bactericera cockerelli*, is problematic because of pesticide resistance and environmental issues. The predatory bug *Engytatus nicotianae* has proven to be an efficient biocontrol agent against TPP in glasshouse grown tomatoes. *Trichoderma* fungi offer numerous benefits to plants and soil, including biocontrol of pathogens, enhanced plant growth, increased nutrient uptake, and improved soil health. Therefore, there could be advantages to the combined use of *Trichoderma* and *E. nicotianae*. However, *Trichoderma* spp. have the potential to alter the behaviour of pests or their enemies by modifying plant defence metabolites such as volatile organic compounds (VOCs). This study investigated the individual and combined efficacy of *Trichoderma atroviride* and *E. nicotianae* in reducing TPP abundance across different developmental stages (eggs, nymphs, adults) and the number of infested leaves in glasshouse grown tomatoes. Plant VOC emissions were also investigated under the different treatments. Across all measured TPP stages all three treatments (*E. nicotianae* alone, *T. atroviride* alone, and *T. atroviride* + *E. nicotianae*) significantly reduced mean TPP counts relative to the control but there were no significant differences in VOC emissions among the treatments. *Trichoderma atroviride* alone was less effective than *E. nicotianae* alone or its combination with *Trichoderma atroviride* to suppress TPP populations. The combined use of *T. atroviride* + *E. nicotianae* did not show significant advantages over the use of *E. nicotiana alone but* did not appear to negatively affect the predatory bug's performance. This suggests that the combined use of these biocontrol agents is feasible and can be considered in the light of other potential advantages of *Trichoderma* to the crop such as growth promotion and pathogen defence.

Does the difference in the aggregation-sex pheromone release pattern between *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) and *Monochamus saltuarius* Gebler ensure reproductive isolation in the cohabitation area?

Prof Il-Kwon Park[1], Dr Min-Jung Huh[1]
[1]Seoul National University, Seoul, Republic of Korea

In our study, we analyzed diel, daily, and weekly pheromone emission patterns to determine whether pheromone release pattern may facilate reproductive isolation between two species that use the same aggregation-sex pheromone, namely *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) and *Monochamus saltuarius* Gebler, (Coleoptera: Cerambycidae) in areas where they are sympatric. The daily emission of monochamol by males of both species showed irregular but constant patterns, with *M. saltuarius* males releasing the pheromone earlier than their *M. alternatus* counterparts after cuticular sclerotization. The first emission of the pheromone occurred, on average, 10.6 and 5 days after sclerotization for *M. alternatus* and *M. saltuarius*, respectively. Weekly patterns of monochamol emission showed a peak in the 3rd week after adult eclosion for both species. *Monochamus saltuarius* released more pheromone than *M. alternatus* from 13:00 to 19:00 during three different time periods throughout the day. Both species continuously released pheromones both before and after copulation. *Monochamus saltuarius* displayed mating behavior by mounting and attempting copulation with a glass rod coated with the female extract of *M. saltuarius*, while they showed no response to the female extract of *M. alternatus*. In contrast, male *M. alternatus* adults did not distinguish conspecifics through substances present on the surface of females. These findings enhance our understanding of the strategies employed to avoid interspecific competition between these two species in cohabitation areas.

Development of second generation passive emanators to reduce mosquito biting behavior

Dr Ingrid Chen[1], Mgeni Tambwe[2], Masudi Suleiman[2], Jennifer Stevenson[2], Sarah Moore[2]
[1]University Of California, San Francisco, San Francisco, United States, [2]Ifakara Health Institute, P.O. Box 74, Bagamoyo, Tanzania

Mosquito-borne diseases are collectively responsible for hundreds of thousands of deaths annually with malaria which is transmitted by *Anopheles* mosquitoes being responsible for the largest global burden of illness, causing an estimated 247 million cases and 619,000 deaths in 2021. Passive emanators (PEs) also known as spatial repellent devices, are a product class under development that aree portable light-weight products that are hung up or placed on the ground indoors or outdoors to diffuse volatile chemicals, creating a protective space from mosquito bites in their vicinity through multiple mosquito behavioral responses collectively known as spatial repellency. PEs show excellent entomological efficacy against *Anopheles*, *Aedes*, and *Culex* mosquitoes in multiple settings and have been shown to reduce malaria and dengue infections in trials. All PE products in development use volatile pyrethroid-class chemicals as active ingredients (AIs) however, placing significant pressure on insecticide resistance. Our project seeks to mitigate this risk, investigating the efficacy of 11 promising AIs in semi-field setups, evaluating their ability to inhibit the feeding of *Anopheles gambiae s.s.* mosquitoes on humans with exposed legs. Results are being applied to product development processes and are part of our growing research to create second generation passive emanators to prevent death and disease from mosquito-borne illnesses.

Plant defense mechanisms in watermelon: Volatile-mediated resistance against whiteflies

Dr Isiaka Owolabi[1,2], Dr Petrina McKenzie-Reynolds[3], Prof Gaelen Burke[1], Dr Alvin Simmons[3]
[1]University of Georgia, Athens, United States, [2]University of Georgia, Charleston, United States, [3]USDA-ARS, Vegetable Laboratory, Charleston, United States

Whitefly (*Bemisia tabaci*) infestations pose a critical threat to watermelon production, necessitating sustainable pest management strategies. This study evaluated the resistance potential of *Citrullus colocynthis* and other *Citrullus* genotypes using oviposition assays, Y-tube olfactometer assays, and gas chromatography-mass spectrometry (GC-MS) analysis of plant volatiles. Among 13 C. *colocynthis* accessions tested, PI-2010-ISRAEL and PI-386024 exhibited the lowest oviposition rates, while PI-386019 and PI-688367 demonstrated reduced attraction to plant volatiles compared to the susceptible cultivar 'Sugar Baby'. GC-MS analysis revealed phenol derivatives and sesquiterpenes as dominant volatiles in resistant accessions, with PI-542616 containing 97.8% phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl], and PI-386019 exhibiting the highest concentration (12.0%) of 2,2'-methylenebis-(6-tert-butyl)-4-ethylphenol acetate. By elucidating the biochemical and volatile-mediated resistance mechanisms in *C. colocynthis*, this research advances integrated pest management (IPM) strategies for sustainable watermelon production while reducing reliance on chemical pesticides.

Harnessing chemical signalling in plant–microbe–insect interactions for improved biological control

Dr Islam Sobhy[1]

[1]School of Biosciences, Cardiff University, Cardiff, United Kingdom

Global demand for food is rising, yet crop production is increasingly threatened by native and invasive insect pests, which reduce yield and quality and pose a risk to food security. While chemical insecticides remain widely used, their future use is under security due to resistance development, non-target effects, and severe decline in availability of active ingredients. This highlights the urgent need for sustainable, ecologically sound pest management strategies. Biological control offers a sustainable alternative to insecticides but remains limited in effectiveness and scale. Enhancing the impact of biological control requires not only increasing populations of natural enemies but also improving their foraging success

One promising approach could be to harness the sophisticated chemical ecology of plant–microbe–insect interactions. In my talk, I will highlight ongoing research efforts to optimize crop plants (e.g. manipulating plant VOC emission), habitat management strategies (e.g. Push-Pull companion cropping system), fermented nectar provisioning and parasitoid/host microbiome characterization, all to enhance the foraging success of natural enemies and its implications for biological control.

Dogs can perform an odour discrimination task using fine scale differences in odour arrival timing

Mr Iwan Downie[1], Dr Timothy Edwards[1], Dr Paul Szyszka[2]
[1]The University of Waikato, Hamilton, New Zealand, [2]University of Otago, Dunedin, New Zealand

Some animals can use time difference between the arrival of two mixed odours to discriminate between odours originating from different sources. Insects, for example, can detect differences in odour arrival as small as a few milliseconds. Whether mammals, including dogs with their proven olfactory prowess, can also use such temporal cues is unknown.

To address this gap, we adapted methods and equipment regularly used for investigations with insects for use with dogs. We used a fast olfactory stimulus delivery device as the core of an olfactometer which can be independently operated by dogs. Initially, the apparatus was tested by training dogs to discriminate between two separate odours. After verifying the apparatus could be operated by dogs, we varied the onset timing of components of odour mixtures, titrating to determine dogs' sensitivity to onset timing differences.

Dogs showed near perfect discrimination between two distinct odours, demonstrating the apparatus suitability for olfactory research. Subsequently, they demonstrated their ability to discriminate between odours delivered simultaneously and at separate times, with the ability to detect odour onset differences of 30ms or higher.

Domestication of blueberries drives performance of an herbivore through changes in constitutive defenses

Jae Kerstetter[1], Dr Cesar Rodriguez-Saona
[1]Rutgers University, New Brunswick, United States

Previous studies have highlighted differences in plant defences between domesticated crops and their wild relatives, particularly in the context of insect-plant interactions. These studies have shown that herbivores tend to perform better on domesticated plants due to reduced defences. However, most research on domestication effects has focused on constitutive defences, with limited attention given to induced defences. Highbush blueberry (*Vaccinium corymbosum*), a perennial shrub native to the northeastern United States, has been domesticated for about a century. This species is rich in secondary metabolites, including phenolic compounds such as proanthocyanidins, which play a key role in direct defence against herbivores. In this study, we examined how domestication affects both constitutive and induced direct defences in blueberries and how these changes influence the performance of the leaf-chewing generalist herbivore, spongy moth (*Lymantria dispar*). We predicted potential trade-offs between these two types of defences, with wild plants relying more on constitutive defences and domesticated plants relying more on induced defences. We found that *L. dispar* performed better on both constitutive and induced leaves of domesticated plants compared to wild plants, showing greater larval mass gain, increased leaf damage, and higher survival rates. While total phenolic content did not differ between plant types, domesticated plants consistently exhibited lower concentrations of proanthocyanidins in both constitutive and induced leaves compared to wild plants. These findings suggest that domestication of blueberries has reduced key chemical defences, such as constitutive proanthocyanidins, thereby enhancing herbivore performance on domesticated plants. However, contrary to our prediction, we did not observe a trade-off between constitutive and induced defences as a result of domestication.

Comparison of methods for assessing the active space of spruce budworm and spongy moth pheromone-baited traps

Jeremy Allison[1], Joel Goodwin, Chris Macquarrie, Jess Kerr, Brooke O'Connor, Sandy Smith
[1]Canadian Forest Service, Sault Ste Marie, Canada

Active space, the area over which a stimulus elicits a behavioural reaction in a receiving individual, is an important yet seldom investigated aspect of insect behaviour. It defines the spatial scale over which insects interact with a stimulus and can influence mating success and population dynamics. From an applied perspective, active space is an important component of the effective range of semiochemical-baited traps used for surveillance of forest and agricultural insects. This study used wingfanning assays, competitive trapping, and portable electroantennography to investigate the active space of spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) and spongy moth, *Lymantria dispar dispar* L. (Lepidoptera: Erebidae) pheromone-baited traps. Wingfanning assays and competitive trapping had similar outcomes for active space distance for both species. For spruce budworm, wingfanning assays estimate an active space of 18.4 m and competitive trapping experiments showed interference at distances less than 20 m. For spongy moth, wingfanning assays suggest an active space of 81.5 m and trap interference has been estimated to occur at 80 m. Portable electroantennography displayed an increase in neural depolarization amplitude at distances less than 30 m for spruce budworm and 130 m for spongy moth. Overall, the three methods used showed similar results for the active space distance of each species.

Effects of drought and flooding on a tri-trophic system, involving aphids and their natural enemies

Jeremy Roberts[1,2], Dr Jason Tylianakis[1], Dr Daniel Stouffer[3], Dr Adriana Najar-Rodriguez[2], Dr Vincent Bus[2]

[1]University of Canterbury, Christchurch, New Zealand, [2]Plant and Food Research, New Zealand, [3]Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

Under climate change, changes in precipitation such as drought and flooding events are predicted to increase in frequency and severity. These conditions are anticipated to impact the chemical interactions among all organisms and disrupt ecosystems, including agricultural systems. The impacts could be from the water stress directly, or impacts can cascade through systems – as their food sources are impacted, herbivores and predators can experience additional indirect effects. Here, we consider one such system, with experiments targeting a biological control system involving three trophic levels: a plant, apple (*Malus ×domestica*); an insect herbivore, woolly apple aphid (*Eriosoma lanigerum*); and two of its natural enemies, the harlequin ladybird (*Harmonia axyridis*) and the green lacewing (*Mallada basalis*). In a first experiment, we considered the impacts of drought and flooding at two severities each on apple plants alone. Then, in a second experiment, we examined how these water treatments affect aphids present on these plants as well, and how aphids from different water treatment host plants impact these natural enemies. A range of data on changes in plant morphology, physiology, and chemistry between stress types (drought/flooding) and stress severities (low intensity/high intensity) was collected. The implications of our findings will be discussed in terms of the different impacts of water stresses on both the plants and the aphids, and the chemical interactions between them and between the insects and their natural enemies.

Identification and behavioral evaluation of VOCs from *Fusarium solani*-infected kidney beans for development of synthetic volatile attractant for gravid female adults of *Bradysia impatiens* (Diptera: Sciaridae)

Ji Hye Oh[1], Seon Ah Jeong[2], 1 Da Hyeon Yu[1], Gwang Hyun Roh[1,2]

[1]Department of Plant Medicine and Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju 52828, Republic of Korea, Jinju, Korea, [2]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea, Jinju, Korea

Olfaction plays a key role in the location of food and oviposition resources by sciarid flies. The fungus gnat *Bradysia impatiens* (Johannsen) is a serious pest in greenhouses and mainly infests the roots of strawberry, tomato, watermelon, and cucumber. The female adults of *B. impatiens* achieve explosive population growth by laying a large number of eggs (100 eggs/female). The newly hatched larvae of *B. impatiens* feed on roots and cause significant damage to the host. Therefore, there is a need to develop control strategies for *B. impatiens* female flies. In this study, we conducted with gravid *B. impatiens* females to evaluate attraction to traps baited with eight host sources (cheonggukjang, enoki mushroom, *Fusarium solani*-infected kidney bean, sterile kidney bean, king oyster mushroom, oyster mushroom, potato, and white mushroom) using laboratory two- and multiple-choice bioassays. In two-choice bioassays, significantly more gravid *B. impatiens* females were captured in traps baited with the eight host sources than in traps baited with control (empty or sterile kidney bean). Among the eight host sources, the greatest number of gravid *B. impatiens* females was captured in traps baited with *F. solani*-infected kidney bean in multiple-choice bioassays. Moreover, the gravid *B. impatiens* females oviposited a significantly greater number of eggs with the *F. solani*-infected kidney bean than the sterile kidney bean. Then, we compared the volatile organic compounds (VOCs) released by *F. solani*-infected kidney bean and sterile kidney bean using gas chromatography-mass spectrometry (GC-MS) and selected 25 compounds (compounds present only or in high amounts in *F. solani*-infected kidney beans compared to sterile kidney bean). Among them, five compounds (dimethyl disulfide, toluene, tetrachloroethylene, 2,4-dithiapentane, and *o*-xylene) attracted (78.6-91.9%) gravid *B. impatiens* females in two-choice bioassays. These results suggest that the findings can be used as agents for population control and monitoring of *B. impatiens* in the greenhouse.

Developing and commercialising a new lure for monitoring and mass trapping *Carpophilus truncatus*, a major pest of almonds

Assoc. Prof John Paul Cunningham[1]

[1]Agriculture Victoria, Bundoora, Australia

Carpophilus truncatus has risen from relative obscurity to become a major pest of Australian almonds, with kernel damage costing the Australian almond industry in excess of $20 million per year. In the last few years *C. truncatus* has also emerged as a pest attacking almonds and other nuts elsewhere around the world, including Argentina, Italy, and most recently in California, where 80% of the world's almonds are grown. This talk describes seven years of research to develop a new semiochemical lure for monitoring and mass trapping *C. truncatus*. The new lure is comprised of the beetle's aggregation pheromone, together with a synergistic "co-attractant" that is based on volatiles emitted by a mutualistic yeast species. In field trials, the new lure placed with a trap catches high numbers of beetles, paving the way for a new tool for growers to monitor populations of this pest. The trap has also been used to study the distribution of *C. truncatus* across eastern Australia, revealing that low level populations exist across the country in non-cropping regions, including national parks. Further studies in the behavioural and chemical ecology of *C. truncatus* have revealed its broader host range beyond nuts, and the crucial role mutualistic yeasts play in host choice and female oviposition behaviour. An industry-wide program has been trialling the new lure and trap as a monitoring device in the 2024/25 growing season. The application of the trap as an attract and kill (mass trapping) technology is also showing promise, with field trials showing damage reduction of around 60%. With the new *C. truncatus* trap entering a pre-commercialisation phase, the talk will also discuss some of the obstacles that have been overcome while attempting to cross the great divide between discovering a new attractant and seeing it commercialised and adopted by industry.

Identification and evaluation of semiochemicals for the control of the cacao mirid bug *Helopeltis bakeri* Poppius

Mr Kris Lord Santos[1], Ms Mary Angelique Tavera[1], Ms Maria Criselda Dela Cruz[1], Prof Divina Amalin[1], **Prof Jose Isagani Janairo**[1]

[1]De La Salle University, Manila, Philippines

The cacao mirid bug (CMB) *Helopeltis bakeri* is one of the major insect pests of cacao in the Philippines. Adults and nymphs feed on the pods and young shoots resulting in lesions which become entry points for rot-causing pathogens. Here we report the semiochemicals (i.e., sex pheromones and kairomones) that can be potentially used in semiochemicals-based trapping systems for CMB. Semiochemicals were extracted using solid-phase microextraction and analyzed using GC-MS. Acquisition of sex pheromones and kairomones was made from virgin females and identified host plants, respectively. α-Pinene and β-ocimene was identified as potential sex pheromones. Both compounds elicited significant EAG responses from male antennae, and were able to attract males in a short-range olfactometer. β-Caryophyllene, on the other hand, was identified as a common volatile in all host plants. The compound was electrophysiologically and behaviorally active towards both male and female CMB. These results show the potential of these compounds for the development of semiochemical-based trapping systems for the sustainable management of CMB.

Recent advancements of semiochemical based blood-sucking pest management

Dr Junwei Zhu[1]
[1]USDA-ARS, AMRU, Lincoln, United States

The impact of blood-sucking pests on livestock operations, companion animals, and human public health has been increasingly recognized as an important part of the 'One Health' concept. Host orientation of those blood-sucking pests are primarily mediated by their perception of volatile compounds associated with host species and host environments via their sensory organs, which of their sensory organs used for chemical odorant detection are mostly located on their antennae. Hundreds of host-associated attractive compounds have been identified and being further developed for surveillance and control. Natural product repellents have been used for hundreds of years to protect humans and animals from arthropod attacks with the repellency of plant-based essential oils including catnip (*Nepeta cataria*), citronella (*Citronella citratus*), geranium (*Geranium spp*.), peppermint (*Zanthoxylum piperitum*), eucalyptus (*Eucalyptus globulus*), and lemongrass (*Cymbopogon citratus*), as well as medium-chain fatty acids from coconut oil. The use of push–pull strategies in integrated pest management was first demonstrated with successful control of *Heliothis* moths in cotton protection but recently has been extended to blood-sucking pest management. The present talk will provide a summary of the last 30 years advancement of blood-sucking pests via semiochemical based knowledge and tools, which focuses on biting flies, including stable flies (*Stomoxys calcitrans* L.) and horn flies (*Haematobia irritans* L.) that are two major blood-sucking pests of livestock, wildlife, and humans worldwide. Future directions for novel strategies in blood-sucking pest control will also be discussed.

Does reproductive status affect behavioural response to conspecific odours in fruit spotting bugs (*Amblypelta* spp. (Stål, 1873), (Hemiptera: Coreidae))?

Mrs Kempsy Adams[1], Mr Stefano De Faveri[1], Dr R. Andrew Hayes[2], Mrs Donna Chambers[1], Mr Ian Newton[1]

[1]QLD Department of Primary Industries, Dutton Park, Australia, [2]University of the Sunshine Coast, Australia

Fruit Spotting Bugs (FSB) *Amblypelta nitida* and Banana Spotting Bugs (BSB) *Amblypelta lutescens lutescens* pose significant threats to various fruit, nut, and berry crops across coastal and sub-coastal regions of eastern and northern Australia.

There is no known pheromone for FSB, and behavioural assays to investigate chemical communication between the insects have been inconclusive until now. Despite being an important variable in behavioural assays, it is unclear when adult females become receptive to mating cues and when males start actively producing pheromones. This uncertainty complicates efforts to determine whether observed behavioural responses are due to genuine disinterest or simply because the insects are not yet physiologically prepared for mating. While the anatomy of *Amblypelta* spp. has been documented, there remains a notable gap regarding the adults' progression from ecdysis through to reproductive maturity. This knowledge deficiency poses a considerable challenge for behavioural studies, particularly to understanding adult responses to pheromones.

To address this knowledge gap, females of different ages were exposed to adult males in a two-choice test. The reproductive development of male and female *A. nitida* were investigated by comparing stages of reproductive organ maturation as the adult insect ages. Behavioural assays indicated an increasing proportion of females choosing the male as they age, and physiological examination confirmed a parallel development of mature reproductive organs.

Reproductively mature females respond significantly more often to males of the same species compared to younger females. Assay results even suggest avoidant behaviour from recently emerged females which would result in false negatives tests examining attractiveness to pheromone compounds. Understanding the reproductive physiology of these species has directly impacted current efforts in pheromone identification of *Amblypelta nitida*.

Functional evaluation of *Bombyx mori* OR3-expressing sensor cells in response to C16 pheromone components

Kent Sato[1], Zhou Rui[1], Yuji Sukekawa[1], Eri Kuroda[1], Takeshi Sakurai[2], Takeshi Fujii[3], Ryohei Kanzaki[1], Hidefumi Mitsuno[1]

[1]The University of Tokyo, Tokyo, Japan, [2]Tokyo University of Agriculture, Tokyo, Japan, [3]Setsunan University, Osaka, Japan

In moths, species-specific sex pheromone blends serve as key signals for mate recognition and reproductive isolation among closely related species. The female sex pheromone signals are detected with high sensitivity and selectivity by specialized sex pheromone receptors on the male antennae. Among them, BmOR3, a pheromone receptor for the silk moth *Bombyx mori,* is highly selective for bombykal [(*E, Z*)-10,12-hexadecadienal], its minor pheromone component. Notably, bombykal and its analogues are also employed as pheromone components in several moths, including pest species. In this study, we employed previously established sensor cells expressing BmOR3 to evaluate its selectivity for bombykal against several structurally similar pheromone components used by closely related moth species. The sensor cells showed strong responses to bombykal, while responses to the analogues were minimal or negligible. Furthermore, when bombykal was co-applied with each analogue at equal molar ratios, no significant suppression of the bombykal-induced response was observed, indicating that these analogues do not competitively inhibit BmOR3-sensor cells' activation. However, when the dose-response relationship for bombykal was measured in the presence of a fixed concentration (100 μM) of a structural analogue, a partial reduction in response was observed at intermediate bombykal concentrations (10–30 μM), suggesting moderate competitive effects. These results demonstrate that BmOR3-sensor cells maintain high ligand selectivity, even under conditions that simulate the presence of background pheromone compounds.

Shedding light on mechanisms underlying the regulation of insect pests in the oilseed rape-faba bean associative system

Mrs Lea Bolis[1,2], Prof Sergio Rasmann[2], Dr Ivan Hiltpold[1]

[1]Agroscope Entomology and Nematology, Nyon, Switzerland, [2]University of Neuchâtel Laboratory of Functional Ecology, Neuchâtel, Switzerland

Associative cropping of winter oilseed rape (*Brassica napus*) with faba bean (*Vicia faba*), traditionally used for weed control and nitrogen supply, has recently been shown to reduce pest pressure from three coleopteran specialists under field conditions. However, the mechanisms underlying these pest-regulating effects remain poorly understood and are likely multifactorial. Recent laboratory and field studies revealed metabolomic shifts in intercropped oilseed rape compared to sole crops, including altered levels of glucosinolates, key defense compounds. Behavioral assays demonstrated that at least one of these differentially expressed chemicals acts as a phagostimulant for the cabbage stem flea beetle (*Psylliodes chrysocephala*), a major pest of oilseed rape, potentially explaining differences in pest pressure between cropping systems. Additionally, olfactometer assays suggest that service cropping influences the emission of volatile organic compounds, which may affect pest behavior and movement at larger spatial scales. Together, these findings shed light on the complex chemical and behavioral interactions driving pest dynamics in diversified agroecosystems.

Fruit scent evolution in plant-seed disperser interactions

Linh M. N. Nguyen[1,2,3], Diary Razafimandimby[4], Rebekka Sontowski[5], Jana Ebersbach[6], John C. D'Auria[7], Darren CJ Wong[8], Renske Onstein[9], Philipp Schluter[10], Nicole M. van Dam[1,2,3,5], Omer Nevo[1,2]

[1]German Centre for Integrative Biodiversity Research (iDiv) Halle- Jena- Leipzig, Leipzig, Germany, [2]Institute of Biodiversity, Ecology and Evolution, Friedrich Schiller University 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, University, Leipzig, Germany, [7]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben), OT Gatersleben, Seeland, Germany, [8]Division of Ecology & Evolution, Research School of Biology, The Australian National University, Canberra , Australia, [9]Naturalis Biodiversity Center, Leiden, Netherlands, [10]Department of Plant Evolutionary Biology, Institute of Biology, University of Hohenheim, Stuttgart, Germany

Fruits have evolved diverse traits to attract seed dispersers, with frugivores shaping their characteristics through selective pressures. Among these traits, fruit scent plays a crucial role in signalling ripeness and potentially indicating fruit quality. However, the specific components of fruit scent that serve as signals for frugivores and the information they convey remain unclear. Aliphatic esters, commonly found in chemically communicative fruit species, are typically concentrated in ripe fruits. Evidence suggests a positive correlation between these esters and sugar content, pointing to a potential coevolutionary link between chemical signals and lemur-dispersed fruits. This study explores whether aliphatic esters function as honest signals of fruit quality and if they represent an adaptive trait in plants. Using up to 20 fig species (*Ficus* spp.; Moraceae) in Madagascar, we aim to: (1) map fig-frugivore ecological networks, (2) reconstruct phylogenetic relationships, (3) analyze chemical signals and nutritional rewards via thermal desorption gas chromatography-mass spectrometry (TD-GCMS) and high-performance liquid chromatography (HPLC), and (4) sequence alcohol acyltransferase (AAT) to assess selection pressures related to dispersal modes. These findings will provide key insights into the evolution of fruit scent in plant-animal interactions and its role in seed dispersal.

Sex as a weapon: Using Fungal sex hormones as novel crop protection

Lisa Humbert[1,2], Dr David Withall[1], Prof Paul Dyer[2], Dr Mike Birkett[1]
[1]Rothamsted Research, Harpenden, United Kingdom, [2]University of Nottingham, Nottingham, United Kingdom

The rapid emergence of resistance in agricultural settings emphasizes the urgent need to develop novel antifungal compounds for the control of plant pathogens. *Pyrenopeziza brassicae*, the causative agent of Light Leaf Spot disease in brassicas, is the most important fungal pathogen of oilseed rape in the UK. The disease is widespread across Northern Europe, Oceania, and Asia and has recently expanded to North America. The pathogen has well-described dispersal mechanisms: asexual sporulation facilitates local spread during the growing season, while sexual spore production enables long-distance transmission via ascospores. Current reliance on broad-spectrum fungicides is increasingly ineffective due to widespread resistance in European strains, necessitating the development of novel targeted biocontrol solutions.

Signaling molecules involved in fungal reproduction, such as sexual hormones, represent a promising source of new antifungals to repress pathogens growth. In *P. brassicae*, hormones referred to as sex factors (SF) produced during sexual reproduction have the ability to significantly suppress asexual sporulation in laboratory conditions. We validated this effect *in planta*, demonstrating reproducible SF activity at a larger scale and highlighting its potential as a novel biocontrol strategy against Light Leaf Spot disease. Through analytical chemistry techniques—including SFC-HPLC, LC-MS, MS/MS and NMR—we isolated, characterised and assigned tentative molecule structures to the sex factors. Furthermore, studies using scanning electron microscopy revealed key mechanistic insights into SF-mediated sporulation repression. Current efforts to decode the genetics behind fungal sexual hormones have also identified putative SF biosynthetic genes and targeted receptors using transcriptomic analysis, and now aim to confirm it through CRISPR-Cas9 gene editing. These findings deepen our understanding of fungal chemical ecology and demonstrate that fungal secondary metabolites may offer more sustainable prospects for future crop protection.

Jellyfish polyps make great neighbours: Larval response to conspecific chemical cues in habitat selection

Mr Lucas Gimenez[1], Dr Anthony Carroll[1,2], Dr Kylie Pitt[1,3]

[1]School of Environment and Science, Griffith University, Gold Coast, Australia, [2]Griffith Institute for Drug Discovery, Griffith University, Nathan, Australia, [3]Australian Rivers Institute, Griffith University, Nathan, Australia

Jellyfish polyp populations form dense aggregations mainly through asexual reproduction. Gregarious settlement may also contribute via larvae selecting to settle in habitats with conspecifics. Conspecific detection in jellyfish is thought to be mediated by chemical cues, but the specific mechanism and the nature of the compounds involved in gregarious settlement remain unexplored. Here, we tested the hypothesis that *Aurelia coerulea* larvae select habitats with conspecifics by detecting organic compounds released by polyps into the water. We conducted choice experiments to confirm that larvae settle gregariously by detecting the chemistry of polyps rather than their physical structure. Then, we carried out settlement induction experiments to explore the mechanism underlying conspecific detection. Specifically, we compared the effects of waterborne and surface-bound compounds from conspecifics as settlement inducers. We also measured the persistence of settlement inducers after 18 h to determine their stability. Finally, we used a solid-phase filtration to assess whether the settlement inducers were organic compounds. Overall, our results advance the understanding of chemical cues involved in jellyfish settlement, shedding light on the mechanisms of conspecific detection. Compelling evidence indicates that *A. coerulea* exhibits a larva-polyp interaction mediated by stable, waterborne organic compounds that lead to gregarious settlement.

Distinctive elementome and biogeochemical niche allows co-development of mutualistic occupants of a fig syconium microcosm

Manasa Kulkarni[1], Dr Jyothilakshmi Vadassery[2], Prof Renee M. Borges[1]

[1]Indian Institute of Science, Bengaluru, India, [2]National Institute of Plant Genome Research, New Delhi, India

Elemental composition dictates the growth and survival of organisms, impacting their ecology. While ratios of carbon, nitrogen and phosphorus have been extensively studied, other elements have received little attention. The elementome, which refers to concentrations of most elements present in an organism and the recently developed biogeochemical niche (BN) provide a better understanding of trophic interactions. BN is the niche occupied by an organism in a multidimensional space formed by the elementome; organisms that coexist or are separated by greater taxonomic distance have non-overlapping BNs. For the first time, we studied the elementome and BN of mutualistic partners that trade services and rewards. Fig trees are pollinated by species-specific pollinator wasps, which also lay eggs inside some female flowers that modify into galls. Seeds of the host and the offspring of pollinator wasps develop within the closed inflorescence of a fig syconium by accessing resources from the host tree. We measured concentrations of important elements of the syconial wall, seeds, and pollinators at the end of their development and constructed BNs using principal component analysis. We also measured trophic stoichiometric ratios (TSR) that quantifies resource mismatch by measuring ratios of carbon and other elements for resource source and resource consumer. Pollinators had higher concentrations of nitrogen, sulphur, phosphorus and zinc compared to seeds or wall tissues, resulting in non-overlapping BNs, likely allowing their coexistence. TSR values of >4 for nitrogen and sulphur for pollinators indicated that they are N- and S-resource limited, highlighting the need of mechanisms to enrich these elements. BN of neotenic wingless male and winged female pollinators were also separated, owing to their anatomical and functional differences. Overall, our study opens up a new dimension in the study of mutualisms, using the concepts of elementome and BN to study resource allocation and trading in these interactions.

Shared sesquiterpenoid pheromones and communication complexity in neotropical stink bugs

Maria Carolina Blassioli Moraes[1], Miguel Borges[1], Raúl A Laumann[1], João V.M. Machado[1], Mirian F.F. Michereff[1], Ashot Khrimian[2]

[1]Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil, [2]Invasive Insect Biocontrol and Behavior Laboratory, USDA-ARS, NEA, Neltsville, USA

The sex pheromones identified in neotropical stink bugs comprise compounds derived either from fatty acids or from sesquiterpenoid molecules. Most species exhibit a species-specific blend of pheromone components. This specificity generally results from differences in the composition of the blend, either using distinct molecules or through variations in the relative ratios of shared compounds. For example, *Nezara viridula* and *Chinavia* species produce the same molecules as sex pheromones but in different ratios. Similarly, a complex of neotropical rice stink bug species—including *Tibraca limbativentris* and *Paratibraca limbativentris*—produces the sesquiterpenoids zingiberenol and sesquipiperitol in their pheromone blends, with identical absolute configurations and similar ratios. However, these species respond preferentially to different isomers of the compounds. Two other rice stink bug species, *Oebalus poecilus* and *Mormidea v-luteum*, also incorporate zingiberenol and sesquipiperitol in their pheromone blends but with different absolute configurations. The similarity in pheromone composition among these species, combined with the frequent occurrence of cross-attraction observed in both laboratory and field experiments, suggests that stink bugs possess a complex communication system. This system likely integrates multiple signaling modalities to distinguish conspecifics from heterospecifics, involving not only long- and short-range chemical cues but also vibrational signals.

Understanding nest marking scents in New Zealand native bees: Insights into nest recognition

Mary Angelique Tavera[1,2], Kye Chung Park[1], Lisa Evans[1], Paul Szyszka[2], Flore Mas[1]
[1]Plant And Food Research, Lincoln, New Zealand, [2]University of Otago, Zoology Department, Dunedin, New Zealand

With the growing threats posed by climate change and biodiversity loss, understanding the ecology of pollinators—upon which a significant portion of global food production depends—is crucial. In Aotearoa New Zealand, solitary bees such as Ngaro huruhuru (native ground nesting bees) are not only effective pollinators of crops but also hold deep cultural significance as *taonga* (treasured species) within Māori tradition. Despite their ecological and cultural value, little is known about their nesting ecology. Often found in large aggregations with hundreds of similarly structured nests, the mechanism by which they identify their own nest among many remains unclear. To investigate this, we examined the glandular and body chemistry of the bees using gas chromatography-mass spectrometry (GC-MS) to identify potential chemical cues used in nest marking. Subsequently, electroantennography techniques, including GC-EAD (gas chromatography-electroantennographic detection) and EAG (electroantennogram), were employed to determine which chemical compounds elicited a response in the bees' antennae. The analysis identified the presence of various compounds such as hydrocarbons, esters, aldehydes and fatty acids common across several species within a genus (*Leioproctus* and *Lasioglossum*) as well as some species-specific compounds. EAG responses varied widely across individuals when individual bees were puffed with their gland and body extracts and GC-EAD suggests that each bee may produce a unique nest marking signature. Behavioural attraction to nest odours was also confirmed. This approach aims to provide deeper insights into the chemical signals that solitary bees use for nest recognition, ultimately contributing to more effective management and conservation of these vital pollinators.

Multiple herbivory by corn leafhopper and fall armyworm shape maize volatile emissions: Insights from conventional and rapid detection methods

MSc Mateus Souza Sanches[1,2], Dr John Caufield[3], Dr Mirian Fernandes Furtado Michereff[1], Dr Miguel Borges[1], Dr Raúl Alberto Laumann[1], Dr Charles Martins Oliveira[4], Dr Paulino Ribeiro Villas-Boas[6], Dr Marina Regina Frizzas[2,5], Dr Maria Carolina Blassioli-Moraes[1]

[1]Semiochemicals Laboratory, EMBRAPA Genetic Resources and Biotechnology, Brasília, Brazil, [2]Graduate Program in Ecology, University of Brasília, Brasília, Brazil, [3]Rothamsted Research, Harpenden, UK, [4]Embrapa Cerrados, Brasília, Brazil, [5]Zoology Department, University of Brasília, Brasília, Brazil, [6]Embrapa Instrumentação, São Carlos, Brazil

In response to insect attack, plants emit herbivore-induced plant volatiles (HIPVs) that can attract natural enemies and repel new herbivores. In the field, plants are rarely attacked by a single herbivore at the same time. In this way, we evaluated the HIPV emissions from maize damaged by *Dalbulus maidis* (DM) and *Spodoptera frugiperda* (FAW), simultaneously and individually. Plants were exposed to four treatments: control – no insects, DM – 90 DM, FAW – two FAW larvae (3rd instar), and MULTI – 90 DM adults for 24 h, followed by two FAW larvae for more 24 h. Each treatment had five replicates with three maize plants each. We also tested a handheld 720 VOC Analyser (RoboScientific Ltd) to assess its potential for detecting herbivory via HIPV emissions. Maize emitted distinct volatile profiles among treatments ($p < 0.001$, PERMANOVA). MULTI differed from both DM ($p = 0.002$) and FAW ($p = 0.006$), releasing fewer volatiles and lacking indole and methyl salicylate, which are typically associated with FAW and DM damage, respectively. These results suggest that simultaneous herbivory compromises the volatile response compared to a single-species attack with less specificity. The VOC Analyser also successfully distinguished all treatments, highlighting its potential as a field tool for rapid detection of herbivore specific volatile signatures. We are conducting bioassays to test insect responses to HIPVs from MULTI plants, as both DM and FAW avoid HIPVs from plants previously damaged by the other in previous experiments.

Semiochemical biomarkers for the identification of sex and reproductive status in Sydney Rock Oyster

Mr Md Abu Zafar[1,2], Dr Saowaros Suwansa-ard[1], Prof Abigail Elizur[1], Prof Scott Cummins[1,2]
[1]Centre for Bioinnovation, University of the Sunshine Coast, Sippy Downs, Australia, [2]School of Science,Technology and Engineering, University of the Sunshine Coast , Sippy Downs, Australia

The Sydney rock oyster (SRO), *Saccostrea glomerata*, represents one of the most important aquaculture species within the Australian oyster industry. Therefore, enhancing SRO aquaculture strategies is essential to secure sustainable farming and production. One of the significant challenges in SRO hatchery production is the sex identification of SRO broodstocks since they do not display any external sexual dimorphism. Current approaches for sex identification are typically performed using invasive and destructive techniques. To explore alternative methods for sex identification, the current study investigated whether SROs may secrete a sex-specific semiochemical profile (specifically proteins). Following proteomic analysis of oyster-conditioned water at different sex and reproductive stages including, ripening, spawning, spent, and regressed stages, we demonstrate that SRO secrete a distinct gender and stage protein profile. Functional annotation of these secreted proteins indicated their potential involvement in metabolic processes, immunity and chemical communication. Semi-quantitative analysis facilitated the identification of candidate sex- and reproductive stage-specific protein markers that were almost exclusive to female oysters. Primary targets for further investigation included an extracellular superoxidase dismutase protein for maturation (ripening and spawning) stages markers and temptin and byssal protein-3-like proteins, both abundantly expressed in the oyster's digestive gland and abundant in the oyster-conditioned water of females. In conclusion, this study has discovered that SROs release a distinctive protein profile into their environment, some of which could serve as markers for determining sex and reproductive stage. This foundation of knowledge paves the way for novel approaches for non-invasive detection of SROs specifically, such as oyster health in general, sex, and reproductive stage, to benefit the aquaculture industry.

Identification of host plant volatile and essential oil compounds for modifying adult pest behaviour of *Liriomyza huidobrensis*

Mr Md Sahadat Hossain[1], Ms Sanjana Akter[1], Mr Lok Nath Aryal[1], Dr Bishwo Mainali[1], Dr Soo Jean Park[1]

[1]Macquarie University, Macquarie Park, Australia

Liriomyza leafminers pose a significant threat to horticultural crops, diminishing productivity and quality through creating mines and thereby impairing the plant's photosynthetic processes. Traditional management relies heavily on synthetic pesticides, but resistance development and environmental concerns highlight the need for alternative strategies. Host plant volatiles play a key role in leafminer behaviour, presenting an opportunity for alternative management strategies. Certain essential oils (EOs) have been found to either attract or repel leafminers. This study investigates specific compounds in host plant volatiles and their EOs to identify candidates for attractant or repellent tools against *L. huidobrensis* in protected cropping systems. Oviposition and stippling assays revealed significantly stronger preference for oviposition on lychnis, petunia and cucumber plants among the nine tested plants, with bean serving as a positive control. Similarly, *L. huidobrensis* significantly preferred petunia and cucumber for feeding. Conversely, salvia and Russian sages were unattractive for oviposition or feeding, suggesting potential repellent properties. Gas chromatography-mass spectrometry (GC-MS) profiling showed that monoterpenes and sesquiterpenes dominated volatile compositions. Gas chromatography-electroantennogram detection (GC-EAD) revealed electro-physiologically active compounds in Russian sage, salvia, celery, chrysanthemum, lychnis and eggplant volatiles, and several EOs including black spruce, clove, basil, garlic, tea tree and citronella. These findings suggest celery, chrysanthemum, lychnis, eggplant, black spruce, clove and basil as promising sources of attractant compounds, while Russian sages, salvia, garlic, tea tree and citronella may contain repellent compounds. These results lay the foundation for developing novel attractant and repellent blends to support sustainable management of *L. huidobrensis*.

Optimizing release devices for non-specific monitoring of invasive and destructive beetles

Dr Michael Fraser[1], Dr Jorge Macias-Samano, Mr Robert Setter
[1]Synergy Semiochemicals, Delta, Canada

The global movement of invasive insects through international ports, often hitching rides in wood packaging, poses a serious ecological and economic threat. Monitoring this diverse array of species is not only logistically complex but also cost-intensive. Current approaches require multiple attractant compounds, frequent trap servicing, and expert identification of captured specimens. Compounding these challenges are the unpredictable interactions among chemical components and their release mechanisms.

Traditionally, compound blends have been dispensed using rudimentary devices such as cotton wicks or rubber septa soaked in solvent. While inexpensive, these methods suffer from short field lifespans, inconsistent release rates, and high maintenance demands, rendering them inefficient for long-term monitoring.

To address this, we evaluated a 10-compound attractant blend released using both conventional cotton wicks and Synergy's polyethylene-based devices (bubble caps and pouches). Release rates were measured gravimetrically under stable temperatures for up to 120 days, with compound ratios monitored via gas chromatography at multiple intervals, testing the ratios of the compounds within the blend release over time.

Field testing in southern British Columbia employed multi-funnel traps baited with ethanol, alpha-pinene, and the test blends. Traps with the compound blends captured a highly diverse range of insects, 60 species from 25 families, comparable to the standard cotton wick treatment, however with much longer device life-time. These findings show that it is not only feasible but also cost-effective to develop advanced release systems with stable, long-lasting performance.

First insights on the chemical ecology of a new invasive mega-pest in Europe

Dr Michael Rostás[1], Dr Karthi Balakrishnan[1]
[1]University Of Göttingen, Göttingen, Germany

The planthopper *Pentastiridius leporinus* has recently emerged as the main vector of two serious bacterial diseases—Syndrome des Basses Richesses (SBR) and Stolbur—in sugar beet, potato, and other vegetable crops, causing significant economic losses for farmers in Germany and Switzerland. Remarkably, this insect, which was once considered a rare native species and even listed as endangered until the 1990s, has become highly invasive in Central Europe. Its rapid spread and the current lack of effective control measures have turned P. leporinus into an existential threat for growers in the region. Against this background, we conducted the first targeted studies on the chemical ecology of this planthopper, aiming to identify volatile organic compounds (VOCs) that could serve as attractants or repellents for future pest management strategies. Here, we report on the host selection behavior of *P. leporinus*. Bioassays were carried out to assess the responses of both aboveground-feeding adults and subterranean nymphs to host plants and individual VOCs. In addition, electroantennography (EAG) experiments were performed to analyze the antennal responses of the planthoppers to selected compounds. As a result, two VOCs were identified that showed clear repellent effects on adult planthoppers in laboratory tests. Whether these substances can also be used effectively in the field for the control of *P. leporinus* remains to be demonstrated in further field trials.

Sesquiterpene biosynthetic gene vir4 from *Trichoderma virens* enhances direct herbivore resistance while maintaining indirect defense

Dr Michael Rostás[1], Noor Agha Nawakht[1], Dr Mohammad Alhussein[1], Dr Artemio Mendoza-Mendoza[2]

[1]University of Göttingen, Göttingen, Germany, [2]Lincoln University, Lincoln, New Zealand

Plants must constantly adapt their defence responses to withstand diverse attackers in agroecosystems. Beneficial root-colonizing fungi such as *Trichoderma virens* may further influence both direct and indirect plant defences. Here, we investigated the impact of *T. virens* and its *vir4* knockout mutant, which is deficient in terpenoid biosynthesis, on maize (*Zea mays*) interactions with the herbivore *Helicoverpa armigera* and its predator *Macrolophus pygmaeus*. Untargeted metabolomics revealed that fungal colonization, particularly by the wild-type strain, induced distinct metabolic shifts in maize shoots, which were further amplified by herbivory. While herbivory was the main trigger for the induction of plant hormones and secondary metabolites, fungal root colonization also contributed to changes in primary and secondary metabolic profiles. Notably, caterpillars feeding on maize colonized by wild-type *T. virens* gained significantly less weight, indicating enhanced direct resistance dependent on the *vir4* gene cluster. Although fungal colonization moderately altered the composition of herbivore-induced plant volatiles, overall volatile emissions and predator attraction were unaffected. These findings highlight the complex interplay between plant metabolism, fungal endophytes, and multi-trophic interactions, and suggest a role for the *vir4* gene cluster in enhancing maize's direct defences against herbivores.

Effects of Zingerone ingestion on the sexual maturation and cuticular profile of male *Bactrocera jarvisi* flies

Most Mottakina Akter[1], Md Forhad Hossain[1], Md Jamil Hossain Biswas[1], Wasala Mudiyanselage Thilini Darshika Ekanayake[2], Brian Thistleton[2], Stefano De Faveri[3], Jodie Cheesman[3], Suk Ling Wee[4], Vivian Mendez Alvarez[1]

[1]Macquarie University, Australia, [2]Biosecurity and Animal Welfare, Department of Agriculture and Fisheries, Australia, [3]Horticulture and Forestry Science, Department of Primary Industries, Australia, [4]Department of Biological Science and Biotechnology, Faculty of Science and Biotechnology, Universiti Kebangsaan Malaysia, Malaysia

Certain phytochemicals are highly attractive to male tephritid flies, offering these males significant mating advantages. Males of *Bactrocera jarvisi*, an endemic Australian fruit fly, are strongly attracted to and feed on the phytochemical zingerone (ZN). The reproductive biology of *B. jarvisi*, and the effects of ZN ingestion on the sexual development and cuticular chemistry of males are not well understood. In this study, we investigated the sexual development of ZN-attracted and unattracted male flies, and the effect of ZN ingestion on sexual maturation and cuticular chemistry of *B. jarvisi* males. The cuticular chemistry of females was also analysed. Our findings revealed that domesticated males began to show attraction to ZN 8 days after emergence, while wild flies started showing attraction at 30 days after emergence. In domesticated and wild flies, ZN-attracted males were more sexually developed than unattracted males; furthermore, ZN ingestion accelerated sexual maturation in both groups. The cuticular chemistry of wild *B. jarvisi* showed significant sexual dimorphism in both immature and mature adults, with ZN-attracted males having higher concentrations of cuticular compounds compared to unattracted males. Although ZN ingestion did not significantly change the cuticular chemistry of the males, we found that ZN was stored in the rectal glands. Our study highlights the close relationship between the cuticular chemical profile of the flies and their sexual development.

Herbivore induced plant-plant signaling via mycorrhizal fungi in Scots pine seedlings

Dr Muhammad Usman Rasheed[1]

[1]University Of Eastern Finland, Kuopio, Finland

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. The semiochemicals have been shown to mediate plant-plant interaction effectively over short distances. Recently, mycorrhizal fungi have been identified as another means of signal transmission across the plants. The signal transmission across the mycorrhizal networks can be inter- or intraspecific and it is likely means of long-distance signal transmission in trees. The nature of signal transmission across mycorrhizal fungi is yet unknown. We designed a dynamic chamber system where potted plants can be placed proximally such that they share the same pot, but the shoots are in separate enclosed spaces. This allowed live herbivore infested emitter plants to interact with receiver plants via belowground channels without the risk of herbivores causing direct damage to the receiver plants and also prevented the exchange of VOCs from the shoots of the emitters to reach the receiver plants. We conducted an experiment to study the responses of two-year-old Scots pine (*Pinus sylvestris*) receiver seedlings connected to emitter seedlings via mycorrhizal fungi belowground. Controls were established such that there were 1) receivers with no soil or root contact with the emitters, 2) receivers with no root but soil contact with the emitters, and 3) receivers with soil, root and mycorrhizal connections with the emitters. The emitters were infested with the larvae of pine sawfly (*Neodiprion sertifer*) for five days. We studied photosynthesis and stomatal conductance in the receiver seedlings as a passive indicator of belowground signaling. We report that the receivers connected to the infested emitters via mycorrhizal fungi upregulated their rate of photosynthesis. These results can only be explained by the signal transmission across mycorrhizal network.

From leaf flush to maturity: Ontogeny-driven intraspecific variation in plant constitutive defence strategies in *Mucuna pruriens* (L.) DC.

Mr Anit Baidya[1], Ms Priyanka Kumari[1], Prof Bhoj Kumar Acharya[2], Prof Renee M Borges[3], Dr Jyothilakshmi Vadassery[4], Dr Arun Kumar Rai[1], **Prof N Sathyanarayana**[5]

[1]Biochemistry and Molecular Biology Laboratory, Department of Botany, Sikkim University, Gangtok-737102, India, [2]Ecology, Biogeography and Conservation Biology Laboratory, Department of Zoology, Sikkim University, Gangtok-737102, India, [3]Centre for Ecological Sciences, Indian Institute of Science, Bangalore-560012, India, [4]National Institute of Plant Genome Research, New Delhi-110067, India, [5]Department of Life Science, Central University of Karnataka, Kalaburagi-585387, India

Plant ontogeny, governed by diverse selective pressures and regulatory pathways, is a major driver of variation in defensive traits. While the individual effects of whole-plant and organ-level ontogeny on defence traits have been studied extensively, their combined influence remains poorly understood. Moreover, it is unclear whether variation in defence traits across leaf ontogenetic stages is dependent on the plant's developmental stage.

To address these gaps, we investigated the constitutive levels of seven defensive traits across three leaf age classes (representing leaf ontogeny) and four plant developmental stages in two varieties of anti-Parkinson's plant *Mucuna pruriens*, using a controlled greenhouse experiment. Our findings reveal that both plant and leaf ontogeny significantly contribute to intraspecific variation in defensive traits. Most traits exhibited non-linear expression patterns across ontogenetic stages, with substantial intraspecific variability. Notably, younger leaves expressed higher levels of both chemical and physical defences compared to intermediate and mature leaves. Furthermore, the relationships among individual defence traits varied across leaf stages and were influenced by the plant's ontogenetic stage.

We conclude that defensive trait expression follows complex, non-linear patterns shaped by the interplay between plant and leaf ontogeny. These patterns likely reflect adaptation to spatiotemporal selection pressures and result in dynamic, multi-trait ontogenetic defence strategies. Our study underscores the importance of incorporating multiple defence traits and ontogenetic dimensions to better understand intraspecific variation in plant defence across ecological contexts.

Smart defense strategies in plants against herbivorous insects: perspectives from FACs biosynthesis and metabolisms in insects.

Assoc Prof Naoko Yoshinaga[1], **Prof Naoki Mori**[1]
[1]Kyoto University, Kyoto, Japan

Fatty acid amino acid conjugate (FAC) was isolated from oral secretions of *Spodoptera exigua* larvae in 1997 [volicitin: *N*-(17-hydroxylinolenoyl)-L-glutamine]. The FACs are known to be elicitors that induce plants to release volatile compounds, and natural enemies of the larvae use the volatiles to locate their hosts. Based on the biosynthetic and degradative enzyme reactions of FACs and the concentration of glutamine in the larvae, we speculated that FACs play an important role in nitrogen metabolism in lepidopteran larvae and that FACs in the midgut lumen act as a reservoir of glutamine. In this study, furthermore, we identified candidate genes encoding a FACs hydrolase in *Spodoptera litura* and then, investigated the importance of FAC hydrolysis on caterpillar performance with CRISPR/Cas9 knock outs. They absorbed 30% less nitrogen from the diet compared to WT caterpillars resulting in a reduction of their body weight of up to 40% compared to wild type caterpillars. This could be very good evidence showing that an important metabolite (FACs) involved in insect nitrogen metabolism, which is not easily discarded by the insects, is used by plants as an indicator of feeding damage. One aspect of the plant's smart defense strategies was revealed.

Physical and chemical stimuli necessary for oviposition of the peach fruit moth: oviposition inhibitory activity caused by shielding by clay mineral coatings

Mr Kunitaro Muroga[1], Dr Yoichi Ishiguri[2], **Dr Naoko Yoshinaga**[1]

[1]Kyoto University, Sakyo, Kyoto City, Japan, [2]Aomori Prefectural Industrial Technology Research Center, Kuroishi, Japan

The lepidopteran pest, the peach fruit moth *Carposina sasakii* poses a serious threat to the apple industry in East Asia due to its cryptic larval development within fruit and its prolonged adult emergence period from spring to fall. Conventional insecticides are ineffective against larvae within fruit, and repeated applications of ovicidal agents increase control costs and the risk of pesticide resistance. Therefore, alternative pest control strategies that do not rely on synthetic chemicals are urgently required.

In this study, we investigated a novel coating-based approach to inhibit oviposition by *C. sasakii*. Application of a water-dispersed bentonite suspension to apple surfaces significantly reduced egg-laying in both laboratory and field settings. Our findings suggest that *C. sasakii* uses volatile compounds emitted by apples to locate hosts and relies on the presence of trichome (fine surface hairs) as a tactile cue for oviposition. When apples were coated with a clay film that physically blocked these trichome the number of eggs laid was markedly reduced. Furthermore, whole-fruit coating was more effective than partial coating, indicating that suppression of volatile emissions by the coating also contributed to the oviposition deterrent effect.

Field trials revealed that the coating's efficacy declined approximately one week post-application. Scanning electron microscopy showed that the film began degrading by day three and was almost entirely removed by day sixteen, leading to re-exposure of trichome and a resumption of egg-laying. To improve coating durability, the additive carboxymethyl cellulose was incorporated into the formulation. This modification effectively suppressed film degradation, maintaining microtrichia coverage and oviposition suppression for at least two weeks.

These results demonstrate the potential of a physical barrier coating as a non-chemical method for controlling *C. sasakii* oviposition in apple orchards.

Rhizobia alter virus–vector–host interactions via host and vector chemistry

Dr Patricia Sanches[1], Dr Hannier Pulido[1], MS Danielle Osoko[1], Prof Dr Consuelo De Moraes[1], Prof Dr Mark Mescher[1]
[1]ETH Zurich, Zurich, Switzerland

Root-associated symbionts often have profound effects on plant growth and health, including effects on plant interactions with antagonists. Rhizobia, for example, can induce plant growth as well as defenses against aphid vectors, which, in turn, mitigate the transmission of aphid-vectored plant viruses. Yet, we have limited understanding of the mechanisms underlying these interactions.

Using metabolomics approaches via GC and LC-MS, we explore how the presence of rhizobia (*Rhizobium leguminosarum*) influences the chemistry of fava beans and pea aphid vectors (*Acyrthosiphon pisum*), which ultimately can explain rhizobia-mediated effects on the transmission of Pea enation mosaic virus (PEMV).

Our results reveal that rhizobia enhance plant resistance by increasing defenses against herbivores and pathogens, including the hormones salicylic acid, jasmonic acid, and abscisic acid. Rhizobia colonization also altered key nutrients available to aphids, even in the presence of PEMV, such as amino acid concentrations in the phloem. Meanwhile, aphids feeding on virus- and rhizobia-colonized plants had reduced levels of estradiol, a critical reproductive metabolite, which, along with reduced nutrient availability, helps explain the negative effects of rhizobia on aphid vector fitness.

Altogether, our results highlight the role of rhizobia in disease dynamics and provide insight into the chemical mechanisms by which they may influence virus–vector–host interactions.

The speed of smell: Temporal resolution and odour source segregation in insects

Paul Szyszka[1], Alpha Renner[2], Alexander Egea Weiss[2], Georg Raiser[2], Aarti Sehdev[2], Yunusa Garba Muhammed[2], Christoph Kleineidam[2], C Giovanni Galizia[2]
[1]University of Otago, Dunedin, New Zealand, [2]University of Konstanz, Germany

In natural turbulent airflows, odour plumes fragment into narrow, odour-laden filaments interspersed with clean air. When these filaments hit an insect's antenna, their spatial structure is converted into a temporal sequence of brief odour encounters and gaps. These encounters can occur at frequencies above 100 Hz, with individual odour pulses lasting only milliseconds.

These temporal odour patterns contain spatial information about the location, number, and separation of odour sources. Yet the limits of temporal resolution in the insect olfactory system remain unclear. We show that olfactory receptor neurons in *Drosophila melanogaster* can resolve odour pulses above 100 Hz, with sub-millisecond spike timing precision – exceeding previous estimates. Behaviourally, *D. melanogaster* can discriminate odour mixtures based on onset asynchronies of just a few milliseconds, allowing segregation of odours from distinct sources. We further present evidence that ephaptic (non-synaptic) interactions between co-housed receptor neurons within a single sensillum contribute to this fine-scale temporal discrimination.

Exploring UV-induced biochemical change in an invasive shrub and its implications for biocontrol

Mr Paul Barrett[1], Dr Arvind Subbaraj[2], Dr Jason Wargent[1], Dr Maria Minor[1], Mr Paul Peterson[3], Mr David Lun[1], Dr Andrea Clavijo McCormick[1]

[1]Massey University, Palmerston North 4410, New Zealand, [2]AgResearch Ltd., Lincoln 7608, New Zealand, [3]Manaaki Whenua - Landcare Research, Palmerston North 4410, New Zealand

Introducing insect biocontrol agents sourced from a plant's native range is an effective, sustainable management strategy for invasive plants. However, many biocontrol agents don't achieve the desired outcome because they either fail to establish or are ineffective when they do. Heather beetle *Lochmaea suturalis,* (Coleoptera: Chrysomelidae) introduced from the United Kingdom (UK) to New Zealand to control the invasive shrub *Calluna vulgaris* (heather) was difficult to establish and reaches low population levels relative to its conspecifics in the native UK range. Difficult establishment and poor performance in biocontrol is often attributed to various abiotic or biotic factors but seldom considers alterations to the target plants biochemical phenotype. Our recent study revealed a significantly different biochemical profile in heather in New Zealand compared with the UK, between which there is considerable difference in the intensity of ultra-violet (UV) radiation. UV is known to drive plant biochemical change, including defensive secondary metabolites and we hypothesized that this factor could enhance heathers' defensive capability and impair beetle establishment and effectiveness. Testing this hypothesis involved exposing heather plants to 20% and 95% UV attenuating screens and using metabolomics to measure plant secondary metabolite responses. Our results demonstrate significant alterations to many compounds derived from the shikimate-phenylpropanoid pathway. However, a bioassay feeding *L. suturalis* on similarly exposed heather foliage revealed no impact on prepupal weight or larval survival. We explore possible reasons for this outcome including the magnitude of UV-induced biochemical changes in relation to specialist insect control agents.

Domestication increases sex pheromone emission and calling effort of Queensland fruit fly males

Dr Jeanneth Perez, Dr Soo Jean Park, Dr Donald Cameron, **Prof Phil Taylor**[1]
[1]Macquarie University, North Ryde, Australia

Sex pheromones play a central role in the mating system of many tephritid flies, including the Queensland fruit fly (*Bactrocera tryoni* aka Q-fly), the most damaging insect pest of horticulture in eastern Australia. Q-fly males spread the pheromone from their rectal glands onto their wings and body, and then rapidly fan their wings to produce audible 'calling songs' that disperse the pheromone. The sterile insect technique (SIT), a key strategy for controlling Q-fly populations, involves mass-rearing, sterilizing, and releasing millions of flies into the field to mate with wild females. However, mass-rearing processes can induce significant changes (domestication) in insect life history, reproductive biology, and communication, potentially affecting the efficacy of SIT programs.

We assessed the effect of domestication on pheromone released by laboratory-reared Q-fly males, comparing young (≤5 generations) and old (≥20 generations) colonies from Brisbane, Cairns, and Sydney. Males from old colonies consistently released more pheromone than males from young colonies. To determine whether this increase was due to higher pheromone production in their rectal glands or increased calling effort, we compared a young and an old colony from the same region (8 and 28 generations, respectively) for the amount of pheromone released, the amount produced in rectal glands, and the characteristics of calling songs. While males from the old colony released more pheromone than males from the young colony, there was no significant difference in the amount of pheromone in the rectal glands between the two colonies. Males from the old colony had calling songs with more pulses per minute than males from the young colony. The increased pheromone emission of domesticated Q-fly males can be attributed to the greater investment in calling songs that disperse the pheromone.

Marking pheromone system of a top wasp predator the Asian giant hornet *Vespa mandarinia* and other *Vespa* species

Dr Ping Wen[1], Dr Jin Chen[1]

[1]Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, China

The Asian giant hornet Vespa mandarinia (AM) is the top predator in indigenous forest food web, positioning as tiger, and predating majorly on other eusocial Hymenoptera species, including sympatric honeybees, wasps. All subspecies of AM showed territory behaviour by group attacking on nests of conspecies in vicinity < 3km, prey marking behaviour by group attacking on colonies of eusocial preys in distance within 10 km. By using field observation, chemical analysis, physiological analysis, and bioassay with synthetic chemical standards, we fully identified the marking pheromone system of AM. Results showed that, communication system in competition and predation of AM was regulated by its marking pheromone system. The venom volatiles and semi-volatiles containing unsaturated ketones, esters, alcohols, amides and terpene alcohols elicited marking and recruitment behaviour in vicinities encoding space and time information, unsaturated keto acids from body surface, secreted by sternal glands of workers elicited long distance marking behaviour. By combining the long and short distance communication signals, group attacking can be regulated effectively according to the prey defence and prey qualities. Based on this novel behavioural ecology principles of top Vespa predators, marking pheromone system of other species were identified as well, including unsaturated ketones in *Vespa velutina*, *V. basalis* and *V. tropica* venom volatiles. This discovery provided basis for behavioural control methods for these notorious pest predators in Apiculture and biodiversity conservation in both indigenous and invaded area globally.

Virulence characteristics of *Nilaparvata lugens* (Stål) reared on resistant rice variety YHY15

Dr Qingsong Liu[1], Miss Yi Meng Li[1], Prof Ted Turlings[1], Prof Yun He Li[1]

[1]State Key Laboratory of Cotton Bio-breeding and Integrated Utilization, School of Life Sciences, Henan University, Kaifeng, China

The brown planthopper (BPH), *Nilaparvata lugens*, is a major insect pest of rice, posing a serious threat to global rice production. Developing and deploying BPH-resistant rice varieties represents one of the most economical and effective strategies for managing this pest. However, the rapid adaptation of BPH to resistant cultivars has led to the emergence of virulent populations, such as biotype Y. Therefore, elucidating the patterns and characteristics of virulence variation in BPH is essential for the sustainable utilization of resistant rice resources. We investigated the virulence traits of two BPH populations, biotype 1 and biotype Y, which differ markedly in their levels of virulence. The results showed that biotype 1 exhibited a significantly longer nymphal development duration and lower fresh weight of newly emerged adults on the resistant rice cultivar YHY15, which carries the BPH resistance gene *Bph15*, compared to the susceptible variety TN1. In contrast, biotype Y showed no significant differences in either nymphal development duration or fresh adult weight between the two rice genotypes. Similarly, biotype 1 females exhibited significantly greater body weight gain and honeydew excretion when feeding on TN1 compared to YHY15, whereas these parameters did not differ significantly for biotype Y between the two hosts. Host preference assays further revealed that biotype 1 females displayed a clear preference for the susceptible TN1 plants and laid significantly more eggs, whereas biotype Y females showed no significant oviposition preference between the two rice varieties. These findings provide first insights into the virulence characteristics of biotype Y on the resistant YHY15 rice, and lay a foundation for investigations into the molecular and ecological mechanisms underlying BPH adaptation to resistant rice.

Using multi-lures to trap forest pest insects in Australia. Is there a benefit of combining lures?

Dr R. Andrew Hayes[1], Dr Emily Lancaster[2], Ms Janet McDonald[2], Dr Helen Nahrung[1]
[1]University of the Sunshine Coast, Dutton Park, Australia, [2]Queensland Department of Primary Industries, Dutton Park, Australia

Invasive insects have a major impact on Australia's softwood plantations, and incursions by new species pose an ongoing risk to the forest industry. Traps with attractant odours can be used to detect new invasions, for general forest pest monitoring, to provide area freedom data, or for delimiting surveys of new detections. We deployed traps in four jurisdictions across Australia (Victoria, NSW, Queensland, Northern Territory), to assess two novel lures designed to target cerambycids and Ips bark beetles. The lures were tested alone, in combination, and compared with control lures for trapping of beetles, especially forestry-relevant families (Anobiidae, Bostrichidae, Buprestidae, Cerambycidae, Curculionidae).

Results varied between locations, with highest numbers of individuals and morphospecies trapped at the combination lure in Victoria, at the Ips lure in NSW and with little difference between the lures in Queensland. Very few forestry-relevant beetles were trapped in the Northern Territory.

In Queensland, 19,116 trapped individuals representing 95 morphospecies belonged to forestry-relevant families. Significantly more bark and ambrosia individuals were caught in Ips lures, either alone or in combination with cerambycid lures, but diversity did not differ between lure types. For cerambycids, trap catches were variable and influenced strongly by trapping location. Significantly fewer individuals were captured in control traps, but there was no difference between cerambycid lure and those containing Ips lure, and no difference in diversity of cerambycids by lure type.

As in global studies, we detected no negative impact of combining the lures, however, we also failed to identify a benefit to doing so. The lack of differences between the lure types was impacted by large numbers of singleton morphospecies (trapped only once) and by a few morphospecies comprising the majority of trapped beetles. Implications for use of generic lures for biosecurity monitoring will be discussed.

Behaviour-modifying volatiles for pest control of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae)

Dr Raimondas Mozūraitis[1], Dr Sandra Radžiutė[1], Dr Laima Blažytė-Čereškienė[1], Dr Violeta Apšegaitė[1], Dr Rasa Čepulytė[1], Prof Dr Habill Vincas Būda[1]

[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania

Sour and sweet cherries are economically important fruit species. Recently, damage caused by the European cherry fruit fly, *Rhagoletis cerasi* to cherry berries increased significantly, exceeding the 2% infestation level allowed in the food market. *R. cerasi* fruit flies are difficult to control by insecticide application since most of the conventional insecticides used have been banned in Europe. Traps with nonspecific attractants are used for monitoring and detection of the beginning of the flight period. Thus, new, more specific attractants for environmentally-friendly pest control are needed. The study aims to determine host-plant volatile organic compounds (VOCs) that modify behaviour of *R. cerasi* fruit flies. Fourteen VOCs, sampled from sour cherry, *Prunus cerasus* foliage, elicited electroantennographic responses in *R. cerasi* females. In a two-choice Y-type olfactometer, virgin *R. cerasi* females significantly preferred the olfactometer arm bearing (*E*)-β-ocimene, or (*Z*)-3-hexenyl 3-methylbutanoate, or (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, or linalool versus the control arm free from a stimulus. Contrarily, *R. cerasi* females avoided the olfactometer arm with (*Z*)-3-hexen-1-ol. Efficiency evaluation of attractive lure, comprised of putative kairomone attractants, including (*E*)-β -ocimene, (*Z*)-3-hexenyl 3-methylbutanoate, and linalool, as well as repellent properties of (*Z*)-3-hexen-1-ol, is conducted in the cherry orchards.

Deciphering the role of silicon and arbuscular mycorrhizal symbiosis in plant defense against herbivory: A benzoxazinoid perspective

Ramalka Kasige[1], Dr Adam Frew[1], Prof Scott N Johnson[1]
[1]Western Sydney University, Richmond, Australia

Maize (*Zea mays*) is a global cereal crop which produces benzoxazinoids (BXDs) for defence against herbivores (e.g. fall armyworm, *Spodoptera frugiperda*; FAW). BXD production is influenced by soil nutrients and symbiotic interactions with microbes. Plant growth and stress tolerance may be enhanced by both silicon (Si) accumulation and associations with arbuscular mycorrhizal (AM) fungi. This study investigated how Si supplementation and AM fungal colonisation influence maize defence against FAW, focusing on foliar BXDs. Maize was grown with or without Si supplementation and inoculated with functionally diverse AM fungal groups – Glomeraceae (associated with plant defence) or Gigasporaceae (associated with nutrient uptake), or a mixed-species AM fungal inoculum representative of natural field diversity or left uninoculated. Defence responses were evaluated by quantifying foliar BXD concentrations (DIMBOA). Both Si supplementation and herbivory exerted strong individual and interactive effects on DIMBOA production. In the absence of AM fungi and Si supplementation, herbivory alone induced DIMBOA synthesis. DIMBOA was high at low Si concentrations. However, Si supplementation elevated DIMBOA levels, particularly in plants subjected to herbivore attack, although this response varied depending on the AM fungal species present. Under high Si, plants colonised by the mixed-species inoculum showed the highest DIMBOA concentrations, indicating that Si and diverse AM fungi can work together to strengthen BXDs. In contrast, without Si supplementation, plants inoculated with the mixed-species inoculum exhibited a significant reduction in DIMBOA compared to non-inoculated controls. Plants colonised by Glomeraceae displayed a slight increase in DIMBOA under high Si, but this effect was not significant, despite a positive trend, whereas plants with Gigasporaceae showed minimal or no impact on DIMBOA levels across Si treatments. Si supplementation and the mixed-species AM inoculum formed the most effective combination for enhancing BXD-mediated defence against FAW, offering a promising avenue for sustainable pest management in cereal cropping systems.

Plant cross-talks: Mechanical stress alters volatile emission and species composition in neighboring grassland plant communities

Rashaduz Zaman[1], Isaac Peetoom Heida[1], James F. Cahill[1]
[1]University Of Alberta, Edmonton, Canada

Plants emit volatile organic compounds (VOCs) in response to herbivory, and these emissions can influence the metabolic responses of neighbouring plants. While plant-plant VOC signalling is well established at individual level, it remains poorly understudied at community scale. We conducted an exploratory study to test localized damage affects VOC emissions and community dynamics in neighbouring, undamaged plants in a multi-species system. Using 24 paired grassland turfs from a native prairie, we clipped one turf in each pair to simulate herbivory, and left the adjacent turf unclipped in greenhouse. We collected aboveground VOCs from both turfs at seven time points over a 28-day period. The results highlight the complex interplay between mechanical stress, temporal progression, and interplant communication via VOCs. The initial convergence of VOC profiles at 24 hours aligns with VOC exchange-induced synchronization, while the divergence at 168 hours underscores treatment-specific metabolic trajectories. Unclipped plants' enhanced VOC output reflects adaptive response to stress-signalling cues from clipped neighbours. Species composition was recorded at Day 1 and 28. Clipped turfs showed modest shifts in species abundances, consistent with direct disturbance. However, unclipped turfs showed species divergence over time suggesting that exposure to clipping-induced VOCs may influence competitive dynamics or recovery patterns at the community level. Combination of VOCs and species shifts indicates a potential feedback mechanism where localized herbivory can alter both the chemical phenotype and plant composition of neighbouring undamaged vegetation. To further test this hypothesis, we plan to scale the experiment to natural prairie patches and assess VOC spillover under field conditions. We also aim to conduct olfactory bioassays with grasshoppers to determine whether herbivore foraging behaviour is influenced by the VOC profiles of clipped versus unclipped turfs. Our results suggest a novel VOC-based feedback process that may shape plant interactions, herbivore behaviour, and ecosystem resilience in grazed grasslands.

Attraction of predatory spotted ladybird to odours associated with fall armyworm larvae and induced sweet corn seedlings

Dr Rehan Silva[1], Dr Jamil Biswas[1], Mr Vaughan Moon[1], Dr Paul Mirzayans[1], Dr Kawsar Khan[1], Dr Sabira Sultana[1], Dr Vivian Mendez[1]

[1]Macquarie University, Sydney, Australia

The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a highly destructive pest of economically important crops such as sweet corn (*Zea mays*) in Australia. Currently, *S. frugiperda* populations are managed using pesticides, however, with growing concerns about pesticide resistance, other management strategies such as biological control will be increasingly important. Predaceous ladybirds (coccinellids) are one of the best-known biocontrol agents for many horticultural and agricultural pests, but their effectiveness against *S. frugiperda* is yet to be evaluated. Here we studied the spotted ladybird *Harmonia conformis* (Coleoptera: Coccinellidae) as a potential predator to assess their ability to kill *S. frugiperda* larvae and to understand how they use olfactory cues to locate *S. frugiperda* larvae. Feeding trials showed that *H. conformis* did kill neonates, first, second and third instar *S. frugiperda* larvae. Two-choice olfactometer experiments showed that there was no evidence of *H. conformis* discriminated between mechanically damaged and intact seedlings but were more attracted to seedlings damaged by *S. frugiperda* larvae. *Harmonia conformis* with prior feeding experience responded faster to seedlings damaged by *S. frugiperda* larvae. Similarly, *H. conformis* were attracted to seedlings with egg masses, however, when given a choice between seedlings with egg masses and seedlings damaged by larvae, H. conformis were more attracted to damaged seedlings. *Harmonia conformis* were also attracted to seedlings with frass from either second or fifth instar larvae, however, when given a choice *H. conformis* were more attracted to seedlings with second instar frass. Volatile compounds collected from intact, mechanically damaged and larval damaged seedlings showed that the larval damaged seedlings released more unique compounds. This study highlights that *H. conformis* is a potential predator for controlling early instar *S. frugiperda* larvae and provides an insight into how *H. conformis* could use volatile cues to locate their prey.

The evolution of olfaction in cetaceans: A genomics approach

April A. Jauhal[1,2], Rochelle Constantine[1], **Richard D. Newcomb[1,2]**
[1]School of Biological Science, University of Auckland, New Zealand, [2] Plant & Food Research, Mt Albert Research Centre, Auckland, New Zealand

Major evolutionary transitions, such as the shift of cetaceans from terrestrial to marine life, can put pressure on sensory systems to change. Relatively little is known about the role of smell in the evolution of mysticetes (baleen whales). While their toothed cousins, the odontocetes, lack the anatomical features to smell, it is less clear whether baleen whales have retained this sense. To address this question, we mined the relevant olfactory genes from available cetacean genomes. For the signal transduction and chaperon genes we examined, all were intact in mysticetes, compared with inactivating mutations observed in many odontocete homologues. For odorant receptor (OR) genes, over 700 were recovered from eight mysticete genomes. While many OR groups had been lost or showed signs of random drift, others exhibited evidence of evolving under purifying or positive selection. One orthologous group in particular from the OR10 family showed signs of relative expansion and purifying selective pressure. Overall, our results support baleen whales having the ability to smell, with evidence of specialisation to a new olfactory landscape. In undertaking this research, we also analysed genome quality assessment methods (BUSCO vs N50) and developed an automated pipeline for identifying odorant receptor genes (GMPipe).

Natural fruit and microbial cues improve selective trapping of *Drosophila suzukii*

Dr Robert Raguso[1], Mr Yaroslav Grynyshyn[2], Dr Binita Shrestha[2], Dr Ajinkya Dahake[3], Ms Ziyin Xiong[4], Mr Afm Khan[1], Ms Claire Makino Duan[4], Ms Gabrielle Brind'Amour[2], Mr Stephen Hesler[2], Dr Gregory Loeb[2]

[1]Cornell University, Ithaca, United States, [2]Cornell University, Geneva, United States, [3]Princeton University, Princeton, USA, [4]Massachusetts Institute of Technology, Cambridge, USA

In the past two decades, the spotted-wing drosophila (SWD; *Drosophila suzukii*) has become a serious invasive pest on soft-skinned fruit worldwide. Present management strategies incur collateral damage due to intensive application of insecticides and/or trapping lures (e.g. Scentry) that attract and kill non-target arthropods. We leveraged the natural history of SWD, from the visual properties of their larval host fruits to the olfactory cues produced by microbes inhabiting these fruits and the flies' guts, to develop a more selective, multimodal trap to monitor and potentially control SWD. First, we cultured bacterial and fungal species identified from wild fruits and SWD guts, collected, identified and ordinated 76 volatile organic compounds (VOCs) emitted by microbial monocultures and bacteria-yeast combinations. We tested these candidates, alone and in combination, as olfactory lures for SWD in lab cafeteria assays, finding one combination that attracted more flies than any other lure and was as attractive as the odorant blend used in the Scentry lure as a positive control. We repeated the lab cafeteria assays combining an odor attractant with color disks, finding that saturated green, yellow and black trapped the most flies, with high contrast combinations of either color with black being the most effective. Using these findings, we developed field traps with yellow-black or all-black entry ports combined with the most effective microbial lure and employed them with negative (sterile media) and positive (Scentry) controls in raspberry fields in central NY, USA. The microbial lure was as effective as Scentry in attracting male and female SWD, particularly with the all-black trap. Moreover, there was no difference in the proportion of gravid female SWD trapped by either lure. However, both black and yellow-black traps collected higher proportions of SWD when baited with the microbial lure than with Scentry, resulting in lower non-target by-catch.

Yeast fermentative volatiles promote illicit foraging behavior in bumble bees

Dr Daniel Souto-Vilarós[1,2], Valerie Martin[1,2], Nicholas Dabagia[2,3], Drew Freshour[1,2], Oriana Gutierrez[2,4], Natalie Rodriguez[2,5], Jade Stryker[2,6], Dr Caitlin Rering[6], Dr Rebecca Irwin[7], **Robert Schaeffer**[1,2]

[1]Utah State University, Logan, United States, [2]Rocky Mountain Biological Laboratory, Gothic, United States, [3]University of Michigan, Ann Arbor, United States, [4]Washington and Lee University, Lexington, United States, [5]University of California Irvine, Irvine, United States, [6]New College of Florida, Sarasota, United States, [7]USDA-ARS Chemistry Research Unit, Gainesville, United States, [8]North Carolina State University, Raleigh, United States

Plant–pollinator mutualisms are widespread in ecological communities; however, their stability is frequently threatened by acts of cheating committed by individual actors. The proximate mechanisms that encourage individual flower visitors to choose to seek benefits without conferring a service in return, such as robbing flowers of their nectar without providing any pollination, remain largely unknown. In this study we combined field and laboratory behavioral assays with gas chromatography mass spectrometry (GC-MS) to test the hypothesis that a nectar-inhabiting yeast can mediate exploitative behaviors displayed by bumble bees with flexible foraging tactics across different floral hosts. In field behavioral assays, *Bombus bifarius* and *B. flavirons* secondary nectar robbers (individuals that take advantage of holes made in flower corollas by primary nectar robbers) discovered robbing holes 2.2 s faster on average and foraged 64% more on *Metschnikowia reukaufii* yeast-inoculated flowers of both *Cordyalis caseana* and *Mertensia ciliata* than controls. Olfactometer assays confirmed a yeast volatile-mediated response, as workers both showed an initial preference for and spent significantly more time in the Y-tube arm assigned to the yeast-treated bouquet for *Corydalis*. Nectar scent was enhanced with fifteen volatile compounds when fermented with yeast for two days relative to unfermented nectar. Collectively, our results suggest an important role for yeast odors in mediating exploitative behaviors displayed by mutualist species, acting as a potential honest signal for resource presence and improving bee foraging efficiency.

Novel lineages of hexapod chemoreceptors establish an origin for insect gustatory and odorant receptors

Dr Robert Mitchell[1], Dr Kevin Moran[2], Dr Duane McKenna[2]

[1]The Pennsylvania State University, University Park, United States, [2]The University of Memphis, Memphis, United States

The insect chemoreceptor superfamily includes three major lineages of gustatory receptors (GRs; sugar GRs, bitter GRs, carbon dioxide GRs) and one lineage of odorant receptors (ORs), which together comprise the majority of the receptor proteins used by insects to smell and taste their environment. However, the evolutionary origin of these lineages has remained obscure, with few direct relationships between the GRs of insects and early-diverging hexapods, and no evidence for non-insect ORs. Here, we resolve these relationships through an intensive manual genomic annotation of chemoreceptors in the earliest extant insects and the non-insect hexapods. We identify novel chemoreceptors that establish a shared origin for bitter GRs, carbon dioxide GRs, and a third lineage that we term the “relict GRs”, which diversified extensively in hexapods before dwindling in modern insects. We also describe a novel lineage related to the sugar GRs that appears to be the direct precursor to the insect ORs.

Floral compound attractants for buzz-pollination by *Amegilla anthophorine* bees

Rogelio Rosales-Garcia[1], Dr Robert Spooner-Hart[2], Dr Simon Tierney[2], Dr Flore Mas[3], Dr James Cook[1]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Penrtih, Australia, [2]School of Science, Western Sydney University, Penrtih, Australia, [3]The New Zealand Institute for Plant & Food Research, Lincoln, New Zealand

Floral scents are complex chemical mixtures, but only a few key compounds are known to help bee foragers identify floral resources, and these are often ubiquitous in many plant species. Most research to date has been undertaken on *Apis mellifera* and broader investigations on other genus of bee species may help unlock their potential as alternate crop pollinators. Here we test the attraction of common floral compounds in the buzz-pollinating bee genus *Amegilla* - a candidate for managed pollination in protected cropping that is common throughout Australia and Afroeurasia. We conducted greenhouse bioassays with four synthetic floral compounds applied to artificial flowers, and recorded visitation by the Australian species *A. pulchra*. In the first experiment, we applied compounds of two benzenoids (Benzaldehyde & Phenylacetaldehyde) and two monoterpenoids (Linalool & β-Ocimene) in low, medium and high concentrations in a randomised array. In a second experiment, we explored possible synergetic effects of the most attractive compounds (Phenylacetaldehyde & Linalool) independently and as a benzenoid-monoterpenoid blend. Results showed that low-concentration of phenylacetaldehyde attracted significantly more visits than the control (visual cue but no chemical cue), but the other compounds were not significantly different from the control. While specific low-concentration synthetic compounds increased artificial flower attraction, visual cues alone elicited frequent *A. pulchra* visitation in our experimental array. In future experiments, we will take into consideration individual bee learning of visual cues. Yet, these preliminary results are encouraging and show the potential application for attracting buzz-pollinating bees to protected horticultural crops.

Plant phenology drives foliar volatiles emission with consequences for arthropod community dynamics in row crops

Rupesh Kariyat[1]

[1]University of Arkansas, Fayetteville, United States

While traditional pest management of field crops revolves around pesticides, novel host-plant resistance strategies have seen an increase in usage and efficiency. Plant phenology- as a function of species, genotype and environment, provides us with data that continues to be critical in developing such strategies, even more important under climate change, where phenology mis match between host and herbivore has been predicted. To improve upon this line of questioning, we used three row crops- soybean, rice and sorghum under various management strategies against a devastating polyphagous pest- fall armyworm (*Spodoptera frugiperda*). We examined the role of plant phenology in mediating plant (morpho-physiology; foliar volatiles) and herbivore response (growth and development). And then investigated if these effects have consequences for arthropod community dynamics in field. Collectively, our results shows that phenology plays a significant, species specific role in plant-herbivore interactions, and has cascading effects on herbivore growth and development. In addition, phenology dependent foliar volatiles had strong consequences for trophic interactions. The implications of these findings for devising management practices are currently under exploration.

Sharing of 4-hydroxy-3-(methylthio)benzaldehyde as a pollination semiochemical between distantly related sexually deceptive orchids

Mr Seeger Van Kints[1,2,7], Dr Gavin Flematti[1], Prof Murray Baker[1], Prof Rod Peakall[3], Dr Ryan Philips[4,5,6], Dr Björn Bohman[1,2]

[1]School of Molecular Sciences, University of Western Australia, Perth, Australia, [2]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden, [3]Research School of Biology, The Australian National University, Canberra, Australia, [4]Department of Environment and Genetics, and Research Centre for Future Landscapes, La Trobe University, Melbourne, Australia, [5]Royal Botanic Gardens Victoria, Science Division, Corner of Ballarto Road and Botanic Drive, Cranbourne, Australia, [6]Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, Australia, [7]Tasmanian Institute of Agriculture, University of Tasmania, Sandy Bay, Australia

Sexually deceptive orchids mimic mating signals from females of their pollinator species to secure pollination. A diverse range of compounds have been identified as semiochemicals employed by sexually deceptive orchids, some of which were previously unknown to science. However, in Australia, multiple genera of orchids sexually deceive thynnine wasps for pollination, raising the possibility of convergent evolution of chemical signals. Here, we use gas chromatography- mass spectrometry (GC-MS) and field bioassays to identify the semiochemical emitted from the labellum of the sexually deceptive orchid *S. ciliata* to attract its thynnine wasp pollinator *Iswaroides* sp. The attractive compound, 4-hydroxy-3-(methylthio)benzaldehyde, was confirmed by peak enhancement in the total ion chromatogram of the co-injection of a synthetic standard and the floral extract of *S. ciliata*. The attractiveness of candidate compounds to male pollinator wasps was assessed using dummies that mimicked the appearance of female wasps.

We demonstrate that this semiochemical alone is sufficient to elicit the full sexual repertoire of *Iswaroides* sp., indicating it is likely the insect's sex pheromone. Interestingly, in plants, (methylthio)phenols were only previously known as semiochemicals from the sexually deceptive orchids *Caladenia crebra* and *C. attingens*, which are pollinated by *Campylothynnus* thynnine wasps. However, these wasps belong to a different subtribe than *S. ciliata*. Our discovery suggests the potential of repeated evolution of the same sex pheromone among thynnine wasps and the orchids that exploit them.

Attractant strategies for beneficial in canola: implementation in pest management

Dr Shovon Chandra Sarkar[1], Dr Sarina Macfadyen[2], Dr Wei Xu[1]

[1]Murdoch University, Murdoch, Australia, [2]Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia

Canola crops face considerable threats from various insect pests, such as the green peach aphid (*Myzus persicae*) and diamondback moth (*Plutella xylostella*), primarily due to their rapid reproduction, short life cycles, and resistance to insecticides. The natural enemy populations within the environment hold great potential for pest control. The effectiveness of these natural enemies in canola crops is closely linked to chemical communication, particularly the volatile compounds released by pest-infested plants. This study aims to develop attractants using herbivore-induced plant volatiles to attract natural enemies within the canola agricultural system. A Y-tube olfactometer study revealed the olfactory preferences of the tested ladybirds and lacewings. Adult ladybirds (*Harmonia conformis*, *Hippodamia variegata* and *Coccinella transversalis*) exhibited a positive response to *M. persicae*-infested canola plants, while their responses to *P. xylostella*-infested plants varied. Lacewing (*Mallada signatus*) adults also showed a significant preference for *M. persicae*-infested plants when compared to non-infested plants or blank controls, but their response to *P. xylostella* infestation was only observed when no alternative plant choice was available. These findings highlight the varying sensitivities of natural enemies to herbivore-induced plant volatiles and their potential implications for biological control strategies in canola crops. Subsequently, we will conduct a series of laboratory, greenhouse, and field trials to observe the electrophysiological and behavioural responses of natural enemies to individual active compounds and volatile blends. The goal of this study is to gain insights into the use of plant volatiles and how they can alter the behaviour of natural enemies, with the aim of improving the biological control of canola pests. This research provides valuable insights for Australia's canola industry, offering an eco-friendly alternative to chemical pesticides.

Improved synthesis and behavioural evaluation of pheromone blends for monitoring the banana-spotting bug

Miss Anna Kell[1], Dr Benjamin Hanssen[1], **Dr Soo Jean Park**[1]
[1]Macquarie University, Sydney, Australia

The banana-spotting bug (*Amblypelta lutescens lutescens*) is a highly polyphagous native pest that poses a significant threat to multi-crop production in northern New South Wales and Queensland. Although a commercially available lure exists, its field efficacy has been inconsistent, often attributed to variable sticky trap performance, yet no systematic evaluation has been conducted. Furthermore, the stereospecific nature of key pheromone components contributes to high production costs, and alternative synthetic routes remain underexplored. This study aimed to improve the lure system by formulating a new pheromone blend based on volatiles collected from mating pair headspace and by developing a more efficient synthetic route to (R,E,E)-α-farnesene-10,11-epoxide, a key pheromone compound. Headspace volatiles were analysed using gas chromatography-mass spectrometry (GC-MS), and the resulting blend was compared with commercial lure extracts in Y-tube olfactometer bioassays. Both male and female BSBs showed significant attraction to the new blend and the commercial extract, with no significant difference in preference, indicating comparable effectiveness. A novel five-step synthetic route to (R,E,E)-α-farnesene-10,11-epoxide was developed, yielding an improved overall efficiency of 45%, substantially exceeding previously reported values. This advancement supports the potential for more cost-effective and scalable production of pheromone-based lures for improved BSB monitoring and management.

Floral scent chemistry and pollination ecology of *Banksia* (Proteaceae)

Dr Stanislaw Wawrzyczek[1,2,4], Dr Bjorn Bohman[2,3], Dr Siegfried L Krauss[4], Dr Robert A Davis[5], Dr Susan Hoebee[1], Dr Gavin Flematti[2], Ms Isabella Butler[2], Dr Ryan D Phillips[1,4]

[1]La Trobe University, Bundoora, Australia, [2]University of Western Australia, Crawley, Australia, [3]Swedish University of Agricultural Sciences, Lomma, Sweden, [4]Kings Park Science, Perth, Australia, [5]Edith Cowan University, Jundaloop, Australia

The evolution of floral traits is largely driven by pollinators, with floral scent as one of the key traits mediating attraction of pollinators to flowers. Because of that, studying the chemical composition of floral volatiles can deepen our understanding of plant-pollinator interactions. *Banksia* (Proteaceae) is an iconic Australian plant genus characterised by large inflorescences and vertebrate-dominated pollination systems. Most banksias are thought to be primarily pollinated by birds; however, a range of non-flying mammals and insects also visit the flowers and may contribute substantially to fruit set. Based on perceived differences in floral scents among different species, it has been predicted that that certain *Banksia* species may be specifically attracting non-avian pollinators by producing strong and unusual, pungent or fruity floral scents. We studied pollination ecology of 10 species of *Banksia* in a region of exceptionally high *Banksia* species richness in south-west Australia. We used GC-MS to analyse their floral volatiles and related the results to the frequency of visitation by different potential pollinators. Preliminary results suggest that species frequently visited by non-flying mammals produce richer floral scent (comprising greater number of volatile compounds) than species visited mostly by birds. In addition, the scents of the species visited most frequently by rodents, include compounds known or suspected to be involved in rodent pheromonal signalling, including nitroalkanes, sulcatone, and 2-methylbutanoic acid. A summer-flowering *B. attenuata* is frequently visited by scarab beetles alongside other insects, birds and mammals. Floral volatiles of this species include 3,6-nonadien-1-ol and 3,6-nonadien-1-yl acetate. A mixture of these compounds was strongly attractive to potentially pollinating beetles in a field bioassay. Our results revealed a previously underappreciated diversity of floral scent traits in *Banksia* and enhanced our understanding of potential adaptations functionally different pollinator groups, including first case among Australian Proteaceae of apparently targeted attraction of pollinating beetles.

Asynchronous dynamics of *Bulbophyllum* orchid-fruit fly pollinator mutualistic interactions and effects of floral fragrance acquisition on male mating enhancement

Dr Suk-Ling Wee[1,2], Dr Keng-Hong Tan[3], Prof Emer Ritsuo Nishida[4]

[1]Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia, [2]Centre for Insect Systematics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia, [3]Academy of Sciences Malaysia, Kuala Lumpur, Malaysia, [4]Graduate School of Agriculture, Kyoto University, Sakyo Ku, Japan

Males of the Oriental fruit fly, *Bactrocera dorsalis*, are specialist pollinators of *Bulbophyllum cheiri* subspecies *cheiri*. The flies are strongly attracted to newly-bloomed flowers and probe on them compulsively. It was previously discovered that the orchid's floral scent contained a significant amount of methyl eugenol (ME). We aim to determine the temporal dynamics of the plant-pollinator interaction throughout a single day; and to explore the underlying causes of floral acquisition on the behavioural and physiological changes in *Ba. dorsalis* males. We found that wild *Ba. dorsalis* visited the orchid flowers from 07:30 to 19:30 h, with a peak attraction between 08:00 and 11:00 h. This pattern was similar when a synthetic ME source was used. Chemical analyses of floral scent emissions collected hourly from 08:00 to 17:00 h revealed that a single *B. cheiri* flower emitted an average between 228 and 449 µg of ME per hour, with no significant peak in emission throughout the day. This indicates that there is no synchronized pattern between the temporal visitation of the flies and the floral scent production in their mutual interactions. Further laboratory bioassays demonstrated that flower-fed *Ba. dorsalis* males experienced extended mating enhancements for at least 10 days compared with untreated males not exposed to the flowers. This suggests that the diurnal attraction of *Ba. dorsalis* males to ME sources, whether natural or synthetic, is regulated by their circadian rhythms. Conversely, the pattern of floral scent emission from *Bu. cheiri* did not align with the foraging behaviour of these specialist pollinators, indicating that there is no circadian regulation modulating the interspecific interactions in this mutualistic relationship.

Use of pheromones for surveillance and monitoring of invasive coconut rhinoceros beetle

Sulav Paudel[1]

[1]AgResearch, Lincoln, New Zealand

The coconut rhinoceros beetle (*Oryctes rhinoceros*, CRB), native to Southeast Asia, is a devastating pest of coconut and oil palms across the Pacific. Its aggregation pheromone, ethyl 4-methyloctanoate (E4-MO), has been used for over three decades in monitoring and trapping programs. However, the recent expansion of CRB's invasive range necessitates a critical reassessment of E4-MO-based strategies. Through case studies, we highlight both the successes and limitations of E4-MO in different ecological and operational contexts, revealing key challenges such as variable trap efficacy and pheromone saturation in high-density infestations. We identify gaps in current practices and propose future research directions to enhance CRB control, emphasizing adaptive strategies for evolving pest dynamics.

Biochemical and antioxidant responses of *Robinia pseudoacacia* l. to infestation by the invasive leaf miner *Parectopa robiniella* (Clemens, 1763).

Dr Svitlana Sytnyk, Prof Dr Kyrylo Holoborodko, Dr Larysa Shupranova
[1]Dnipro State Agrarian and Economic University, Dnipro, Україна

Robinia pseudoacacia, despite its controversial reputation as an invasive species, remains one of the key forest-forming species in Ukrainian forestry. Due to its fast growth, drought tolerance, and ability to thrive in poor soils, this tree species plays an important ecological and economic role, contributing to land rehabilitation, soil erosion control, and the production of local energy crops in the Steppe zone of Ukraine. However, in recent decades, a serious phytosanitary threat has emerged across Europe due to the spread of the invasive North American leaf-miner *Parectopa robiniella* (Lepidoptera: Gracillariidae), which damages *R. pseudoacacia* and disrupts its physiological functions. Our study aimed to investigate the impact of *P. robiniella* infestation on photosynthetic activity and the protein-antioxidant protective system of *R. pseudoacacia* at different tree ages. The infestation resulted in significant reductions in chlorophyll fluorescence parameters (background, stationary, maximum, and variable fluorescence), especially in trees younger than 15 years, indicating greater sensitivity of younger trees to insect herbivory. Increased levels of catalase, benzidine peroxidase, and guaiacol peroxidase activity were found in the leaves infested by the herbivore, which were measured using spectrophotometric methods. Additionally, infestation altered the expression pattern of benzidine peroxidase isoforms: in 5-year-old trees, five isoforms decreased while four increased. In contrast, 15-year-old trees exhibited an overall reduction in the number of synthesized isoforms, indicating age-related variation in enzymatic response. The increase in soluble protein content and antioxidant enzyme activity suggests the activation of defense mechanisms in response to oxidative stress. The restructuring of the peroxidase isoenzyme system confirms the involvement of benzidine and guaiacol peroxidases in the protection of *R. pseudoacacia* against *P. robiniella*. These findings contribute to our understanding of adaptations of this forest-forming species to invasive insect herbivore pressure and may inform future strategies for monitoring or enhancing resistance in forest ecosystems in Ukraine.

The role of anthocyanin in belowground conspecific interaction: A case study using *Dioscorea alata* (water yam) cultivars with varying anthocyanin contents

Mr Takuto Kaneko[1,2], Asst Prof Yu Nishizawa[2], Prof Michio Onjo[2], Prof Akira Yamawo[1]
[1]Center for Ecological Research, Kyoto University, Otsu, Japan, [2]Kagoshima University, Kagoshima, Japan

Anthocyanins (AnC), a group of flavonoids, are water-soluble compounds found in almost all terrestrial plants. While they are primarily known for their roles in the aboveground tissues of plants such as contributing to seed dispersal and stress tolerances, AnC are also contained in belowground organs in many plant species. However, the ecological functions of belowground AnC remain largely unexplored. In this study, we investigated the potential role of belowground AnC in mediating conspecific interactions using three *Dioscorea alata* (water yam) cultivars that differ in AnC contents in both above- and belowground parts. A competition experiment was conducted, in which biomass and other traits were compared across treatments. Previous study found that cultivar A does not contain AnC in both above- and belowground tissues, and cultivar S contains AnC in aboveground but does not AnC in belowground tissues. In contrast, cultivar P contains AnC in both parts. Our results showed that the biomass of cultivar A and S was significantly reduced when grown in competing with cultivar P. However, no significant biomass reduction was observed when cultivars competed with cultivar S. These findings suggest that belowground AnC may function as a competitive trait, potentially suppressing the growth of conspecific competitors.

Lung cancer detection with dogs: Method development and accuracy estimation

Dr Timothy Edwards[1], Mr Linguo Ji[1], Dr Catherina Chang[2], Dr Michael Jameson[3]
[1]University of Waikato, Hamilton, New Zealand, [2]Waikato Hospital, Hamilton, New Zealand, [3]Waikato Clinical Campus, University of Auckland, Hamilton, New Zealand

We trained dogs for lung cancer detection using breath and saliva samples obtained from over 1,000 patients visiting Waikato Hospital's respiratory clinic. Dogs were trained and tested using a fully automated apparatus, thereby eliminating human cuing and other common issues associated with applied scent-detection procedures. In the process of training the dogs, we conducted three evaluations: (1) we compared the dogs' performance with breath and saliva samples; saliva has not been evaluated as a substrate for canine scent-detection of lung cancer and could have some advantages over breath for this purpose; (2) we evaluated the dogs' accuracy with re-used breath samples; it is common practice to re-use samples to some extent during training, but the effect of sample re-use on accuracy has not been systematically evaluated; and (3) we developed and evaluated methods of determining when to transition from training to blind testing; this information is absent in the relevant literature but has major implications for blind testing outcomes. Following training, we conducted a blind test using operationally viable methods, that is, methods that could be applied directly for lung cancer screening if dogs are capable of identifying individuals with lung cancer with sufficiently high accuracy. Following the blind test, we completed a re-training exercise, given that there was significant evidence of disrupted scent-detection performance under the operational blind testing conditions. A brief summary of the findings from each stage of this programme of research is presented herein.

Multi-omics driven investigation of the principles of intraspecific molecular communication (particularly autotoxicity) within the holobiont of the red seaweed, *Asparagopsis taxiformis*

Dr Tomas Lang[1], Prof Scott Cummins[1], Prof Nicholas Paul[1], A/Prof Alexandra Campbell[1]
[1]University of the Sunshine Coast, Sippy Downs, Australia

Interspecific and intraspecific communication via semiochemicals is a widespread phenomenon in all photosynthetic organisms; however, its principles are more thoroughly understood in terrestrial plants in comparison to marine macrophytes including seaweeds. This also applies to the red seaweed *Asparagopsis taxiformis*, which has gained significant attention as it can be introduced to cattle diet to partially prevent methane biosynthesis in cattle rumen, which is due to its ability to store antimethanogenic brominated compounds. Consequently, there are increasing efforts to grow *Asparagopsis* at industrial scale, which will likely include land-based monoculture where *Asparagopsis* (and its associated microbiome) will undergo intensive interactions with conspecifics. The exudates of *Asparagopsis* are allelopathic towards a range of other species; however, there remains a lack of data on their autotoxic capacity. To address this research gap, we utilised a combined metabolomic and transcriptomic approach to investigate allelopathy in *Asparagopsis* and its associated microbiome. After a 5-day closed-system culture in seawater pre-conditioned with *Asparagopsis* exudates, the growth rates of *Asparagopsis* dramatically declined, particularly at low stocking densities. An exometabolomic analysis then revealed candidate molecules (e.g. myristic acid and geranylgeranyl diphosphate) that could become autotoxic upon accumulation above an autotoxicity threshold in a closed system. Further, *Asparagopsis* cultured in pre-conditioned water showed increased expression of various stress-related genes, including those involved in the production and scavenging of reactive oxygen species. Altogether, these results form a foundation for further research on chemical communication in *A. taxiformis* and provide valuable information for developments of land-based *Asparagopsis* aquaculture.

Optimisation and evaluation of an external trap as a mass trapping and monitoring device for small hive beetles.

Dr Umar Lubanga[1], Dr Daniel Geiberras[1], Dr R. Andrew Hayes[2], Dr Paul Cunningham[1]
[1]Agriculture Victoria, Bundoora, Melbourne, Australia, [2]University of Sunshine Coast, Sunshine Coast, Australia

The small hive beetle (SHB) (*Aethina tumida)* is a significant threat to Australia's honeybee industry, valued at $14.2 billion, and to native bees essential for pollination services in agriculture. SHB damages hives by consuming honey and brood while introducing a symbiotic yeast (*Kodamaea ohmeri*) that ferments hive contents into a "slime," compelling bees to abandon their hives. Current control methods, including internal traps, entomopathogenic fungi and chemical control, show promise but can disrupt hives and raise concerns about chemical toxicity. The situation may be exacerbated by climate change and the spread of varroa mites (*Varroa destructor),* recently detected in Australia*,* which is predicted to weaken and increase the vulnerability of hives to attack.

SHB are attracted by specific volatiles emitted by the hive and yeast, presenting an opportunity to develop external traps to intercept them before they invade. While honey fermentate has been used successfully as an attractant in external traps, its effectiveness diminishes within 3-4 days. The goal of our research was to develop an optimized honey fermentate-based synthetic lure with enhanced attractiveness and extended longevity for better SHB monitoring and potential for attract-and-kill traps.

In our study, we employed gas chromatography-mass spectrometry (GC-MS) to analyse how the volatile profile of the honey fermentate changed as fermentation progressed and identify which compounds were likely to be attractive to SHB. We conducted gravimetric experiments in a controlled wind tunnel to tune the release rates of 25 volatile compounds (identified from our laboratory studies and literature) from low-density polyethylene (LDPE) sachets of varying sizes and thicknesses. LDPE sachets significantly prolonged the release of most targeted volatiles, although adjustments were necessary for highly volatile compounds and less volatile compounds. Synthetic prototype lures were formulated based on these findings. However, honey fermentate outperformed all synthetic lures in field trials against SHB.

More brew than bruise: a nectar yeast transforms nectar scents of multiple wildflower species and shifts inflorescence volatiles more than nectar robbing

Valerie Martin[1], Dr Daniel Souto-Vilarós[2], Dr Caitlin Rering[3], Jade Stryker[4], Molly Long[5], Natalie Rodriguez[6], Dr Rebecca Irwin[7], Dr Robert Schaeffer[1]

[1]Department of Biology, Utah State University, River Heights, United States, [2]University of Utah, Salt Lake City, United States, [3]USDA-ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville, United States, [4]Department of Entomology, University of Georgia, Athens, United States, [5]Data Science Institute, University of Chicago, Chicago, United States, [6]Environmental Studies Department, Dartmouth College, Hanover, United States, [7]Department of Applied Ecology, North Carolina State University, Raleigh, United States

Microbes inhabiting floral nectar have the potential to reshape plant–pollinator interactions by altering floral traits used in forager decision-making. Floral microbes, including nectar-specialist yeasts dispersed by flower visitors, may modify floral scent directly through fermentation and metabolism of plant volatiles or indirectly by triggering plant physiological responses. Despite growing interest in floral microbiomes, few studies have characterized how microbes influence the volatile profiles of real floral nectar across multiple plant species, rather than synthetic nectar analogs. Using SPME and GC-MS, we found that inoculation with the nectar yeast, *Metschnikowia reukaufii*, altered nectar headspace volatiles in three wildflower species, with a core set of yeast-produced volatiles and reduced emissions of plant-produced nectar scents found across yeast-fermented nectar samples. In addition, little is known about how microbial cues scale up to alter whole-flower or inflorescence-level scent, particularly in comparison to the effects of floral antagonists such as nectar robbers. Using SPE and GC-MS, we found that microbial inoculation influenced inflorescence scent in three of four plant species, while robbing alone affected scent in only one. Our findings suggest that microbial colonization can have strong and species-specific effects on inflorescence-level scent, in most cases exceeding the influence of mechanical damage. By integrating microbial, chemical, and behavioral ecology, this work highlights nectar microbes as underappreciated agents capable of modifying floral signals and potentially influencing foraging decisions by flower visitors.

The mechanism of BPH17-mediated resistance to brown planthopper in rice

Wen-Po Chuang[1]
[1]National Taiwan University, Taipei City, Taiwan

Rice (*Oryza sativa* L.) is a major global crop and a crucial staple in Asia. The brown planthopper (BPH, *Nilaparvata lugens* Stål) is one of the most destructive insect pests of rice, causing hopperburn and severe yield losses during outbreaks. In addition to direct damage, BPH also transmits several rice viruses, making effective pest management vital. However, the emergence of new BPH biotypes has rendered many resistant rice varieties ineffective. Therefore, understanding the molecular mechanisms underlying resistance genes is essential. The BPH resistance gene *BPH17* was initially identified in the indica variety Rathu Heenati and consists of a cluster of lectin receptor kinase genes (*OsLecRK1*, *OsLecRK2*, and *OsLecRK3*). Despite its importance, the detailed mechanism of *BPH17*-mediated resistance remains largely unclear. In this study, we employed a near-isogenic line (NIL-*BPH17*) and its recurrent parent IR24 to investigate candidate components involved in BPH resistance. Proteomic analysis identified 192 differentially expressed proteins. NIL-*BPH17* showed elevated expression of proteins related to primary metabolism, defense responses (e.g., L-ascorbate peroxidases), and biosynthesis of the insect-toxic compound tricin. Phytohormone profiling revealed that NIL-*BPH17* maintained higher levels of IAA, ABA, JA, and JA-Ile regardless of BPH infestation. Additionally, NIL-*BPH17* accumulated higher levels of tricin and lignin, suggesting enhanced physical and chemical defenses. Further analysis confirmed that *BPH17* regulates defense responses through abscisic acid and jasmonic acid signaling pathways and contributes to increased tricin and lignin levels, both of which are important for structural resistance. Moreover, BPH infestation was found to influence tricin production in rice. This study advances our understanding of how *BPH17* confers resistance against BPH and provides insights into the molecular interaction between rice and its insect pests.

Landscape context influences natural enemy attraction to herbivore-induced plant volatiles

Yahel Ben-Zvi[1], Dr Cesar Rodriguez-Saona[1]
[1]Rutgers University, New Brunswick, United States

In response to herbivory, plants emit herbivore-induced plant volatiles (HIPVs), many of which attract the natural enemies of herbivores. One such compound, methyl salicylate (MeSA), has been used in agroecosystems to enhance biological control by recruiting natural enemies and reducing pest populations. In crops like the American cranberry (*Vaccinium macrocarpon*), MeSA has proven effective at attracting beneficial insects such as syrphid flies, lacewings, and coccinellid beetles. However, the broader agroecosystem context, including surrounding landscape composition, can strongly influence insect community dynamics. Adjacent natural habitats may provide critical resources and nesting sites for natural enemies, potentially modifying their responses to HIPVs. In this multi-year study, we investigated the interactive effects of MeSA and landscape composition on natural enemy communities across 50 georeferenced sites on three commercial cranberry farms in New Jersey (USA), which together represent approximately 70% of the state's cranberry production acreage. At each site, we deployed two yellow sticky traps—one baited with MeSA and one unbaited — to monitor natural enemies. Landscape composition was quantified within 100 m, 250 m, 500 m, and 1500 m buffers using the most recent land-use data from the New Jersey Department of Environmental Protection. Our results show that MeSA significantly increased the abundance and diversity of natural enemies. Moreover, landscape effects were more pronounced at larger spatial scales, and different land cover types influenced distinct natural enemy taxa. Notably, interactions between MeSA and landscape composition further shaped natural enemy responses. These findings offer new insights into how landscape context mediates the efficacy of HIPVs like MeSA, emphasizing the importance of incorporating landscape ecology into biological control strategies in agroecosystems.

Carbon dioxide drives oviposition in *Helicoverpa armigera*

Prof Yang Liu[1], Dr Qiuyan Chen[1,2], Prof Hetan Chang[2], Prof Guirong Wang[1,2]

[1]Institute Of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

As a vital constituent of the atmosphere, carbon dioxide (CO_2) holds a pivotal position in maintaining life on earth. CO_2 is also a powerful greenhouse gas, which have risen dramatically over the past century, leading to widespread ecological effects on plants and animals alike, including insects that serve vital roles in many food webs. Elevated atmospheric CO_2 is anticipated to affect insect biodiversity by influencing essential behaviors, although the mechanisms remain poorly understood. Here, we demonstrate that female *Helicoverpa armigera* use plant-emitted CO_2 as a primary cue for egg-laying, showing a preference for younger leaves with higher CO_2 gradients to enhance offspring survival. Elevated environmental CO_2 disrupts this preference, reducing females' attraction to optimal egg-laying sites. Employing genome editing tools, we assessed the CO_2 receptors in this species and proved three gustatory receptors—HarmGR1, HarmGR2, and HarmGR3—that form a trimeric complex in the sensory neurons of the labial palp organ, essential for CO_2 detection. These neurons project to the labial pit organ glomerulus (LPOG) in the antennal lobe, which mediates CO_2-responsive behavior. Genetic disruption of any of these receptors impairs CO_2 sensing and alters oviposition behavior. Our findings underscore the essential role of CO_2 in moth reproductive behavior and reveal that rising anthropogenic CO_2 levels may have significant ecological and agricultural repercussions.

Chemical and molecular mechanisms underlying the ovicidal defense of rice against phloem-feeding insects

Prof Dr Yonggen Lou[1], **Dr Quin Gao**[1]
[1]Zhejiang University, Hangzhou, China

In response to herbivore oviposition, plants perceive signals associated with herbivore eggs and initiate a defense-related signaling network mediated by mitogen-activated protein kinase (MPK) cascades and pathways mediated by jasmonic acid (JA), salicylic acid (SA), and ethylene (ET). The activated signaling network induces the production of defensive compounds including volatile and non-volatile compounds, which decrease the survival of herbivore eggs directly and indirectly by attracting natural enemies of herbivore eggs. However, which signals associated with herbivore eggs elicit this defense, which phytohormone pathways regulate it and which defensive compounds dominate it remains largely unknown. In this study, using rice, rice planthoppers, and their egg parasitoid, *Anagrus nilaparvatae*, as a research system, we explored the chemical and molecular mechanisms underlying the ovicidal defense of rice against planthoppers. We found that the small N-terminal subunit of vitellogenin (VgN) in planthopper eggs triggered strong defense responses in rice by activating defense-related signaling pathways. The defenses induced by VgN not only decreased the hatching rate of planthopper eggs, but also induced volatile emissions in plants, which rendered them attractive to the egg parasitoid. Moreover, impairing the ABA signalling pathway, especially the JA signalling pathway in rice enhanced the survival of planthopper eggs. In vitro bioassays revealed that some flavonoids, such as naringenin and sakuranetin, promote the ovicidal effect of benzyl benzoate, an ovicidal compound, on planthoppers. The results demonstrate that the rice ovicidal defense against planthoppers elicited by elicitors in planthopper eggs, such as VgN, was jointly regulated by JA and ABA signalling pathways and that benzyl benzoate, as well as some other compounds, such as naringenin and sakuranetin, contribute to the mortality of planthopper eggs.

Food-derived odors modulate chemical communication in the termite *Reticulitermes flavipes*

Dr Yuki Mitaka[1,2], Dr Anjel Helms[2], Dr Edward Vargo[2]
[1]Nagoya University, Nagoya, Japan, [2]Texas A&M University, College Station, USA

Division of labor in social insects is characterized by a sophisticated communication system based on semiochemicals. In many social insect species, workers cooperate to find new food sources and share information about food identity, quality, and location. However, semiochemicals that communicate food identity and recruit receivers to a particular food source have not been previously documented. In this study, we discovered that foraging workers of the Eastern subterranean termite *Reticulitermes flavipes* use a chemical blend comprising aggregation pheromone and food-derived odors to communicate food source information to their nestmates and attract nestmates who previously ate the same food type. Gas chromatography-mass spectrometry analyses (GC-MS) and two-choice bioassays demonstrated that workers were attracted to aggregation pheromone secreted by their nestmates. However, when pheromone was combined with food-derived volatiles, which varied according to the type of food eaten, workers were more strongly attracted and preferred the blend secreted from nestmates on the same food that they had previously experienced. Comparison of chemical profiles between the blends and extracts of food wood using GC-MS analysis also suggests that almost all of the food-dependent volatiles in the blend are produced in the worker body. Furthermore, RNA-seq of antennae of the workers on different foods revealed that change from one food to a different food triggers remarkable upregulated or downregulated expression of chemoreceptor genes, suggesting that the foraging workers specialize in the chemical environment of the food they are currently feeding on by tuning their own olfaction and gustation through changes in chemoreceptor expression profiles. Our study opens new avenues for understanding the chemical communication system and foraging behaviors of social insects that can be modulated by additional odor cues to transmit more complex information than simple intraspecific chemical communication.

Convergent and divergent functional evolution of terpene synthase enzymes in butterflies

Dr Zarley Rebholz[1]

[1]Indiana University Bloomington, Bloomington, United States

Terpenes are a prolific, structurally diverse class of specialized metabolites used by all kingdoms of life in myriad chemical interactions. While their biosynthesis in plants and microorganisms is well-characterized, knowledge of their biochemical origins in insects remains limited. My work, and that of others, has demonstrated that in insects, primary metabolic enzymes have been recruited for novel terpene synthase function. However, little is known about the protein substitutions and structural changes that underly the gain of terpene synthase function and changes in their specific product profiles in insects. We characterized entire terpene synthase gene families in phylogenetically diverse butterfly species with often overlapping specialized metabolite profiles. In this work, we found enzymes with both high protein sequence similarity and very divergent function in addition to those with low similarity and convergent enzyme function. Furthermore, terpene synthase function evolved at least twice in butterflies within different protein families. Within these two evolutionarily distinct terpene synthase families, we find both convergence of products between the two families and functional divergence within the same families. We also find that the enzymes vary greatly in their degree of product specificity, with some producing a single terpene while others produce many. These functional data offer a unique opportunity to probe the mechanistic determinants of catalytic function in these novel enzyme families through the construction of mutational libraries.

An egg parasitoid assesses host egg quality from afar using oviposition-induced plant volatiles

Zhiqiang Tian[1]

[1]Henan University, Kaifeng, China

Parasitoids of herbivores exploit inducible plant volatiles to find plants with potential hosts from a distance, whereas at close range they typically use host-derived cues to pinpoint and identify suitable hosts. Here we show, however, that the egg parasitoid *Trichogramma japonicum* assesses host egg quality far more efficiently by remotely using oviposition-induced plant volatiles (OIPVs). In olfactometer assays, female *T. japonicum* wasps showed a strong preference for the odor of rice plants carrying 2-d-old eggs of the rice leaf folder *Cnaphalocrocis medinalis*, over the odor of plants with younger or older eggs, a preference that correlated with higher parasitism rates. In accordance with the preference-performance hypothesis, the offspring of *T. japonicum* showed superior performance in 2-d-old eggs, including shorter development times and higher eclosion rates. Volatile analysis revealed significantly increased emission of D-limonene and α-pinene from plants with 2-day-old eggs, and we found that synthetic versions of these two monoterpenes were highly attractive to the wasp. Knockout rice plants deficient in D-limonene and α-pinene synthesis lost their appeal to the wasps, but attraction could be restored by dispensing synthetic versions of the attractants alongside the knockouts. These findings reveal a novel and highly efficient host-assessment strategy in egg parasitoids, whereby plant-provided cues inform the wasps about host quality from afar. This discovery is illustrative of the clever strategies that have evolved out of plant-insect interactions and offers fresh ideas to optimally exploit plant traits for biocontrol approaches against *C. medinalis*, a major rice pest.

Posters

In alphabetical order (by first name of presenter)

Associative learning of host-associated volatiles in a Drosophilid parasitoid

Dr Santiago Masagué[1], Federico Triñanes[1], Belén Puch[1], Dr Gerardo de la Vega[2], **Prof Andrés González**[1]

[1]Chemical Ecology Group. Faculty of Chemistry. Universidad de la República., Montevideo, Uruguay, [2]Insect Population Ecology Group, Institute for Forest and Agricultural Research, INTA/CONICET, Bariloche, Argentina

Parasitoids of frugivore insects may utilize fruit volatiles as host-associated chemical cues. We have previously shown that fruits infested by *Drosophila suzukii* (Diptera: Drosophilidae), an invasive worldwide pest of fine fruit crops, emit volatiles that differ from non-infested fruit. While these volatiles may not be attractive to naïve parasitoids, they may be learned associatively from previous experience, thus facilitating host finding. In this study, we tested the associative learning ability of *Trichopria anastrephae* (Hymenoptera: Diapriidae), a pupal parasitoid of drosophilid species. Female wasps were exposed to volatiles from strawberry pulp as conditioned stimulus, along with pupae of *D. suzukii* as unconditioned stimulus. The wasps were subsequentially tested in olfactometer bioassays for attraction to the volatile stimulus. Control treatments included naïve female wasps, as well as wasps exposed to either the conditioned or unconditioned stimuli, and wasps exposed asynchronically to both stimuli. Experienced wasps subjected to associative learning treatment were significantly attracted to strawberry pulp volatiles in olfactometer tests. Naïve wasps were not attracted, nor were wasps from all other control groups, thus indicating learning capacity for host-associated volatiles by *T. anastrephae* female wasps. Chemical analysis of the fruit pulp volatiles showed typical fruit esters such as ethyl hexanoate, which we have shown to trigger electroantennogram responses in *T. anastrephae* female antennae. As preliminary proof of concept of the application potential of *T. anastrephae* learning ability in biological control, we tested the control capacity of the wasp in *D. suzukii* infested strawberry guava (*Psidium cattleianum*, Myrtaceae), and the attraction of female wasps to volatiles from infested fruit. While the wasps reduced fly emergence in more than 98%, volatiles from infested guava were not attractive, showing promise for associative learning as a tool for increasing host finding efficiency. The results are discussed in the context of targeted inundative biocontrol purposes.

Pheromone candidates of the endangered Katipō spider (*Latrodectus katipo*)

Andrew Twidle[1], Nigel Joyce[1], Lisa Pilkington[2,3], Thomas Sullivan[1], Meikura Arahanga[4], Nicola Sullivan[1,5], Tara Murray[6], Cor Vink[5]

[1]The New Zealand Institute for Plant and Food Research Limited, Lincoln, New Zealand, [2]University of Auckland, Auckland, New Zealand, [3]Te Pūnaha Matatini, Auckland, New Zealand, [4]Bioprotection Aotearoa, Lincoln, New Zealand, [5]Lincoln University, Lincoln, New Zealand, [6]Department of Conservation, Dunedin, New Zealand

Katipō spiders (*Latrodectus katipo*) are the native widow spider of Aotearoa New Zealand. They inhabit coastal sand dunes, with their webs built amongst the native sand-binding sedges and grasses. Destruction of sand dune habitat by human activities along with the invasion of exotic plants has led to population decline of these spiders, which are now classified as 'At Risk Declining' and are protected organisms under local conservation law. With a small sample of katipō spiders held by Lincoln University for breeding and genetics studies, we took the opportunity to identify potential pheromone candidates on the silk of these spiders. Preliminary investigations of virgin female katipō silk by solid phase microextraction versus sub-adult female silk and virgin male silk showed four compounds abundant in the headspace of the mature virgin female silk. These compounds were identified as acetic acid, 2-methylpropanioic acid, (*S*)-2-methylbutanoic acid and 2-pyrrolidone by comparison with synthetic standards via gas chromatography coupled with mass spectrometry. Further analysis of the silk by solvent extraction and liquid chromatography coupled with mass spectrometry indicated two contact pheromone candidates *N*-3-methylbutanoyl-*O*-(*S*)-2-methylbutanoyl-*L*-serine and *N*-3-methylbutanoyl-*O*-2-methylpropanoyl-*L*-serine, both confirmed via comparison with synthetic standards. The identification of these compounds allows for the development of possible live capture lures for population monitoring and increases the knowledge of pheromone chemistry within the *Latrodectus* genus.

Both host plant volatiles and sex pheromones are required for finding mates in the codling moth

Dr Anna Laura Erdei[1], Dr Maria Sousa, Dr Francisco Gonzalez, Prof Marie Bengtsson, Prof Peter Witzgall

[1]Swedish University of Agricultural Sciences, Department of Plant Protection Biology, Alnarp, Sweden

Host plant shifts are key drivers of insect speciation, even in the absence of geographical barriers. In phytophagous insects, finding a mate and locating a host both rely on pheromones and kairomones, which are subject to sexual and natural selection, respectively. While it is recognised that the interaction between sexual signals and habitat cues affects reproductive isolation, the underlying mechanisms are not fully understood. We investigated the potential impact of habitat cues on the codling moth (*Cydia pomonella*), a specialized pest of apple and other rosaceous fruits. Our results demonstrate that the male response to the sex pheromone codlemone is dependent on the presence of pear ester, a kairomone emitted by host plant fruits. In non-host environments, such as birch, male attraction to the pheromone alone is greatly reduced; however, this is fully restored when supplemented with pear ester. Our study demonstrates how host-associated cues can modulate pheromone elicited behaviors, providing insight into the connection between host discrimination and sexual communication.

Effects of xenobiotics on associative learning and peripheral olfaction of honeybees

Prof Carmen Rossini[1], Guillermo Bragunde[1,2], Carolina Sosa[1,2], Prof Andrés González[1], Dr Belén Branchiccela[3], Pablo Juri[4], Dr Ciro Invernizzi[5]

[1]Laboratorio de Ecología Química, Facultad de Química, UDELAR, Uruguay, Montevideo, Uruguay, [2]Programa de Posgrados de la Facultad de Química, UdelaR, Montevideo, Uruguay, [3]Sección Apicultura, Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay, [4]Animales de granja, Facultad de Veterinaria, UdelaR, Montevideo, Uruguay, [5]Sección Etología, Facultad de Ciencias, UdelaR, Montevideo, Uruguay

Honeybees (*Apis mellifera*, Hymenoptera: Apidae) rely on chemical cues to locate food sources, particularly floral volatiles, which they can detect via antennal olfactory receptors and subsequently associate with floral rewards through learning. One key behavioral mechanism involved is the innate proboscis extension reflex (PER), wherein bees extend their proboscis upon perceiving a rewarding stimulus such as sucrose. This reflex can be conditioned to respond to external stimuli, such as floral odors, thereby serving as a proxy to evaluate olfactory learning and memory.

To assess potential sublethal effects of xenobiotics on olfactory function and learning in honeybees, two varroacides were tested: thymol (commonly used to treat Varroosis, a major parasitic disease) and an essential oil (EOEb) extracted from *Eupatorium buniifolium* (Asteraceae). Peripheral olfactory responses were analyzed via electroantennography (EAG), and olfactory learning was assessed through classical PER conditioning in bees exposed to these substances.

The study included laboratory and in-hive trials across different age groups. No significant differences in EAG responses were found among treatment groups (Laboratory: 9-10 days old: thymol N=9, EOEb N=8, control N=12; 14-15 days old: thymol N=4, EOEb N=9, control N=19. In-hive: 14-17 days old: thymol N=12, EOEb N=12, control N=14; ANOVA Split-plot, $P > 0.05$). Similarly, associative learning and memory performance were not significantly affected by exposure to either compound (Laboratory: 5–6 days old: thymol N=57, EOEb N=63, control N=78; 12–13 days old: thymol N=55, EOEb N=48, control N=51. In-hive: 14–17 days old: thymol N=36, EOEb N=30, control N=31; ANOVA with post-hoc tests, $P > 0.05$).

These findings indicate that thymol and EOEb do not impair peripheral olfactory perception or associative learning, supporting their safe use as varroacides without compromising pollination behavior in honeybees.

Spatial repellent and attractant effects of natural products against *Halyomorpha halys* (Stål) and *Plautia stali* Scott (Hemiptera: Pentatomidae): electrophysiological responses and field tests

Da Hyeon Yu[1], Ji Hye Oh[1], Seon Ah Jeong[2], Gwang Hyun Roh[1,2]

[1]Department of Plant Medicine, Gyeongsang National University, Jinju, South Korea, [2]Institute of Agriculture & Life Science, Gyeongsang National University, Jinju, South Korea

Halyomorpha halys and *Plautia stali* are native to Northeast Asia and are major economic pests of leguminous crops and fruits. The increased use of insecticides for pest control to prevent crop damage has raised concerns regarding pesticide resistance and environmental contamination. Therefore, recognizing the need for alternative pest management strategies, we conducted the behavioral responses of natural products using electroantennogram (EAG) recordings and field tests. In EAG screening using fifteen natural compounds, four compounds (*A*, *B*, *K*, and *M*) exhibited significant EAG responses at a 1 mg dose in both male and female adults of *H. halys* and *P. stali*. In subsequent field tests, we evaluated the behavioral responses of the EAG-active compounds (500 mg dose) on *H. halys* and *P. stali* to the aggregation pheromone, methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT), compared with MDT alone (positive control) and blank control (negative control). Compound *A* decreased trap catches of *H. halys* by 30.7%, whereas trap catches of *P. stali* increased by 45.6% relative to the positive control. In addition, compound *B* significantly reduced trap catches of *H. halys* by 47.2% compared to the positive control. Therefore, our findings suggest that natural products have considerable potential as commercial repellents or attractants and provide useful information for the development of future control strategies using a push–pull approach in the integrated management of *H. halys* and *P. stali*.

The New Zealand floral volatilome

Dr Flore Mas[1], Lee-Anne Manning[1], Tom Moore[1], Brian Cutting[1], Lisa Evans[1]
[1]The New Zealand Plant and Food Research Institute, Christchurch Mail Centre, New Zealand

For insect pollinators searching for floral food resources in the landscape, floral scent may be the most prevalent long-distance sensory cue. Across the large-scale monocultural crops and intertwined patches of diversified native or wild exotic plants, complex blends of floral volatile organic compounds (FVOCs) are emitted. To better understand this complex floral volatile landscape, i.e., the floral volatilome, we sampled 113 plant species at their blooming stage over three seasons, across different landscapes on the South and North Islands of Aotearoa-New Zealand, representing 23 orders and 42 plant families.

Sampled floral plant species spanned from major horticultural crops cultivated in Aotearoa-New Zealand, such as fruit crops (kiwifruit, apple, avocado, plum, cherry, apricot, and peach), arable crops (Brassicaceae, carrots, radish), or ornamentals, including invasive weeds representing 79 exotic species and 34 native plants. *In situ* headspace volatile collections were performed for each species, and solvent extractions were processed via gas chromatography coupled with mass-spectrum analysis. About three hundred compounds were tentatively identified, from 458 detected.

We identified the most common (frequent) and prevalent (relative proportion) FVOCs across Aotearoa-New Zealand flora and among the four different agricultural regions. We compared the compositional chemical diversity in FVOCs among native, exotic crop, or wild plants and discussed this in relation to their spatio-temporal emission as well as known insect visitors' preference.

We found that D-limonene, caryophyllene, benzaldehyde, and cis-3-hexenyl acetate were the most common floral compounds across all plants. Native plants were dominated by terpenoids, in contrast to exotic crops, which produce more fatty acid derivative compounds. Richness, evenness, and disparity in FVOCs were significantly different between native and exotic plants. Knowledge of the differences in floral chemical composition, including spatio-temporal variation, can inform landscape management to maintain a diversified floral volatilome that can attract pollinators while repelling herbivores.

Combining semiochemicals with ecological intensification to harness aphid biocontrol in apple crops

Dr Gaëlle Ramiaranjatovo[1], Teun Dekker[1,2], Tibebe Biasazin[1]

[1]Chemical Ecology Unit, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Box 102, SE-230 53, Alnarp, Sweden, [2]National Species Management, Kosterweg 1W, 6702 AA, Wageningen, Netherlands

Effective management of aphids, particularly *Dysaphis plantaginea*, in apple orchards remains challenging when reducing chemical inputs to preserve biodiversity and ecosystem functions. In aphid control, ecological intensification involving flower strips has been promoted to support natural enemies, thus enhancing ecosystem services. However, aphid-ant mutualism (myrmecophily) complicates this approach, as ants aggressively defend aphid colonies, reducing predator effectiveness. Recent studies demonstrate that diverting ants with honeydew mimic solution effectively allows predators to control aphids. Although diverting ants enhances predator abundance, their response is often delayed compared to the exponential growth of aphid, suggesting the need for early-season predator attraction. This study explores the combined effectiveness of flower strip management, ant diversion and semiochemical-based predator attraction to optimize aphid control. Two field trials were conducted in apple orchards. Firstly, we investigated the impact of flower strip and honeydew mimic applications. Predator populations increased significantly in non-mowed flower strips receiving late honeydew mimic treatments. This effect likely results from an increase in aphid abundance due to delayed ant diversion, leading to a higher concentration of aphid volatile emissions, which attract predators. Secondly, we evaluated predator responses to general insect lures applied directly to apple trees. These lures significantly attracted predators not only to treated trees but also to neighbouring ones, indicating broad spatial efficacy. Furthermore, to identify specific cues driving predator attraction, antennal responses of four predator species to volatiles from aphid-infested apple leaves were analysed using gas chromatography-electroantennography detection. Despite distantly related, all predator species responded to a shared subset of volatile compounds. A synthetic blend of these bioactive compounds is currently tested in laboratory olfactometer and will be evaluated under field conditions as part of an integrated approach combining targeted predator attractant, ant diversion and flower strip management. This strategy will ensure a sustainable and timely aphid control in apple orchards.

Sex pheromone of *Carposina coreana* (Lepidoptera: Carposinidae), a key pest of traditional Chinese medicinal plant *Cornus officinalis*: Component identification, field evaluation, and trap optimization

Han Li[1], Haili Qiao, Pengfei Lu
[1]Beijing Forestry University, Beijing, China

As an important Chinese medicinal herb, *Cornus officinalis* not only has medicinal value, but also relates to the economic sources of multiple planting areas and rural areas. *Carposina coreana* Kim (Lepidoptera: Carposinidae) is a severe fruit-boring pest that inflicts devastating damage on *C. officinalis*. There is an urgent need to develop attractants to prevent and control this pest. In order to develop a sex pheromone attractant suitable for trapping *C. coreana*, this study identified and screened (*Z*)-7-eicosene-11-one and (*Z*)-7-tricosene as active substances to attract male moths for locating females by Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Electroantennogram Detection (GC-EAD) analysis. Then, the optimal concentration ratio of (Z)-7-eicosene-11-one: (Z)-7-tricosene =1000 μg: 500 μg was determined by studying the influence of 7 factors, such as different doses, core and trap types, pasteboard trap of different area, color, hanging height and hanging distance, on the amount of *C. coreana* captured. And the best scheme for field trapping is to hang green triangle-shaped traps 1.5-2.0 meters above the ground and 15 to 20 meters apart. This study provided technical support for the monitoring and control of *C. coreana*, achieving a complete prevention and control method from scratch.

Transcriptional regulation of γ-octalactone induced expression of odorant binding protein 83g-2 in *Bactrocera dorsalis* (Hendel)

Prof Hongbo Jiang[1], Dr Xiaofeng Chen[2]
[1]Southwest University, Beibei, China, [2]Yunnan Agriculture University, Kunming, China

Odorant binding proteins (OBPs) are majar components of the insect olfactory system, which is involved in the detection of environmental chemical cues. It seems that the expression alterations of OBPs induced by odorant exposure are conserved in many species. It presents an intriguing initial screening tool when searching for novel OBP-odorant interaction pairs. However, the transcriptional regulation mechanism that causes altered expression of OBPs still remains unclear. Here, we reported a case study on the transcriptional regulation mechanism of an invasive species, *Bactrocera dorsalis* upon γ-octalactone induction. The γ-octalactone has been proven to be a host volatile that strongly attracts its females to lay eggs. In the current study, we identified OBP83g-2 as a key OBP was involved in γ-octalactone perception by *in vitro* and *in vivo* functional assay. In addition, we found transcription factor Adf-1-like positively regulated expression of *OBP83g-2* upon γ-octalactone induction through expression pattern analysis, dual-luciferase reporter system, EMSA and RNAi. Based on the results, we proposed a model for the transcriptional regulatory mechanism of OBP gene in *B. dorsalis*. Our data not only highlights the significant role of OBP83g-2 in γ-octalactone mediated oviposition behavior, but also provides a theoretical foundation for a deeper understanding of the transcriptional regulation of OBPs triggered by external odorants in insects.

Olfactory adaptations underlying ecological divergence in *Drosophila melanogaster* and *Drosophila suzukii*

Hyemin Kim, YeongHo Kim[1], Gwang Hyun Roh[2], **Young Ho Kim**[1,3]

[1]Department of Ecological Sciences, Sangju-si, Republic of Korea, [2]Department of Plant Medicine and Institute of Agriculture & Life Sciences, Jinju-si, Republic of Korea, [3]Research Institute of Invertebrate Vector, Sangju-si, Republic of Korea

The closely related species *Drosophila melanogaster*, which typically inhabits fermenting fruits, and *Drosophila suzukii*, which prefers fresh, ripening fruits, exhibit distinct ecological preferences. These ecological differences suggest potential divergence in olfactory adaptations. To investigate this, we conducted a comparative study using both behavioral and physiological approaches to examine species-specific responses to key fruit-derived volatile compounds. Y-tube olfactometer assays were employed to evaluate behavioral preferences for 2-phenylethanol, ethanol, and acetic acid across a range of concentrations. Electroantennogram (EAG) recordings were subsequently performed to assess antennal sensitivity to the same volatiles. Both assays were conducted on males and females of each species, considering mating status as an additional variable. By comparing behavioral responses and antennal sensitivity between the two species, this study aims to elucidate how ecological specialization is reflected in olfactory processing. Our findings are expected to enhance the understanding of host selection mechanisms and inform the development of environmentally sustainable pest control strategies, particularly targeting *D. suzukii*, a pest of significant agricultural concern.

Bird vs cats…paw: Why don't brown honeyeaters like catspaw nectar?

Isabella Butler[1], Bjorn Bohman[1], Siegy Krauss[2]

[1]University Of Western Australia, Rockingham, Australia, [2]Department of Biodiversity Conservation and Attractions, Perth, Australia

Previous studies have found catspaw (*Anigozanthos humulis*) was repeatedly only visited by honeyeater the western spinebill (*Acanthorhynchus superciliosus*), even though Brown honeyeaters (*Lichmera indistincta*) were abundantly found in the reserve. Catspaw produces large amounts of nectar and is well known for the role of birds in its pollination. This led us to asking is there something in catspaw nectar that brown honeyeaters find distasteful? Brown honeyeaters are known to drink from Pilbara foxglove (*Quoya zonalis*) and a woollybush (*Adenanthos cygnorum*) so by comparing the nectar composition between catspaw *Q. zonalis* and woollybush we hypothesise there is something in catspaw nectar brown honeyeaters find distasteful. Other species of *Anigozanthos* such as *Anigozanthos pulcherrimus* and *Anigozanthos flavidus* were also included as a comparison of catspaw to other species in its genus. Nectar is an important food reward for pollinators as it contains sugars and amino acids that provide them with nutrients and energy. Nectar is composed largely of sugars such as the disaccharide sucrose a complex sugar and the monosaccharides fructose and glucose. Due to sucrose's complexity some birds cannot digest it. To test our hypothesis nectar samples were collected from the five study species followed by nectar derivatisations and analysis with Gas Chromatography Mass Spectrometry. The results of this study found catspaw had no measurable amounts of sucrose. This was consistent across the genus. Pilbara foxglove and woollybush on the contrary largely consisted of sucrose. This study has important implications for understanding bird pollination in Western Australia. With the rise of interest from the public to have native gardens in residential areas to provide resources for native pollinators.

Antennal olfactory receptor neurons for host plant volatiles and sex pheromone in *Dioryctria abietella* (Denis & Schiffermüller, 1775) (Lepidoptera: Pyralidae)

Jaewoo Lee[1], Il-Kwon Park[1]
[1]Seoul National University, Seoul, South Korea

Dioryctria abietella (Denis & Schiffermüller, 1775) (Lepidoptera: Pyralidae) is considered a major pest affecting seed production of various *Pinus* species in the Republic of Korea. In this study, we investigated the response profiles of antennal olfactory receptor neurons (ORNs) in adult *D. abietella* to 15 host plant volatiles and two sex pheromone components using the single sensillum recording (SSR) technique. We identified seven types of olfactory sensilla along with various types of ORNs in the antennae of *D. abietella*. Of these seven olfactory sensillum types, two were present in both sexes, whereas five were sex-specific. The most abundant type contained one or two ORNs, which exhibited high specificity for *α*-pinene enantiomers and *β*-pinene, with minor responsiveness to geranyl acetate and (−)-bornyl acetate. Another common sensillum type contained one specialized ORN responsive to *β*-caryophyllene and *α*-humulene. In females, a pheromone-sensitive sensillum type contained two highly specialized ORNs, one responsive to (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene and the other to (9*Z*,11*E*)-tetradecadienyl acetate. Another female-specific sensilla type responded to *α*-phellandrene, terpinolene, and (±)-limonene. In males, a pheromone-sensitive sensillum type contained two ORNs, of which only one responded to (9*Z*,11*E*)-tetradecadienyl acetate. Similarly, a male-specific sensillum type also contained two ORNs, but only one responded exclusively to geranyl acetate. Another male-specific sensilla type contained two ORNs, with one responsive to *α*-pinene enantiomers and *β*-pinene, while the other was responsive to (*E*)-2-hexenal. These findings suggest that *D. abietella* possesses a set of specialized ORNs for host plant volatiles and sex pheromone compounds, providing initial understanding into its olfactory communication system. However, further experiments are necessary to comprehensively identify ORNs of this species.

Identification of pheromone binding protein genes in *Dioryctria abietella* (Denis & Schiffermüller) (Lepidoptera: Pyralidae)

Ji Hwan Han, Jae Woo Lee[1], Myung Hee Jung[3], Sung Chan Lee[4], Il Kwon Park[1,2]

[1]Department of Agriculture, Forest, and Bioresources, College of Agriculture and Life Sciences, Seoul National University, Seoul, South Korea, [2]Research Institute of Agriculture and Life Science, College of Agriculture and Life Sciences, Seoul National University, Seoul, South Korea, [3]Bioinformatics Group, R&D Center, Insilicogen, Inc., Republic of Korea, Yongin-si, South Korea, [4]Forest Entomology and Pathology Division, National Institute of Forest Science, Seoul, South Korea

Dioryctria abietella is a major cone-boring pest of coniferous forests across Asia and Europe and it has recently been reported to reduce seed yields in conifer seed orchard. Despite its ecological importance, the molecular mechanisms underlying pheromone detection in this species remain poorly understood. This study aimed to identify pheromone-binding proteins (PBPs) responsive to sex pheromones and to infer their roles in pheromone perception based on gene expression patterns and predicted binding affinity.

De novo assembly of antennal transcriptomes was performed to investigate the molecular basis of pheromone detection, with a particular focus on PBPs. In total, 181 olfaction-related genes were identified, including odorant receptors (ORs), odorant-binding proteins (OBPs), chemosensory proteins (CSPs), and PBPs. Five candidate PBPs were selected based on differentially expressed genes (DEGs) analysis, gene ontology (GO) enrichment, and BLASTP homology with known lepidopteran PBPs. Multiple sequence alignment revealed conserved cysteine motifs and signal peptides, and their three-dimensional protein structures were computationally predicted. Binding affinities of the candidate PBPs were assessed through molecular docking with two pheromone components, 9*Z*,11*E*-tetradecadienyl acetate and C25 pentaene.

Expression patterns in response to pheromone exposure were analyzed using quantitative real-time PCR. Three PBPs (DabiPBP2, DabiPBP3, and DabiPBP4) showed significantly upregulated expression in male heads following exposure, indicating a role in male-specific pheromone detection. DabiPBP1, which showed the highest predicted binding affinity to both sex pheromone components, exhibited slightly higher expression in female bodies than in male heads, indicating a potential role in pheromone autodetection.

These findings demonstrate sex- and tissue-specific expression of PBPs in *D. abietella*, supporting functional divergence among the PBPs involved in pheromone detection. Understanding the molecular mechanisms of pheromone detection provides a foundation for developing species-specific and environmentally sustainable pest management strategies targeting olfactory communication.

Morphology and distribution of olfactory sensilla on the antennae of the bronze beetle, *Eucolaspis brunnea*

JiAe Kim[1], Hyun-woo Oh[1], Kunhyang Park[2], Kye Chung Park[3]

[1,2] Department of Core Facility Management Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, 34141, Daejeon, Republic of Korea [3] New Zealand Institute for Plant and Food Research, New Zealand

The morphology and distribution of olfactory sensilla on the antennae of the bronze beetle (*Eucolaspis brunnea*), a key pest of apple trees in New Zealand, were examined using scanning electron microscopy, to better understand the species' olfactory communication system. The antennae, composed of scape, pedicel, and nine flagellomeres, showed a significantly higher density of sensilla on the distal five flagellomeres compared to the others. Each of these terminal segments exhibited a distinct distal region containing 20 – 30 short basiconic sensilla of various types. The presence of numerous nanoscale pores on the surface of these basiconic sensilla suggests a primary function in olfaction. In addition, other regions of antennae contained various long trichoid sensilla, many of which also displayed multiple nanoscale pores, further supporting an olfactory role. At the tip of the terminal flagellomere, a cluster of short basiconic sensilla was observed, lacking multiple surface pores but exhibiting terminal pores, indicating their gustatory sensory function. These findings suggest that *E. brunnea* possesses a well-developed chemosensory system, supporting the potential use of semiochemical-based tools for the management of this species.

Endophytic entomopathogenic fungus, individually and in combination with rhizobacteria, enhances resistance in wild and cultivated tomatoes to *Tuta absoluta*

Paolo Salazar-Mendoza[1], Diego M Magalhães[1], Marvin Pec[1], Kamila EX Azevedo[1], Italo Delalibera Jr[1], **José Maurício Bento**[1]

[1]University of Sao Paulo -USP/ESALQ, Department of Entomology and Acarology, Brazil

Several beneficial microbes have been shown to activate defensive mechanisms in plants, enhancing their resistance against herbivores. However, it remains unclear whether different beneficial microbes can synergize to improve defenses in wild plants, similar to their effects in cultivated plants against insect pests. Here, we investigated the effect of the endophytic entomopathogenic fungus *Metarhizium robertsii,* both individually and in combination with the growth-promoting rhizobacteria *Bacillus amyloliquefaciens,* on plant growth and volatile emissions in the cultivated *Solanum lycopersicum* and its two wild parents, *S. pimpinellifolium* and *S. habrochaites*. We also assessed the ovipositional preference of the destructive pest *Tuta absoluta* and the olfactory responses of its natural enemy, the mirid predator *Macrolophus basicornis,* toward these treatments across each tomato species. Both wild and cultivated plants inoculated with *M. robertsii* exhibited enhanced growth and emitted higher levels of specific volatile compounds than non-inoculated plants. Furthermore, *T. absoluta* females laid fewer eggs on *S. lycopersicum* and *S. habrochaites* inoculated with *M. robertsii*. Additionally, the inoculation of this beneficial fungus resulted in increased attraction of *M. basicornis* to the volatiles of *S. lycopersicum* and *S. pimpinellifolium*. Interestingly, the combined inoculation of *B. amyloliquefaciens* and *M. robertsii* generally did not yield an additive effect on volatile emissions and resistance against *T. absoluta* compared to *M. robertsii* alone in wild and cultivated tomato plants. These results suggest that the inoculation of *M. robertsii* could be a promising tool for protecting tomato plants against *T. absoluta* and enhancing the attraction of its natural enemy, *M. basicornis*.

When the fungus takes control: Manipulation of insect sexual signaling and mate choice by *Fusarium verticillioides*

Amanda C. Túler[1], Arodi P. Favaris[1], Flávia P. Franco[1], **José Maurício Bento**[1]
[1]University of Sao Paulo, Department of Entomology and Acarology, Brazil

Microorganisms associated with insects have been shown to influence host behavior in manifold ways, often enhancing their own dispersal potential through interactions with the insect host. In this study, we demonstrate that contamination of the sugarcane borer, *Diatraea saccharalis* by the phytopathogen *Fusarium verticillioides* alters specific aspects of the insect's sexual behavior. While female calling rhythm and latency remained unaffected, mating preferences were significantly influenced. In flight tunnel behavioral assays, males exhibited increased attraction to sex pheromone-like odors released by contaminated females compared to non-contaminated individuals. Moreover, although non-contaminated females showed no discernible mating preference, contaminated females mated more frequently with contaminated males, suggesting that mate choice was modulated by infection status. Chemical analysis of gland extracts from contaminated females revealed elevated levels of Z9-16:Ald, a minor component of the species' sex pheromone blend. These findings highlight the capacity of plant-associated pathogenic fungi to modulate insect sexual behavior, particularly by influencing mate choice dynamics. This study provides mechanistic insight into how microbial interactions within a multitrophic system can shape behavioral outcomes relevant to insect reproductive ecology, and offers a clear example of a fungus modulating mating preferences in *D. saccharalis*.

Rearing history, larval density, and ontogeny affect volatile- and light-mediated diel hiding behavior in *Mythimna unipuncta*

Kaori Shiojiri[2], **Junji Takabayashi**[1], Jeremy McNeil[3]
[1]Kyoto University, Otsu, Japan, [2]Ryukoku University, Otsu, Japan, [3]Western University, London, Canada

Earlier work showed that plant volatiles and light jointly shape the diel hiding behavior of *Mythimna unipuncta* larvae. We now tested how rearing history, larval density, and developmental stage modulated this response under controlled laboratory conditions. Third-instar larvae from a newly established field-derived colony hid more frequently in the presence of maize volatiles, regardless of light regime, whereas third instars from a long-term laboratory colony (>5 years) showed no volatile-induced change. Density during the second instar also altered responsiveness: low-density cohorts from the new colony increased hiding in response to volatiles, whereas high-density cohorts decreased it. Developmental stage further conditioned the reaction. Second instars from the new colony consistently increased hiding when exposed to volatiles, independent of light. By contrast, fifth–sixth instars hid more under light plus volatiles but less under darkness plus volatiles, indicating an interaction between age, volatiles, and photoperiod. Our results demonstrated that behavioral plasticity in *M. unipuncta* was shaped not only by immediate environmental cues but also by the larvae's rearing history and social context. This research provides novel insights into the behavioral adaptations underlying the diel activity patterns of herbivorous insects.

Temporal variations in the floral volatile emissions of cocoa and the abundance of its pollinators

Mr Kris Lord Santos[1], Prof Dr Divina Amalin[1], Prof Dr Jose Isagani Janairo[1]
[1]De La Salle University, Manila, Philippines

Cocoa (*Theobroma cacao* L.) is known to be exclusively pollinated by insects particularly Ceratopogonid midges. Pollination is an important limiting factor in production, but little is known still about the complex interactions between cocoa and its pollinators. For instance, cocoa flowers are only receptive for one day and are pollinated by midges only during the day. This short window suggests some mechanisms used by the plant to attract midges for pollination. Here we assessed the potential correlation between floral volatile emission and the abundance of pollinators in a cacao orchard. Floral volatiles were collected by SPME at different time intervals during the day, and analyzed by GC-MS. On the other hand, abundance of floral visitors at different time intervals was determined by trapping using an insect glue. Results showed that floral volatile emission and abundance of floral visitors both vary significantly throughout the day. This observation suggests some correlation between floral volatile emission and pollinator abundance and highlights the need for further studies aimed at studying the chemical ecology of cacao pollination.

Comparative transcriptomic analysis of chemosensory recognition in *Frankliniella occidentalis* and *Thrips palmi* Karny

Kun Hyang Park[1], Dr Dae-Soo Kim[2], Ms Jiae Kim[1], Dr Sugi Lee[2], Ms DoYoung Kwak[2], Dr Hyun-woo Oh[1]

[1]KRIBB, Yuseong-gu, Daejeon, South Korea, [2]Department of Digital Bio Technology Innovation, Korea Research Institute of Bioscience & Biotechnology (KRIBB), Yuseong-gu, Daejeon, South Korea

The western flower thrips (*Frankliniella occidentalis*) and the melon thrips (*Thrips palmi*) exhibit distinct host plant preferences, suggesting potential differences in their olfactory mechanisms. To investigate whether variations in odor perception contribute to these differences, we performed transcriptome sequencing and analysis of *T. palmi*. Through RNA-seq data generation and de novo assembly, we identified 44,751 genes in *T. palmi*. For *F. occidentalis,* 48,889 genes were obtained from publicly available data in NCBI, and a comparative analysis was conducted between the two species. Focusing on odorant-binding proteins (OBPs), which play a key role in insect olfaction, we identified and compared OBP gene families across both species. To further explore the chemosensory system in thrips, we conducted antennal transcriptome analysis of *F. occidentalis* and whole-body RNA analysis of *Frankliniella intonsa*. Comparative analysis revealed nine orthologous OBPs and four chemosensory proteins (CSPs) shared between the species. Among the four CSPs, three showed high sequence similarity, while one exhibited low similarity, particularly in the insect pheromone-binding protein domain. Similarly, of the nine OBPs identified, four displayed high sequence similarity, whereas the remaining five showed notable sequence divergence between the species. These findings suggest that differences in OBPs and CSPs may reflect species-specific evolutionary adaptations, potentially shaped by differing host plant preferences and feeding competition.

Species-specific cuticular hydrocarbons as potential sex pheromones in Chrysidid wasps

Dr Laima Blažytė-Čereškienė[1], Dr Sandra Radžiutė[1], Dr Violeta Apšegaitė[1], Dr Eduardas Budrys[1], Dr Anna Budrienė[1], Prof Vincas Būda[1]

[1]Nature Research Centre, Vilnius, Lithuania

Cuckoo wasps (Hymenoptera: Chrysididae), commonly known as jewel or gold wasps, are kleptoparasites and parasitoids that exploit the nests of other wasp species. Effective mate location and recognition are essential for their reproductive success and are mediated by chemical signalling, particularly cuticular hydrocarbons (CHCs). In this study, we investigated the role of CHCs as sex pheromones in virgin females of three species: *Chrysis angustula, C. fulgida,* and *C. iris*.

Using coupled gas chromatography-electroantennogram detection (GC-EAD), we identified 16 hydrocarbons with chain lengths ranging from C_{23} to C_{27} that elicited electrophysiological responses in conspecific males—6 in *C. angustula,* 8 in *C. fulgida,* and 13 in *C. iris females*. Each species exhibited a unique blend and ratio of compounds, indicating species-specific chemical profiles involved in sexual communication. Behavioural observations also suggest that females may alter the production of certain unsaturated CHCs after mating, which likely act as sex pheromones. This post-mating shift may play a role in signalling reproductive status and preventing unnecessary mating attempts. These findings provide new insights into chemical communication and mate recognition in chrysidid wasps.

Functional characterization of two olfactory receptors in *Oedaleus asiaticus*

Ling Li[1], Haibin Han[1], Haichao Wang[1]
[1]Inner Mongolia Agricultural University, Hohhot, China

The band-winged grasshopper, *Oedaleus asiaticus* Bei-Bienko, is one of the most dominant and economically important grasshopper species in the steppe grasslands in northern China. Its occurrence not only leads to a large reduction in forage production, but also causes grassland degradation and desertification, which seriously threatens the ecological security in northern China. Insects perceive the information substances in the external environment through olfaction. Olfactory receptors play an important role in the process of insect olfactory perception. In this study, functional analysis of olfactory receptor gene OR17 and co-receptor ORco were performed by using RNA interference in *O. asiaticus*, and combined with Electroantennogram and Y-type olfactometer. The results showed that after OasiOR17 was silenced, the EAG activity of females in response to 5 volatile substances was significantly decreased, including trans-2-hexenal, (Z)-3-hexen-1-ol, heptaldehyde, phenylacetonitrile and 4-vinyl anisole. In males, EAG responses were significantly reduced to only two odors, including heptaldehyde and phenylacetonitrile. When OasiORco was silenced, the electrophysiological responses of females to 10 volatile compounds were significantly reduced, including trans-2-hexenal, hexanal, (Z)-3-hexen-1-ol, butyl acetate, methylacrolein, 2-methylbutyraldehyde, 2-ethylhexanol, heptaldehyde, phenylacetonitrile and 4-vinyl anisole, while the EAG responses of males to 5 odors were significantly decreased, including trans-2-hexenal, hexanal, (Z)-3-hexen-1-ol, heptaldehyde and phenylacetonitrile.

The results of Y-type olfactometer showed that after interference with OR17, the selection rates of (Z)-3-hexen-1-ol and 4-vinyl anisole increased, and others decreased obviously. After interference with ORco, the selection rates of both sexes were significantly lower than control group. These results indicate that OasiOR17 may be involved in the recognition of 5 odors, such as trans-2-hexenal, (Z)-3-hexen-1-ol, heptaldehyde, phenylacetonitrile and 4-vinyl anisole. OasiORco is the key receptor for the olfactory perception of *O. asiaticus*. These results can lay a foundation for finding molecular targets for green control of *O. asiaticus*.

Sugar response and gustatory gene expression stingless bees

Dr Maria Sol Balbuena[1]

[1]University of Buenos Aires - CONICET, CABA, Argentina

Chemosensory reception is critical for foraging success in bees, influencing their ability to locate and exploit nectar and pollen sources. Here, we examined the sugar detection and gustatory responsiveness of the stingless bee *Tetragonisca fiebrigi* to the main sugars found in nectar (sucrose, glucose, and fructose). In addition, we investigated the expression of the gustatory receptor genes *TfieGr1* and *TfieGr3* in two sensory tissues (antennae and proboscises) of forager bees. Proboscis extension assays revealed that returning pollen foragers (PF) exhibited lower sugar response thresholds and higher sensitivity to sucrose, glucose, and fructose than returning non-pollen foragers (NPF), suggesting that NPF could be mainly engaged in finding high-quality nectar sources. Moreover, 60% of PF responded to all sugars tested, while NPF showed a strong preference for sucrose and glucose, with only 4% responding to fructose. Molecular experiments revealed no significant differences in *TfieGr1* and *TfieGr3* expression levels between both groups of foragers in either sensory tissue. These results suggest that other gustatory receptors or mechanisms, such as central processing or internal nutrient sensing, may underlie the observed behavioral differences. Our findings highlight potential species-specific adaptations in sugar detection mechanisms and underscore the importance of integrating behavioral, molecular, and ecological approaches to understanding foraging strategies in stingless bees.

Ontogeny of maize shapes emission of herbivore-induced plant volatiles but depends on who attacks – A case study with fall armyworm and the corn leafhopper

MSc Mateus Souza Sanches[1,2], Dr Mirian Fernandes Furtado Michereff[1], Dr Miguel Borges[1], Dr Raúl Alberto Laumann[1], Dr Charles Martins Oliveira[3], Dr Marina Regina Frizzas[2,4], Dr Maria Carolina Blassioli-Moraes[1]

[1]Semiochemicals Laboratory, Embrapa Genetic Resources and Biotechnology, Brasília, Brazil, [2]Graduate Program in Ecology, University of Brasília, Brasília, Brazil, [3]Embrapa Cerrados, Brasília, Brazil, [4]Zoology Department, University of Brasília, Brasília, Brazil

Ontogenetic variation influences plant defense traits, with early-stage plants generally investing more in defense due to their lower tolerance to damage. Herbivore-induced plant volatiles (HIPVs) are compounds emitted by plants in response to herbivore attack, contributing to their defense system. Maize crops are susceptible to pests; in Brazil, two of the most common are the fall armyworm *Spodoptera frugiperda* (FAW) and the corn leafhopper *Dalbulus maidis* (DM). We investigated HIPV emissions under herbivory by FAW and DM, considering two vegetative stages (V4 and V6, a ten-day developmental gap). To do this, we collected volatiles using headspace systems, with each maize stage either infested or non-infested (NI) by each insect. FAW-infested plants received two 2nd instar larvae per plant, while DM-infested plants received 30 leafhoppers per plant. The ontogenetic stage affected HIPV emissions in maize injured by 30DM ($p < 0.001$; PERMANOVA) and by FAW ($p < 0.001$; PERMANOVA), with emissions differing depending on the herbivore species. However, the emission patterns varied: V4 plants under DM attack released four times more total HIPVs than the V6-DM plants, while for FAW damage, the opposite was observed: V6-FAW plants emitted twice as many volatiles as V4-FAW plants. Although a greater HIPV emission was expected from younger plants (V4), the results depended on the herbivore. The strong response in V4-DM plants may reflect adaptation to DM damage, which occurs in the earlier stages with more impact if the insects carry mollicutes. Despite early-stage FAW damage being more severe and higher volatile production at V6, the result suggests possible physiological constraints limiting HIPV production in younger maize. These findings contribute to understanding the modulation of the defense system in maize under different pressures.

Regional variation of sex pheromone in the Australian Fall armyworm population

Dr Md Jamil Hossain Biswas[1], Vaughan Moon[1], Rehan Silva[1], Vivian Mendez Alvarez[1]
[1]Applied BioSciences, Macquarie University, Macquarie Park, Australia

The fall armyworm is a highly migratory, polyphagous moth native to the Americas, known to feed on over 350 host plant species. Since 2016, this invasive pest has spread rapidly across Africa, Asia, and the Pacific and arrived in Australia in 2020, creating significant challenges to crop protection. Sex pheromones are critical for mating behaviour and species-specific communication in moths, and regional variation in female pheromone blends affects the efficacy of pheromone-based monitoring and control tools. We have identified regional differences in female pheromone gland composition across Australia and are using this information to improve lure specificity and pest management strategies. Female moths were reared from wild-collected eggs and larvae on organic corn leaves and kernels in cohort rearing and pupae from eleven field sites across Queensland, New South Wales (NSW), the Northern Territory, and Western Australia. Pheromone gland extracts were analysed using gas chromatography-mass spectrometry (GC-MS), revealing nine components in characteristic pheromone blends for Australian populations, including the two major compounds Z9-14:OAc and Z11-16:OAc and the minor compound Z7-12:OAc. The common compound (Z)-9-dodecen-1-yl acetate (Z9-12:OAc) was not found in any of the gland extractions from all locations. The identified compounds were present in varying ratios and combinations across regions. Further testing, including EAG male responses from each location and wind tunnel bioassays, will inform on the attraction of males to their respective regional blend. This research will help determine which combinations and concentrations of pheromone compounds are most effective at attracting fall armyworm moths in each region in Australia and reduce the bycatch of non-target moths.

Reproductive isolation in sympatric *Philodendron* species: do floral odours act as private communication channels?

Bastian Hourdé[1], Iliana Bertome[1], Yannick Estevez[2], Louise Brousseau[3], Mathieu Chouteau[1], Marc Gibernau[4], **Melanie McClure**[1]

[1]UAR 3456 LEEISA, Cayenne, France, [2]UMR 745 ECOFOG, Kourou, France, [3]UMR 123 AMAP, Cayenne, France, [4]UMR 6134 SPE, Ajaccio, France

The immense diversity of angiosperms is often attributed to the interaction between flowers and their pollinators. As such, the study of floral traits responsible for attracting these pollinators is clearly of great importance. Although many studies have focused on how the visual signal, such as colour and shape, diversifies and potentially acts as barriers to gene flow, comparatively little has been done on the role of floral odours. Yet floral odours abound, are highly diverse, and likely act as specific and private communication channels. *Philodendron* species (Araceae) are an ideal study system to test this as they are highly diverse, and closely related species can exist in sympatry. As they are pollinated by nocturnal *Cyclocephala* scarab beetles, floral odours play a vital role in attracting pollinators. Here we present preliminary data on floral odours and pollinators of 8 different sympatric *Philodendron* species flowering simultaneously in French Guiana. We found that floral volatiles differed significantly between species, although some overlap was found for some species. Pollinators they attracted were consistent with previous findings when known, but were not always exclusive to a single *Philodendron* species. Interestingly, those *Cyclocephala* species that were found in different *Philodendron* were not found in those with similar odours, which suggests that certain minor compounds or ratios may be important for pollinator specificity and that some pollinators may pollinate multiple species. Reproductive isolation in *Philodendron* is therefore likely ensured by differentiation of floral odours and pollinator specificity in some cases, but may also depend on other reproductive barriers, such as gamete incompatibilities.

Elucidation of the biosynthesis pathway of sex pheromones in *Cnaphalocrocis medinalis* and functionally investigation of the key enzymes

Mengyu Liu[1], Baojian Ding[1], Christer Löfstedt[1]
[1]Xianghu Laboratory, Hangzhou, China

The rice leaf folder, *Cnaphalocrocis medinalis*, is one of the most destructive insect pests in the paddy field. (Z)-13-Octadecanal (Z13–18:Ald) and (Z)-11-Octadecanal (Z11–18:Ald), as the main components of *C. medinalis* sex pheromones, have been used as attractants, which is widely considered as one of the environmentally friendly pest control agent. Therefore, analyzing the biosynthetic pathway of sex pheromone in *C. medinalis* is of great significance. By conducting RNA-seq analysis on different tissues of *C. medinalis*, we screened some fatty acid desaturase genes and carbon chain elongation genes related to sex pheromone biosynthesis, and verified the biological functions of candidate genes by using *Nicotiana benthamiana* transient expression system. These results confirmed the biosynthesis pathway of the intermediate products of sex pheromone in *C. medinalis*. This study will provide theoretical basis and technical support for *C. medinalis* sex pheromone biological production.

Electrophysiological responses of antennal sensilla in *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) to pheromone, bark beetle pheromone, and host volatiles

Minjung Huh[1], Jaewoo Lee[1], Il-Kwon Park[1]
[1]Seoul National University, Seoul, Republic of Korea

We compared electrophysiological responses of male and female *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) to pheromone and kairomones. Electroantennogram (EAG) and single sensillum recording (SSR) were conducted on six individuals of each sex using 22 semiochemicals, including aggregation-sex pheromones (2-undecyloxy-1-ethanol), two bark beetle pheromones, and 19 host volatiles. Among the tested compounds, 14 volatiles elicited significantly stronger EAG responses in males, while 15 volatiles induced significantly stronger responses in females. Both sexes exhibited the highest EAG responses to ipsdienol and *α*-terpineol. Within the two types of olfactory sensilla - thin (SBaA) and thick (SBaB) sensilla basiconica - 10 subtypes of olfactory sensory neurons were identified and clustered based on their functional responses to specific volatiles. These findings are expected to advance our understanding of the chemical ecology of *M. alternatus* and suggest promising candidates for behavioral attractants.

Preliminary identification of a sex pheromone candidate in the ginseng stem fungus gnat, *Bradysia procera* (Diptera: Sciaridae)

Min-Woo LEE[1], Il-kwon PARK[1,2]

[1]Department of Agriculture, Forestry and Bioresources, Seoul National University, Seoul, South Korea, [2]Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, South Korea

The ginseng stem fungus gnat, *Bradysia procera*, is a major pest of cultivated ginseng (*Panax ginseng*) in Korea. Larval feeding damages stems and roots, leading to plant wilting and significant yield losses. Due to the insect's cryptic behavior and the limited efficacy of chemical control, it poses a serious threat to ginseng production.

To explore environmentally friendly control strategies, we investigated the presence and characteristics of sex pheromones in *B. procera*. Male behavioural responses to female-derived cues were tested using Y-tube olfactometer assays. Males showed a significant preference for female hexane extracts and virgin females, suggesting that females emit sex pheromones.

To identify candidate compounds, hexane extracts were prepared from pooled female adults. Gas chromatography coupled with electroantennographic detection (GC-EAD) using male antennae revealed a consistent antennal response to a specific compound across all replicates. An additional compound also induced antennal activity but was not detected in the FID chromatogram, suggesting it may be present at trace levels.

These findings provide preliminary evidence for the presence of female-emitted sex pheromones in *B. procera*. Ongoing chemical identification and behavioural assays will further elucidate the role of the candidate compounds. This study contributes foundational knowledge toward the development of pheromone-based monitoring or control strategies to support sustainable ginseng cultivation.

Mapping sagebrush chemotypes along an elevational gradient

Prof Richard Karban[1], **Dr Muhammad Usman Rasheed**[2], Dr Mikaela Huntzinger[1], Dr Patrick Grof-Tisza[2,3,4], Prof James Blande[2]

[1]Department of Entomology and Nematology, University of California, Davis, USA, [2]Department of Environmental and Biological Sciences, Kuopio, Finland, [3]Institute of Biology, University of Neuchatel, Neuchatel, Switzerland, [4]Department of Natural Sciences, Converse University, Spartunburg, USA

Plants form the foundation of most terrestrial ecosystems; therefore, plant chemistry can have important and far-reaching consequences for many ecological interactions and processes. Despite this, few studies have explicitly explored the spatial variation in plant chemistry. We mapped the distribution of volatile emissions of the mountain subspecies of sagebrush in 25 meadows north of Lake Tahoe. This plant exhibits several distinct volatile emission profiles (chemotypes) that are part of the system involved in stimulating induced defenses to different herbivores and pathogens. Sites varied in the volatile chemotypes that were present although three chemotypes were most common. The three common volatile chemotypes sorted out along an elevational gradient. This chemotypic pattern sets the stage for interactions such as those that result in different abundances of herbivores.

Screening, identification, and functional study of olfactory-related genes in the teak defoliator moth, *Hyblaea puera*

Identification and Function of Study of Olfactory Genes In Hyblaea puera Cramer Qi Dong[1]
[1]Beijing Forestry University, Beijing, China

The teak defoliator moth, *Hyblaea puera* Cramer, exhibits a broad host range and poses severe threats to plants such as *Tectona grandis* and *Avicennia marina*. Due to its high growth and survival rate, and robust reproductive capacity, it has recently caused significant ecological damage to coastal regions in China. The insect's highly sensitive and specialized olfactory system enables precise perception of environmental cues, triggering corresponding physiological responses. This process involves a series of olfactory-related proteins, including odorant binding proteins (OBPs), chemosensory proteins (CSPs), odorant receptors (ORs), and ionotropic receptors (IRs), which interact synergistically to transmit signals and drive adaptive behaviors, forming the basis of the insect's remarkable environmental adaptability. In this study, high-throughput transcriptome sequencing and phylogenetic analysis preliminarily identified members of five chemosensory protein families in *H. puera*. Based on expression profiling, three key olfactory-related genes—*HpuePBP1*, *HpuePBP2*, and *HpuePBP5*—were selected via quantitative real-time PCR (qPCR) due to their significantly high expression in antennal tissues. To further investigate the olfactory recognition functions of these genes, molecular docking techniques were employed to elucidate the specific binding patterns between these three pheromone-binding proteins and sex pheromones, unveiling their molecular recognition mechanisms. This research marks the first comprehensive transcriptome sequencing and analysis of the olfactory system in *H. puera*, successfully identifying and screening multiple key olfactory functional genes. These findings lay a critical foundation for subsequent functional validation of olfactory receptors and the development of targeted behavioral regulators for pest control. The results provide theoretical and technical support for advancing novel green control strategies against *H. puera* based on olfactory interference.

Entomopathogenic nematode responses to host-derived volatiles: Behavioral and emission patterns of 1-nonene

Dr Rasa Čepulytė, Deimantė Tiškevičiūtė, Dr Violeta Apšegaitė, Evelina Osinska, Prof Vincas Būda

[1]Nature Research Centre, Laboratory of Chemical and Behavioral Ecology, Vilnius, Lithuania

Entomopathogenic nematodes (EPNs) are insect-parasitic organisms used in biological control, relying on chemical cues to locate their hosts in the soil. Volatile compounds released from infected cadavers may serve as signals guiding EPN behavior, yet their role remains underexplored. We investigated the behavioral responses of infective juveniles of three EPN species with different foraging strategies - *Steinernema kraussei* (cruiser), *S. feltiae* (intermediate), and *S. carpocapsae* (ambusher) - to 1-nonene, a volatile emitted by EPN-infected hosts. Additionally, we quantified 1-nonene emission from *Galleria mellonella* larvae infected with each of the species.

Behavioral assays revealed that *S. kraussei* was repelled by 1-nonene at concentrations ≥20 mM. *S. feltiae* was attracted at low concentrations but repelled at higher concentrations, while *S. carpocapsae* was attracted to high concentrations only. Emission data showed that *S. feltiae*-infected larvae released the least amount of 1-nonene, while emissions were 15× higher from *S. kraussei*-infected larvae and 76× higher from *S. carpocapsae*-infected larvae. These species-dependent behavioral and emission patterns suggest that volatile signal (both its reception and release) is linked to the foraging strategies of EPNs. *S. kraussei* and *S. feltiae* exhibited similar responses distinct from *S. carpocapsae*, reflecting ecological divergence in cue use. Reactions to the compound released by the EPN-infected insect cadaver demonstrate that the studied EPN species behave not only as predators (a trait necessary for EPNs when used in biocontrol) but also as scavengers.

Dynamic releaser as a suitable tool for chemical ecology and behavioral approaches

Dr Rodrigo Magnani[1], Dr Haroldo Volpe[1], MSc Rejane Luvizotto[1], Mrs Tatiana Mulinari[1], Dr Thiago Agostini[1], Dr Jairo Bastos[2], Dr Victor Ribeiro[2], Dr Michele Carmo-Sousa[1], Dr Nelson Wulff[1], Dr Leandro Peña[1,3], Dr Walter Leal[4]

[1]Department of Research and Development, Fund for Citrus Protection (Fundecitrus), Araraquara, Brazil, [2]School of Pharmaceutical Sciences, University of São Paulo (USP), Ribeirão Preto, Brazil, [3]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones, Valencia, Spain, [4]Department of Molecular and Cellular Biology, University of California-Davis, Davis, USA

Developing improved methods for the controlled release of volatile organic compounds (VOCs) from blended formulations, ensuring consistent emission over time, is essential for understanding complex trophic interactions and for advancing integrated pest management (IPM) strategies. These strategies include monitoring, mating disruption, and push–pull–kill techniques. We first compared the emission rate of *β*-caryophyllene from a static system (2 ml open glass vials containing 100 µl hexane solutions) with a dynamic releaser, inspired by “wick-bait” designs, which enables steady semiochemical release. To calibrate the system, *β*-caryophyllene emissions from the static setup (n = 17) were compared with those from dynamic devices loaded with solutions of 0.05, 0.1, 0.3, and 0.8 µg/µl (n = 3 per dose). VOCs were collected using Tenax® TA traps and analyzed by thermal desorption gas chromatography–mass spectrometry (TD-GC-MS).These analyses showed that a static device loaded with a 1 µg/µl solution released an equivalent amount of *β*-caryophyllene as a dynamic releaser loaded with 0.17 µg/µl. Further measurement indicated that our dynamic device, loaded with this 0.17 µg/µl active dose, released *β*-caryophyllene at a rate of 0.044 µg/min. To assess the stability of the dynamic system, we evaluated the temporal release profile of a sesquiterpene mixture (*α*-copaene:*β*-caryophyllene:*α*-humulene at a 1:100:10 ratio). VOCs from the dynamic device were sampled every three minutes over a 15-minute period. TD-GC-MS analyses confirmed a consistent release of each compound, maintaining the original blend ratio in the airborne phase. This stability indicates the device's suitability for behavioral assays involving semiochemical mixtures.

Cryptostylines: Natural plant alkaloids involved in herbivory defence in Australian *Cryptostylis*?

Saskia ter Horst[1], Dr Björn Bohman[2], Assoc Prof Gavin Flematti[2], Dr Ryan Phillips
[1]University of Amsterdam, Amsterdam, the Netherlands, [2]University of Western Australia, Perth, Australia

Unique tetrahydroisoquinoline (THIQ) alkaloids, named cryptostyline I, II, and III, were first isolated and structurally elucidated from the orchid *Cryptostylis arachnites* over 50 years ago. THIQ alkaloids form one of the largest plant metabolite subgroups and are known for a range of biological activities, including antioxidant, anti-fungal, insecticidal and antibacterial effects. They also have several pharmaceutical and industrial applications. Cryptostylines are unique, as they are one of the few known plant alkaloids to contain a phenyl backbone. Very little is known about their biological role or their distribution across taxa and plant tissues.

Recently, preliminary analysis of solvent extracts of *Cryptostylis ovata*, a sexually deceptive orchid endemic to Western Australia, showed a range of compounds with mass spectra indicative of cryptostylines, including some of the compounds identified from *C. arachnites*. These results indicate that Australian *Cryptostylis* may have several novel structurally related compounds. Based on preliminary field data, we hypothesise that cryptostylines act as deterrents against herbivores. Unusual for a southern Australian orchid, *Cryptostylis* leaves are perennial, meaning they are present during the dry, hot summer period. Intriguingly, field observations have shown that *Cryptostylis ovata* leaves rarely display herbivory damage, even during the summer period where few herbs are available as forage.

Within the next year we aim to establish herbivory rates in *Cryptostylis ovata* and compare those to co-occurring orchids. Extracts from several Australian *Cryptostylis* species and tissues will be collected, and cryptostylines in these extracts will be isolated using semi-preparative HPLC and identified with GC-MS and NMR spectroscopy. These experiments will provide further evidence on the distribution and biological function of cryptostylines and their involvement in herbivory defence.

Olfactory activities of sex pheromone and structurally related compounds in *Spodoptera litura*: GC-EAD and field trapping study

Seon Ah Jeong[1,2], Oh Hyun-Woo[3], Park Doo-Sang[4], Byun Bong-Kyu[2], Roh Gwang Hyun[1,5], Park Kye Chung[6]

[1]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, South Korea, [2]Department of Biological Science and Biotechnology, Hannam University, Daejeon, South Korea, [3]Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea, [4]Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, South Korea, [5]Department of Plant Medicine, Gyeongsang National University, Jinju, South Korea, [6]Bioprotection, New Zealand Institute for Plant and Food Research, Lincoln, New Zealand

Spodoptera litura, commonly known as the tobacco cutworm moth, is a serious agricultural pest with a global distribution, infesting a wide range of crops. Although polyphagous, it exhibits species-specific host plant preferences. Like other groups of moths, some of the female sex pheromone components of *S. litura* are structurally similar among congeneric species within the genus *Spodoptera*. In this study, we investigated the electrophysiological and behavioral responses of *S. litura* to a total of 17 compounds, including its four known female sex pheromone components and 13 structurally related analogues. In gas chromatograph-electroantennogram detection (GC-EAD) analysis, both sexes of *S. litura* exhibited consistent EAD responses to all 17 compounds, suggesting that this species is capable of detecting sex pheromones emitted by other species in *Spodoptera*. To evaluate the behavioral activities of the EAD-active compounds, field trapping experiments were conducted in various fields in Korea. Some EAD-active compounds significantly inhibited male attraction to conspecific female sex pheromones, whereas some others significantly enhanced attraction. Although female *S. litura* exhibited significant EAD responses to the pheromone and related compounds, these compounds did not show any behavioral attraction to female *S. litura* in the field trapping test. These findings add new insights into the sex pheromone-related chemical communication system in *S. litura*, providing a valuable foundation to elucidate the behavioral and ecological significance of these compounds and to develop environmentally friendly behavioral control strategies using semiochemicals. Further studies, such as single sensillum recording, would be useful in understanding the profile of olfactory sensory neurons responsive to these EAD-active pheromone-related compounds, which can provide useful information on the mechanism of species isolation in *Spodoptera*.

Electrophysiological identification of host plant- and protein bait-derived volatiles by the antennae and maxillary palps of *Bactrocera depressa* for the development of female-targeted attractants

Seon Ah Jeong[1], Jang Eun Su[2], Oh Ji Hye[2], Yu Da Hyeon[2], Roh Gwang Hyun[1,2]

[1]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, South Korea, [2]Department of plant medicine, Gyeongsang National University, Jinju, South Korea

The pumpkin fruit fly, *Bactrocera depressa*, is a significant pest of Cucurbitaceae crops, yet effective monitoring and control tools remain limited. Unlike many other *Bactrrocera* species, no male-specific attractants have been identified for *B. depressa*, and available female attractants are non-specific and weakly attractive. In this study, we aimed to identify olfactory-active compounds that may serve as potential attractants for female *B. depressa*, by using a coupled gas chromatograph-electroantennogram detection (GC-EAD) and a coupled gas chromatograph-electropalpogram detection (GC-EPD) technique. The electrophysiological recordings from the antennae and maxillary palps of *B. depressa* exhibited the presence of olfactory-active compounds in the headspace samples of various host plants and protein baits. The results exhibited distinct profiles of olfactory-active compounds between the two sensory organs, indicating that the antennae and maxillary palps house different populations of olfactory receptor neurons (ORNs). Several compounds also exhibited sex specific responses, suggesting the presence of sexually dimorphic ORN populations. These findings highlight the complexity of the olfactory sensory system in *B. depressa* and its potential for selective odor detection. Ongoing efforts are focused on structurally identifying the EAD- and EPD-active compounds and evaluating their behavioral relevance. This work provides a foundation for developing species-specific and sex-specific attractants, which could significantly enhance monitoring and management strategies for *B. depressa*.

The symbiont *Stenotrophomonas maltophilia* mediates ivermectin resistance via xenobiotic metabolism in *Haemonchus contortus*

Phd Candidate Simin Wu[1]

[1]Huazhong Agricultural University, Wuhan, China

The emergence of anthelmintic resistance in parasites poses a global threat to livestock and human health. While parasitic genomic adaptation has been extensively studied, recent evidence suggests bacterial symbiont may modulate parasite physiology and drug responses. This study investigates the novel hypothesis that *Stenotrophomonas maltophilia* (SM1), a symbiont of *Haemonchus contortus(H. contortus)*, mediates ivermectin resistance through xenobiotic degradation—a previously unexplored mechanism in helminth drug resistance. Full-length 16S rRNA sequencing revealed a 3.4-fold higher abundance of SM1 in ivermectin-resistant *H. contortus* strains compared to susceptible counterparts. Gentamicin depletion of symbiont significantly increased larval susceptibility to ivermectin, while SM1 enrichment exacerbated resistance phenotypes. In vitro assays demonstrated SM1's capacity to degrade ivermectin via oxidative pathways. Whole-genome sequencing identified distantly homologous genes of CYP3A4 and CYP3A5 in SM1, and KEGG pathway analysis confirmed the enrichment of cytochrome P450-mediated drug metabolism.These findings suggest SM1 mediates ivermectin resistance via xenobiotic metabolism in *H. contortus*.Our study provides the first experimental evidence that symbionts directly regulate anthelmintic resistance through xenobiotic biotransformation. This "microbiota-mediated resistance" paradigm challenges traditional host-centric models and redefines our understanding of drug resistance evolution in parasitic nematodes.

Assessment of diversity in volatiles of World Rice Core Collection (WRC) as basis for the understanding of crop indirect defense

Mrs Thanh Nhan Ho[1], Mr Tomonori Shinya[1], Mr Ivan Galis[1]

[1]Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama, Japan

Natural plant defense against insect herbivores is important for intrinsic damage levels in crops but large-scale studies that investigate defense mechanisms at crop variety level are scarce. In particular, field studies with insects are difficult due to environmental factors and year-by-year oscillations in pest densities. Recently, a World Rice Core Collection (WRC) was developed to study agronomical traits in rice by molecular techniques, including Genome-Wide Association Studies (GWAS). Taking advantage of this cutting-edge rice resource, we are now investigating the volatile production in each WRC line to identify the main factors (compounds) that govern indirect plant defenses, as well as behavior of pests and their natural enemies in the field. In 2024, we conducted a pilot field study that confirmed the highly variable insect damage levels in the WRC collection. In particular, 69 WRC accessions showed a substantial diversity in brown planthopper infestation under natural pesticide-free conditions. Simultaneously, WRC lines showed a high diversity in the production of monoterpenes, sesquiterpenes and green leaf volatiles determined by GC-MS under control laboratory conditions. Our current objective is focused on the identification of genes that control innate production of rice volatiles in the individual rice varieties, and therefore contribute to indirect defense, by GWAS.

Repellent activity of α-copaene and copaiba oil against the Asian citrus psyllid

Dr Thiago Trevisoli Agostini[1], Dr Haroldo Xavier Linhares Volpe[1], Dr Rodrigo Facchini Magnani[1], MSc. Rejane Angélica Grigio Luvizotto[1], Tatiana Aparecida Mulinari[1], Dr Jairo Kenupp Bastos[2], Dr Victor Pena Ribeiro[2], Dr Michele Carmo-Sousa[1], Dr Nelson Arno Wulff[1], Dr Leandro Peña[3], Dr Walter S. Leal[4]

[1]Department of Research and Development, Fund for Citrus Protection (Fundecitrus), Araraquara, Brazil, [2]School of Pharmaceutical Sciences, University of São Paulo (USP), Ribeirão Preto, Brazil., [3]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones, Valencia, Spain, [4]Department of Molecular and Cellular Biology, University of California-Davis, Davis, USA

The Asian citrus psyllid (*Diaphorina citri*, ACP) is the insect vector of *Candidatus* Liberibacter asiaticus, the bacterium responsible for Huanglongbing (HLB), the most devastating citrus disease worldwide. We assessed the repellence effect of sesquiterpenes (*α*-copaene, *β*-caryophyllene and *α*-humulene) and *Copaifera* oil against ACP using a 4-arm olfactometer. A dynamic releaser device was employed to maintain consistent blend ratios and stable airborne concentrations. In dose-response behavioral assays of single compounds, *β*-caryophyllene repelled ACP only at the dose of 0.17 µg/µl (*n*=99, *p*=0.0152), when tested in a range from 0.13 to 0.22 µg/µl. *α*-Copaene (0.1–2.9 ng/µl) was repellent within the 0.9–2.1 ng/µl range, with ACP spending more time in the control zones at the following concentrations: 0.9 ng/µl (*n*=129, *p*=0.0042), 1.3 ng/µl (*n*=126, *p*=0.0052), 1,7 ng/µl (*n*=106, *p*=0.0168) and 2.1 ng/µl (*n*=117, *p*=0.0210). In contrast, *α*-humulene showed no significant repellency at either 0.013 µg/µl (n=128, *p*=0.8751) or 0.017 µg/µl (n=101, *p*=0.3145). Testing binary mixtures revealed that combining *β*-caryophyllene (0.17 µg/µl) and *α*-copaene (1.7 ng/µl) at a 100:1 ratio did not produce a synergistic effect (n=100, *p*=0.6841). However, a lower-dose blend of *β*-caryophyllene (0.13 µg/µl) and α-copaene (1.3 ng/µl) was significantly repellent (n=108, *p*=0.0391). Notably, a tertiary mixture (*α*-copaene: 1.3 ng/µl, *β*-caryophyllene: 0.13 µg/µl, *α*-humulene: 0.013 µg/µl, 1:100:10 ratio) enhanced ACP repellency. Chemical analysis of copaifera oil, a natural source of sesquiterpenes, revealed high concentrations of *β*-caryophyllene (517.09±9.54 mg/ml), *α*-humulene (60.33±0.71 mg/ml), and *α*-copaene (28.26±0.50 mg/ml). When diluted 3,500× to match the active concentration of *β*-caryophyllene used in olfactometer assays, the oil significantly repelled ACP (n=115, *p*=0.0005). No repellency was observed at 3,000× or 4,000× dilutions (n=122, *p*=0.1402; n=99, *p*=0.3939, respectively). These results identify *α*-copaene and *β*-caryophyllene as key repellents against ACP and support the potential use of copaifera oil as a sustainable, slow-release repellent for ACP management in citrus production.

Behavioural and peripheral olfactory responses to male attractants across Tephritidae (Diptera): A comparative study

Vincent Jacob[1], Emma Persyn, Nicolas Slomiany, Emanuelle Jacquin-Joly
[1]Cirad UMR PVBMT, Saint-pierre, Réunion

The diverse male attractants used for Tephritidae pest control play a peculiar ecological role: males that ingest plant-born natural versions of these compounds enrich their sex pheromonal blend, making them more competitive than other males for reproduction, as a result of female preference in mate choice. To investigate the evolutionary origin of this sexual selection, we compared the olfactory responses of males from nine Tephritidae species*, Bactrocera dorsalis, Bactrocera zonata, Ceratitis capitata, Ceratitis catoirii, Ceratitis quilicii, Dacus ciliatus, Dacus demmerezi, Neoceratitis cyanescens,* and *Zeugodacus cucurbitae*, to a set of 15 compounds known to attract males in at least one species of the Tephritidae family. The behavioural responses were screened in five selected species (n=32 individuals per compound and species), using a bioassay specifically designed for high-throughput screening of insect orientation behaviour, the Flywalk olfactometer. Additional experiments were performed with a six-choice olfactometer for comparison. We observed species-specific attractant preferences, analysed through partial-least square discriminant analysis (PLS-DA). It mostly matched documented preferences, but minor differences were also observed, such as the attraction of *B. dorsalis* but not *B. zonata* to isoeugenol. Using three-point electroantennography in all eight species (n=8 individuals each) and electropalpography in five (n=5 each), we found that most compounds elicit similar peripheral responses in males across all species. Interspecific variability in olfactory detection assessed through PLS-DA was small and displayed only a weak connection to behavioural preferences. These results point to a potential evolutionary decoupling between conserved peripheral detection and divergent behavioural relevance of these compounds, suggesting that male attraction may have arisen from central reprocessing of ancestral olfactory signals. Overall, our work provides comparative data across species with standardised protocols, which document the olfactory mechanisms underlying responses to semiochemicals used in pest management.

Regional variation in the efficacy of FAW pheromone-based lures in Australia

Dr Vivian Mendez Alvarez[1], Tania Yonow[2], Darren Kriticos[2], Rehan Silva[1], Md Jamil Hossain Biswas[1], Phil Taylor[1], Sabrina Sultana[1]

[1]Macquarie University, Ryde, Australia, [2]Cervantes Agritech, Australia

The fall armyworm (FAW), *Spodoptera frugiperda*, a highly invasive noctuid moth pest that is capable of long-distance flights and the ability to thrive on a wide range of host plants, arrived in Australia in 2020. It has since spread to all the states except for South Australia. FAW has severely impacted Australian growers, particularly affecting sweet corn, sorghum and maize but also pulses, grasses and other vegetables and grains. Monitoring FAW has proved to be difficult. Although there are commercial pheromone-based lures available in Australia, a direct correlation between crop damage and number of moths captured in traps has not yet been established. Additionally, the lures attract a significant number of non-target species, particularly *Leucania loreyii*, the False armyworm. We tested effectiveness of four commercial lures available in Australia: Frugilure (Chemtica), Pherolure (Insect Science), Pherocon (Tréce) and Iscalure (ISCA), across 30 field sites in Western Australia, Northern Territory, Queensland and New South Wales. We compared lure efficacy based on the number of Fall Armyworm and non-target species captured in the traps. The four commercial lures performed similarly in the number of FAW attracted but there was regional variation in the attraction of False armyworm. In the northern regions, Pherolure and Tréce attracted fewer False Armyworm but this trend shifted in the southern areas, with Tréce, Pherolure and Iscalure attracting more False Armyworm. We also observed a strong effect of crop by location, influenced by climate, on the capture rates of FAW, with sweet corn and maize showing the highest numbers of FAW, as expected. Using the information we have collected on regional variation of FAW female sex pheromone, we have developed region-specific pheromone blends for use in commercial lures across Australia. These are currently being tested in the field.

Identification and functional characterisation of *Bactrocera tryoni* odorant receptors

Mr W. Stephen Penrose[1,2], Dr Alexander Piper[2], Assoc Prof J. Paul Cunningham[2,3], Prof Coral Warr[1]

[1]School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, Australia, [2]AgriBio Centre, Agriculture Victoria Research, Bundoora, Australia, [3]School of Applied Systems Biology, La Trobe University, Bundoora, Australia

Bactrocera tryoni, the Queensland Fruit Fly, is one of Australia's most economically important pests, costing the horticulture industry an estimated $300 million annually. Following the withdrawal of the traditional organophosphate insecticides used to control this pest, there is urgent industry demand for novel control methods that are compatible with Integrated Pest Management (IPM). A promising avenue involves attract and kill approaches using semiochemical lures, however, despite extensive chemical ecology research, current lures that target the fruit damaging female Qfly have varied efficacy dependent on available hosts. Here we take a different approach, focusing instead on the chemosensory machinery responsible for the detection of these volatiles by the insect, specifically the olfactory receptors (ORs). In this study we identified ~90 putative OR genes in the Qfly genome and characterised their sex-specific expression patterns across antennal and palp tissues. Comparative analysis across Tephritid pests with similar ecological niches revealed an expansion of OR genes known to be involved in the detections of esters, volatiles produced by fruits. Moving forward, key receptors will be functionally characterised through transgenic expression in the 'empty neuron' system of *D. melanogaster*, a well-established model for determining the ligands of odorant receptors. Through an improved understanding of the molecular basis of Qfly olfaction, we can improve OR response prediction models, expanding the range of potential lure components and overcoming the limitations of traditional chemical ecology approaches.

Synthesis of pheromones of various nettle caterpillars, and their field application

Mr Yuki Miyake[1], Mr Yasuhiko Kutsuwada[1], Ms Erina Ohno[1], Mr Tatsuya Fujii[2], Dr Takeru Watanabe[1], Dr Takeshi Kinsho[1]

[1]Shin-Etsu Chemical Co., Ltd. Specialty Chemicals Research Center, Joetsu-shi, Japan, [2]Shin-Etsu Chemical Co., Ltd. Organic Chemicals Division Fine Chemicals Department, Chiyoda-ku, Japan

Nettle caterpillars are destructive defoliators of oil palm and sanitary pests in Asia. Most of the caterpillars are covered with rows of poisonous spines and the poisons cause an allergic reaction in people. *Setothosea asigna*, *Setora nitens* and *Darna* spp. are serious oil palm pests in Indonesia and *Parasa lepida* is dangerous sanitary pest in Japan. Insecticide application against Nettle caterpillars has been used, however, it is sometimes unfavorable in terms of effects on human health and negative impact on the environment. Pheromone-based control methods against these serious pests have been strongly desired.

The sex pheromones of nettle caterpillars contain unique terminally conjugated diene structures and functional groups are aldehyde, alcohol and ester. For example, female produced sex pheromone of *S. asigna* is (9*E*)-9,11-dodecadienal and (9*E*)-9-dodecenal, *S. nitens*'s is those geometric isomers, (9*Z*)-9,11-dodecadienal and (9*Z*)-9-dodecenal and *P. lepida*'s is (7*Z*)-7,9-decadien-1-ol. In our synthesis these conjugated diene was built by dehydrochlorination from homoallylic chloride. We will report details of our synthesis for these sex pheromones.

The field attraction trials for *S. asigna* and *S. nitens* have been conducted in Indonesia and for *P. lepida* in Japan. With all three species, increasing pheromone loading increased trap catches of males in our trial. Our tube type lure was effective for several months. In mating disruption (MD) trial for *P. lepida* in Japan, we have deployed tube-type dispensers to thicket of broadleaf trees. MD efficacy was demonstrated through suppression of male trap capture and the trap shutdown was almost perfect in MD plots. We think pheromone-based MD for Nettle caterpillars will be one of the promising options for controlling these pests.