AWARD PRESENTATIONS

CHEMICAL ECOLOGY WITH A CAST OF THOUSANDS AND RECOGNITION OF ONE

Walter S. LEAL[1] et al.

[1]*University of California-Davis, CA, USA*

Professor Thomas Hartmann once said that students, postdoctoral scholars, visitors, collaborators, and other co-workers perform the scientific work that leads to professors' honors and awards. Speaking for myself, the Silver Medal lecture is no exception. As I've discussed multiple times with Professor Tom Baker, in academia we are privileged that taxpayer dollars are entrusted to us to do fascinating work: to ask intriguing questions, to let students and postdocs find the answers, and to go around the world spreading the good news. Thanks to the support from multiple funding agencies and other sponsors, I have been cheerleading current and past lab members, collaborators, and many others to unravel the intricacies of the insect olfactory system and to advance the field of chemical ecology. As I will discuss is this presentation, my major interest is in the fundamental aspects of insect chemical communication, but I will never forget Society's investment in my research. As insects' lives intertwine with ours by damaging our crops and inflicting human suffering by vectoring pathogens, it is possible to serve two Gods: basic and applied biology/entomology. I am interested in understanding the molecular mechanisms of chemical communication. At the onset, "molecular" meant to me pheromones and other semiochemicals. Then they led me to "large molecules" – the ones involved in the reception of the smaller ones. I will briefly discuss our research on the carries, the odorant-binding proteins; the detectors, the odorant receptors; and the terminators, the odorant-degrading enzymes. Together, they form the pillars of a sophisticated insects' olfactory system for the reception of semiochemicals, but as we gain a better understanding of the molecular basis of interactions between small and large molecules, their sophistication may become an Achilles' heel. Hopefully, these studies, along with research in many other laboratories throughout the world, will lay the foundation for the rational design of eco-friendly, green chemicals for controlling populations of insects of medical importance and agricultural pests while preserving beneficial insects.

SEMIOCHEMICALS - THEIR STEREOCHEMICAL DIVERSITY AS REVEALED BY ORGANIC SYNTHESIS

Kenji MORI[1]

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Do the two enantiomers of a chiral semiochemical show different bioactivities? In 1973, when I started my pheromone synthesis, no one could answer this question. My long-standing purpose of pheromone research is to clarify the significance of chirality in pheromone science by determining the absolute configuration of pheromones through their synthesis. Various stereoselective synthetic methods were used to synthesize the following bioactive natural products.
(1) The male aggregation pheromone (4,8-dimethyldecanal) of *Tribolium castaneum*. The natural pheromone is a stereoisomeric mixture.
(2) The male sex pheromone [CH503, (3*R*,11*Z*,19*Z*)-3-acetoxy-11,19-octacosadien-1-ol] of *Drosophila melanogaster*. The bioactivity of natural CH503 is weaker than that of the unnatural stereoisomers.
(3) A cytotoxic metabolite miyakosyne A [(3*R*,4*E*,14*S*,24*E*,26*R*)-14-methyloctacosa-4,24-diene-1,27-diyne-3,26-diol] of a marine sponge *Petrtosia* sp. A concise synthesis was achieved by two-directional approach employing olefin cross metathesis and lipase-catalyzed acetylation.
Diversity is the hallmark of the relationship between stereochemistry and bioactivity. The existing dogma- "only a single enantiomer is bioactive"- must be modified.
Mori. 2007. *Bioorg.Med.Chem*., *15*, 7505-7523
Mori. 2011.*Chirality*, *23*, 449-462.

CHEMICALLY MEDIATED INTERACTIONS AMONG PLANTS, INSECTS, AND PATHOGENS

Consuelo DE MORAES[1]

[1]*Penn State University, University Park, PA, USA*

Olfactory cues mediate diverse interactions among plants and other organisms. Over the past two decades a key focus of chemical ecology has been on elucidating the role of constitutive and induced plant volatiles in conveying information to insect herbivores and their natural enemies. It is now well established that volatile blends can carry complex information about the identity and status of emitting plants, and that insects and other organisms perceive and respond to such cues in sophisticated and sometimes surprising ways. A great deal of research has documented the role of volatiles in tri-trophic interactions and explored implications for the control of herbivorous pests in agricultural ecosystems, and recent work is extending these insights in a variety of exciting directions. These include increasing attention to population-level and evolutionary processes occurring in natural communities, to the role of volatile chemistry in mediating interactions in complex environments in which plants simultaneously interact with a diverse community of other organisms (including microbial symbionts and pathogens as well as insects), and on the perception of olfactory cues by plants themselves. In this talk, I will describe these developments and discuss recent work from our program in each of these areas. Specifically, I will discuss recent studies exploring (i) the effects of inbreeding and genotypic variation within plant populations on community level plant-insect interactions, (ii) the influence of pathogen-induced changes in host plant odors on interactions with vector and non-vector insects, and (iii) plant responses to olfactory cues from other plants and from insects.

PLENARY PRESENTATIONS

KOALAS AND *EUCALYPTUS*: THE CHEMICAL ECOLOGY OF A SPECIALISED MAMMAL PLANT INTERACTION

William FOLEY[1]

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Dietary specialization, although common amongst insects, is considered rare in vertebrate herbivores. The classic example of a mammalian dietary specialist is the Australian arboreal marsupial, the koala (*Phascolarctos cinereus*). Koalas feed almost exclusively on the leaves of *Eucalyptus* trees and how they manage to maintain themselves in this dietary niche has long been a matter of speculation. Importantly, they are not the only marsupials to exist solely on *Eucalyptus* foliage; the greater glider (*Petauroides volans*) similarly feeds almost exclusively on *Eucalyptus* whereas the common ringtail (*Pseudocheirus peregrinus*) and brushtail possums (*Trichosurus vulpecula*) consume large amounts of *Eucalyptus* in many parts of their range. This talk will describe the variation in the composition of *Eucalyptus* foliage and the chemical factors that permit one tree to be eaten by koalas (and other folivorous marsupials) yet another of the same species is rejected. The most important group of foliar toxins is the formylated phloroglucinol compounds (macrocarpals and sideroxylonals). These are potent antifeedants that stimulate the nausea system and occur at vastly different concentrations even amongst neighbouring trees. Animals are able to tradeoff the concentrations of these against the concentration of available N (the integrated effects of tannins on protein digestion). Terpenes are not proximal deterrents but contribute to the metabolic load that must be excreted. The large amounts of terpenes that are ingested by these marsupials require them to carefully manage the rate and nature of their feeding to avoid toxicosis. This is achieved through rapid feedback that allows them to match feeding with their metabolic load and the rate of liver activity. The variation in the chemical composition of *Eucalyptus* across the landscape influences the reproductive success of populations and will be a major factor affecting the survival of koalas as climates change.

HOW A NATIVE PLANT SOLVES LIFE'S CHALLENGES WITH A SOPHISTICATED USE OF CHEMISTRY

Ian Thomas BALDWIN[1]

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

Plants are rooted at the base of most food chains on this planet, but being passive food for the rest of the planet's heterotrophs is not the role they have chosen. This talk will describe two decades of research into how a native tobacco plant, *Nicotiana attenuata*, that lives in the Great Basin Desert of the SW USA has been developed into a model system for the study of all types of plant-ecological interactions, particularly those biotic interactions that dominate the agricultural niche. This plant recognizes attack from specific herbivore species by the particular chemistry of the herbivore's saliva, and uses this recognition to tailor a complicated 5-layered defense response that requires a remodeling of the plant's transcriptome, metabolome and proteome, as well as some of its life history traits. The science writer, Michael Pollan, inverted the relationship between humans and their domesticated plants to argue that it was plants that domesticated humans, and not vice versa. *Nicotiana attenuata* has had designs more Machiavellian than domestication for the heterotrophs that feed on it.

START OF INSECT LIFE: INSECT EGGS AND THEIR INTERACTIONS WITH PLANTS

Monika HILKER[1]

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Insect eggs are highly vulnerable life stages that cannot escape from danger. Insect parents take care of them by hiding them inside a substrate, endowing them with toxins, covering them with protective material or even guarding them with the parental body. Parents may even affect the health of the insect embryo by transgenerational immune priming and thus influence how efficiently the egg will cope with attack by egg parasitoids. However, insect eggs laid on leaves face counteractions by the plant. An egg-laden plant is able to defend against the upcoming danger of feeding damage by hungry larvae. Plants can react to insect egg deposition by producing toxins killing the eggs, forming neoplasms or necrotic tissues that lead to detachment of eggs from the plant. Plants may even take the egg deposition as a warning signal for upcoming future larval feeding damage; larvae feeding on a plant where they hatched from eggs show reduced performance compared to larvae feeding on egg-free plants. In addition to such defensive plant responses acting directly against the eggs or the hatching larvae, plants are known to "call" egg parasitoids when eggs have been laid onto leaves. Insect egg deposition on leaves induces the emission of leaf volatiles that attract parasitoids or induces changes of leaf surface chemistry that facilitates the parasitoid´s search for eggs. Interestingly, some egg parasitoids need to learn to respond to the egg-induced plant changes, whereas others, the highly specialized ones, can respond innately to them. The high species specificity of the tripartite interactions between plants, insect eggs and egg parasitoids requires fine-tuned mechanisms of adaptation in each participant of this infochemical web.

Hilker & Meiners. 2011. *Phytochemistry* 72:1612-1623.
Beyaert et al. 2012. *Proc. Roy. Soc. London*, Ser. B 279:101-108.

THE CHEMISTRY AND EVOLUTION OF POLLINATION BY SEXUAL DECEPTION IN AUSTRALIAN ORCHIDS

Rod PEAKALL[1], Michael WHITEHEAD[1], Vasiliki FALARA[3], Ranamalie AMARASINGHE[1], Russell BARROW[2], Eran PICHERSKY[3]

[1]*Research School of Biology, Australian National University, Canberra, Australia*
[2]*Research School of Chemistry, Australian National University, Canberra, Australia*
[3]*Department of Molecular, Cellular, and Developmental Biology, University of Michigan, USA*

It has long been hypothesized that pollinators have played a key role in plant evolution. Yet, despite some evidence for pollinator-driven speciation, we still have much to learn about this potentially important process. Sexually deceptive orchids may offer an ideal system for exploring the role of chemistry and pollination in speciation. These orchids lure male pollinators to the flower by emitting floral odors that mimic the specific sex pheromone of the pollinator. In Australian sexually deceptive *Chiloglottis* orchids we have evaluated wasp pollinator specificity in the field; identified the compounds involved in pollinator attraction; mapped our chemical findings onto a phylogeny of the orchids and explored the population genetics of species boundaries. We discovered that the specific interaction between orchids and pollinators involve one, two or three compounds from a pool of six related chemical variants representing a new class of natural products, all 2,5-dialkylcyclohexane-1,3-diones or 'chiloglottones'. We have also established that pollinator specificity has a strong chemical basis, confirmed species boundaries are well defined by chemistry, and discovered that speciation is often associated with pollinator switching, usually underpinned by minor chemical change. Research attention has now shifted to uncovering the biochemical and molecular basis of chiloglottones. Surprisingly, continuous UV-B light is required for chiloglottone synthesis. Inhibition experiments indicate that chiloglottone precursors are derived from the fatty acid biosynthetic pathway. Collectively, this research indicates that plant speciation can be both rapid and potentially achieved by minor chemical and genetic changes.

THE EVOLUTION OF CHEMICAL ECOLOGY AND CHEMICAL ECOLOGISTS OVER THE DECADES

Jeremy N. MCNEIL[1]

[1]*Western University, London, Canada*

Chemical ecology has evolved very rapidly over the last 50 years. In this presentation I will give an overview of the advances made in the different sub-disciplines of chemical ecology, and pay tribute to some of the scientists around the world who have contributed to these advances. I apologise in advance for any omissions, particularly when it comes to mentioning key players, that members of the audience may think I have made: this is the result of time constraints and should not be taken as lack of appreciation of the excellent work being carried out in chemical ecology.

THE CHEMICAL ECOLOGY OF BACTERIAL DEVELOPMENT

Roberto KOLTER[1]

[1]*Harvard Medical School, Boston, MA, USA*

The molecular processes that become manifest as bacterial development are both fascinating and beginning to be well understood. The vast majority of this understanding has been derived from molecular genetic studies. Such studies have focused on analyzing development in the context of pure cultures of model bacteria, e.g. *Bacillus subtilis*, *Myxococus xanthus* and *Streptomyces coelicolor*. But these processes have evolved in the context of multi-species communities. Yet, our understanding of how development of one species is affected by the presence of other species is rudimentary at best. Clearly, small molecules released by neighboring species can have dramatic effects on the overall physiology of bacteria, including their development. These released small molecules thus mediate the chemical ecology of bacterial development. We have begun to apply the latest technological advances of mass spectrometry, including nano-scale desorption/ionization mass spectrometry and imaging mass spectrometry, to open new windows in order view the chemical interactions taking place between different species of soil bacteria. Our latest findings suggest that interspecies interactions trigger massive system-wide changes in the secreted metabolome of bacteria and that this secreted metabolome does indeed affect development. Our results illustrate that the chemical interactome of the terrestrial bacteriosphere is surprisingly vast and dynamic.

SYMPOSIA

SYMPOSIUM TOPIC 1: INTERACTIONS BETWEEN PLANTS AND ANIMALS IN THE AUSTRALIAN BIOTA

Topic Coordinators:
William Foley (Australian National University, Australia) and **Ben Moore** (University of Western Sydney, Australia)

COLOUR PREFERENCE IN *EUCALYPTUS*-FEEDING PSYLLIDS AND IMPLICATIONS FOR INSECT- PLANT ASSOCIATIONS

Kevin FARNIER[1], Adrian G. DYER[2], Richard A. PETERS[1], Martin J. STEINBAUER[1]

[1]*Department of Zoology, La Trobe University, Melbourne, Australia*
[2]*School of Media and Communication, RMIT University, Melbourne, Australia*

Psyllids that feed on eucalypts are diverse and abundant in Australian ecosystems. In native ecosystems, most psyllids are monophagous or narrowly oligophagous specialized on a limited range of *Eucalyptus* species. Psyllids are important taxa in which to study host specificity given their economic importance worldwide, however, knowledge of psyllid host location and acceptance of a eucalypt host is almost non-existent. Consequently, the mechanisms shaping psyllid-eucalypt associations can presently only be inferred from our understanding of behaviours in related taxa. This situation needs to be rectified if we are to understand phenomena such as host shifts when psyllids are introduced overseas. We used a multiple-choice arena to assess psyllid attraction to different colour stimuli and video recorded their movements to document how responses change with stimuli. Four species exhibited unique colour preferences suggesting that vision plays a central role in host discrimination. Interestingly, two species showed a very unusual attraction to long wavelength “red stimuli”. While similar attraction has been observed in some aphids, it has been attributed to achromatic vision (attraction to brightness), rather than due to true colour vision. Our investigations provide the first behavioural evidence for the potential existence of a long wavelength receptor in these two psyllid species; this has never been observed previously in Hemiptera. Our results demonstrate the prominent role vision plays in host location and suggest that psyllids utilize plant pigments and leaf reflectance to assess the quality of the foliage of a potential host (i.e. leaf type and age).

INTEGRATING THE RESPONSES OF AUSTRALIAN INSECTS TO EUCALYPT TERPENOIDS AND EPICUTICULAR WAXES INTO GENERAL CONCEPTUAL FRAMEWORKS OF HOST PLANT SELECTION

Martin J. STEINBAUER[1], Helen F. NAHRUNG[2]

[1]*Department of Zoology, La Trobe University, Melbourne, Victoria, Australia*
[2]*University of the Sunshine Coast, Maroochydore, Queensland, Australia*

Insect host plant selection proceeds via a catenary process of behavioural responses that enable individuals at distance from a plant to orientate toward, find and then assess the plant for its suitability as a host. The responses of insects throughout this process are mediated by the visual, olfactory, mechano-sensory and gustatory plant cues they perceive. This process has ecological and applied significance because it underpins insect host plant specificity and utilization. The leaves of eucalypts (*Eucalyptus*, *Angophora* and *Corymbia*) are renowned for their scented and waxy foliage. Olfactory and mechano-sensory stimuli play key roles in host plant selection by herbivorous insects and their relative significance is unique to each specific insect-plant association. Nevertheless, general trends are apparent according to the order of insect to which species belong. To advance our understanding, we consider it essential that future research addresses insect-eucalypt interactions using general conceptual frameworks such as outlined by Schoonhoven, van Loon and Dicke (2005, *Insect-Plant Biology*, Oxford University Press). We discuss how terpenoids and epicuticular waxes mediate the interactions of Australian insects with their host eucalypts, and integrate our examples into models of host location and acceptance using examples from Lepidoptera, Coleoptera and Hemiptera. We show that insect-eucalypt interactions are not oddities pertinent only to Australasia but provide excellent models of host plant selection by insects that utilize oleaginous and waxy plants, e.g. conifers and plants endemic to Mediterranean climates. We also consider the influence of waxes on visual and mechano-sensory plant cues.

EXPLORING THE CHEMICALLY-MEDIATED INTERACTIONS BETWEEN A MARSUPIAL HERBIVORE AND ITS ENVIRONMENT, AND HOW THIS CONTRIBUTES TO ITS ADAPTABILITY

Natasha WIGGINS[1,2], Stuart MCLEAN[2]

[1]*Boise State University, Boise, USA*
[2]*University of Tasmania, Hobart, Australia*

Herbivores detect and respond to chemicals in their environment, from plant chemical defenses to the scent chemicals they produce to communicate with other individuals. This research aimed to quantify the compounds, or mixtures thereof, that elicit chemically-mediated interactions between a generalist marsupial herbivore, the common brushtail possum *Trichosurus vulpecula*, and its environment. Possums are one of the most widely distributed marsupials in Australia, and we examined two chemical interactions that may contribute to their adaptability. Firstly, we measured the functional responses of possums to the defensive chemicals in their *Eucalyptus* diet using captive feeding trials. Secondly, we characterized the chemicals involved in scent marking in possums by analyzing with gas chromatography-mass spectrometry the compounds secreted from their cloacal glands. Possums tightly regulated their ingestion of dietary chemical defenses, enabling them to mitigate the effects of plant defences and avoid toxicosis. Possums fed more efficiently on chemically diverse diets, which may contribute to their exploitation of a variety of vegetation types across diverse landscapes. Their successful adaptation may also be attributed to their ability to alter their behaviour and social organization. The scent chemicals identified from possum cloacal glands included a large number of fatty acids and alcohols, esters, sulfur compounds and alkylglycerol ethers. These chemicals collectively act as a signature mixture of semiochemicals that likely provide an important means of communication between individuals. This research offers insight into how chemically-mediated interactions between a marsupial and its environment contribute to its adaptability across a diversity of diets and habitat types.

DIFFERENTIAL OVIPOSITION ON SUBSPECIES AND GENOTYPES OF *EUCALYPTUS CAMALDULENSIS* BY *LEPTOCYBE INVASA*, AN AUSTRALIAN GALL-FORMING WASP

Beryn A. OTIENO[1, 2], Baldwin TORTO[3], Martin J. STEINBAUER[1].

[1]*Department of Zoology, La Trobe University, Melbourne, Australia*
[2]*Kenya Forestry Research Institute, Nairobi, Kenya*
[3]*International Centre for Insect Physiology and Ecology, Nairobi, Kenya*

Herbivorous insects selectively choose the plant species and plant parts on which to lay their eggs. This choice is crucial in gall forming insect herbivores such as *Leptocybe invasa* (Hymenoptera: Eulophidae), as the survival of the immobile larvae rely on the transformed plant cells inside the galls. Since *L. invasa* adults do not feed on the hosts, mechano-sensory cues and chemical cues on the surface could indicate favourable conditions for larval development during host evaluation and determine the insect oviposition behaviour. *Eucalyptus camaldulensis,* which comprises seven sub-species, has shown variability in severity of galling caused by *L. invasa*. We sought to establish the role of leaf characteristics of subspecies and genotypes of *E. camaldulensis* to ascertain whether their differences can influence host discrimination during oviposition. The number of egg deposition marks on shoots of seedlings raised from seeds from the Australia Tree Seed Centre, was used to rank hosts on their susceptibility as assessed by wasps. While the genotypes varied on the specific leaf weight (SLW) and leaf water content, SLW was the leaf physical attribute that most influenced oviposition on the different genotypes. The genotypes of *E. camaldulensis* subspecies *simulata* and *refulgens* were least preferred for oviposition. Investigation of the influence of leaf terpenoid composition on oviposition preference of the different genotypes is underway. We discuss our findings in relation to natural distribution of *L. invasa* and *E. camaldulensis* in Australia.

PLANT QUALITY IS MORE THAN JUST CHEMISTRY: LINKING MORPHOLOGY, PHYSIOLOGY AND BEHAVIOUR TO NUTRITIONAL OUTCOMES

Fiona CLISSOLD[1]

[1]*University of Sydney, New South Wales, Australia*

Interactions between plants and their herbivores sit at the nexus of all food webs, and how well an animal matches its demand for nutrients with supply from the environment affects not only that individual, but has population- and community-level consequences. To understand trophic interactions within food webs, we need to be able to predict the nutritional consequences for a herbivore of ingesting a particular plant; however, grinding up plant tissues and measuring their chemical composition does not provide an indication of the quality of that plant to herbivores. While there is a growing understanding of which nutrients are required by insect herbivores, and in what amounts and relative proportions, as well as the consequences of restriction to imbalanced diets, this knowledge has come from studies using chemically defined artificial diets. The next challenge is to understand the realised nutritional outcomes for insects eating real plants. The ability of an insect herbivore that chews plant leaves to absorb nutrients depends on how these nutrients have been packaged within the plant, in interaction with the insect's food processing tools (typically the mandibles and gastrointestinal tract) and its behavioural and physiological flexibility. I will present experiments that set out to bridge this gap between plant composition and nutritional quality, using locusts as a model herbivore.

EUCALYPTUS SECONDARY CHEMISTRY IN A WARMER WORLD

Ben MOORE[1], Paul RYMER[1], John DRAKE[1], Mark TJOELKER[1]

[1]*Hawkesbury Institute for the Environment, University of Western Sydney, Richmond, Australia*

Globally increasing temperatures are fundamentally changing the constraints and challenges faced by plants, which, in many cases, exhibit local adaptation to pre-industrial climatic conditions. Of particular concern in Australia is how the widespread, ecosystem-dominant tree genus *Eucalyptus* will respond to warming and what the consequences will be for herbivores that rely on this resource. We investigated spatial patterns in foliar nutritional and secondary chemical traits, including the contributions of genotype and environmental temperature, in two widespread eucalypt species, *Eucalyptus grandis* and *E. tereticornis.* For each species, we grew trees from ten different provenances spanning their Australian distributions (northern NSW to north Queensland for *E. grandis* and Victoria to north Queensland for *E. tereticornis*). We grew each provenance in two glasshouse chambers, one with its climate controlled to match that of the trees' site of origin, and one with the temperature raised by 4 degrees. We monitored the growth and physiology of trees until they were harvested, at which stage we measured leaf traits (size, thickness, specific leaf area) and commenced foliar chemical analysis. Secondary chemistry considered included types and concentrations of terpenes and formylated phloroglucinol compounds, foliar nitrogen and the protein-digestibility-reducing effects of condensed tannins. Tree growth varied with latitude and trees from cooler-latitude provenances showed stronger growth responses to increased temperatures. We will present results of foliar chemistry against this background and that of changes in leaf thickness and specific leaf area.

INTERACTIVE, DIRECT AND PLANT-MEDIATED EFFECTS OF ATMOSPHERIC CO_2 AND TEMPERATURE ON INSECT HERBIVORES OF *EUCALYPTUS*

Markus RIEGLER[1], Tara MURRAY[2], Andrew GHERLENDA[1], David TISSUE[1], David ELLSWORTH[1]

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[2]*New Zealand School of Forestry, University of Canterbury, Christchurch, New Zealand*

Understanding interactive, direct and plant-mediated effects of elevated CO_2 and temperature on insect herbivores, and how changes in defoliation subsequently alter nutrient cycling and plant productivity, is essential to predict herbivore responses to a range of potential climate change scenarios. Few studies so far have measured interactive effects of CO_2 and temperature on insect herbivores, in particular over extended time periods, and have so far not included low pre-industrial CO_2. Hardly any have contrasted direct and indirect effects, both combined and separately, to estimate their relative importance. In a series of experiments we assessed both the interactive effects of CO_2 and temperature on foliar quality, and the interactions between direct and plant-mediated effects of CO_2 and temperature on the feeding activity and development of cup moths, common insect herbivores of Australian eucalypts. Eucalypt herbivores may be particularly sensitive to climate-driven shifts in plant chemistry as foliage is naturally low in N. Overall, insects performed best on foliage grown at pre-industrial CO_2. As expected, rising CO_2 increased leaf mass per area and leaf carbohydrate concentration, subsequently reducing leaf N. Lower leaf N induced compensatory feeding and impeded insect performance, particularly by prolonging larval development. Importantly, elevated temperature dampened the negative effects of rising CO_2 on larval performance. Therefore, rising CO_2 over the past 200 years may have reduced forage quality for eucalypt herbivore insects, but concurrent temperature increases may have partially compensated for this, and may continue to do so in the future. These results highlight the importance of assessing plant-insect interactions under multiple climate-change factors due to the potentially opposing effects of different factors within and between trophic levels.

DOES METHYL JASMONATE PRIME TERPENE DEFENSES IN NORWAY SPRUCE AGAINST BARK BEETLES AND FUNGI?

Tao ZHAO[1], Anna-Karin BORG-KARLSON[1], Paal KROKENE[2]

[1]*Royal Institute of Technology, Stockholm, Sweden*
[2]*Norwegian Forest and Landscape Institute, Ås, Norway*

Exogenous application of methyl jasmonate (MeJA) on Norway spruce stems is known to increase terpene levels in the phloem and sapwood, and make the trees much more resistant to attack by insects and pathogens. However, our recent results suggest that MeJA application may also prime Norway spruce defenses against subsequent invaders. Priming is the process by which defense responses to a challenge are accelerated or enhanced by prior stimulation. To characterize the possible priming effect of MeJA on terpene defenses in Norway spruce, we sprayed 100 mM MeJA on the stem bark of three Norway spruce clones. Water-sprayed ramets of the same clones were used as controls. Four weeks after MeJA treatment, we wounded 1×1 cm bark sections on each tree using a push pin and collected bark and sapwood samples from wounded and unwounded tissues over a 48 hour time series. Bark and wood samples were analysed separately for terpene contents using an Agilent 2D-GC-MS system. MeJA treatment alone had little effect on terpene levels in the bark and sapwood of these three clones. Mechanical wounding induced strong terpene responses in all trees, and MeJA-treated trees had much stronger terpene responses than control trees. In MeJA-treated trees, total terpene levels (sum of 169 terpenes) in wounded bark were 20.1-46.5 fold higher than in intact bark, compared to 9.1-10.6 fold higher levels in wounded vs. intact control bark. These results suggest that MeJA treatment may prime induced terpene defenses in Norway spruce.

SYMPOSIUM TOPIC 2: AQUATIC CHEMICAL ECOLOGY

Topic Coordinators:

Tilmann Harder (University of New South Wales, Australia) and **Justin Seymour** (University of Technology, Sydney, Australia)

CHEMICAL MEDIATION OF INTERACTIONS BETWEEN SEAWEEDS AND THEIR DIVERSE MICROBIAL COMMUNITIES

Peter STEINBERG[1,2,3]

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Marine eukaryotes such as seaweeds, sponges or corals are increasingly viewed as “holobionts”, in which the host and its associated microbial community are both necessary for normal functioning. One manifestation of the disruption of the normal functioning of holobionts is disease. Host secondary metabolites can play a key role in mediating disease and other interactions between marine eukaryotes and their associated microbes, and here I focus on how seaweed chemical defenses mediate the interplay between environmental factors, seaweeds and complex microbial communities. The Australian red seaweed *Delisea pulchra* suffers from a bacterial “bleaching” disease, particularly in summer when up to 80% of the population can be affected. Bleaching is associated with elevated temperatures, low levels of host halogenated furanones, and strong shifts in bacterial communities. Experimental manipulation of temperature or furanones enhances bleaching, and also influences the sequence of colonisation of bacteria on the host, which in turn influences the rate of bleaching. We have now characterized bleaching or related putative disease phenotypes in other Australian seaweeds such as kelps, along with shifts in their complex microbial communities and antibacterial host compounds. This research suggests that the effects of environmental factors, including anthropogenic ones, on marine eukaryotes such as seaweeds may be due to disruption of host-microbe interactions, rather than a direct physiological effect on the host, with environmental modulation of chemical signals from the host or microbes important in mediating these interactions.

CHEMICAL DEFENCES INFLUENCE MICROBES IN THE HABITAT-FORMING KELP *ECKLONIA RADIATA*

Rebecca NEUMANN[1,2], Tamsin PETERS[1,2], Ezequiel MARZINELLI[1,2], Peter STEINBERG[1,2], Tilmann HARDER[1,2]

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Despite the long-standing recognition of disease as a key process driving declines of marine habitat-forming species in the tropics, comparatively little is known about disease in temperate systems. *Ecklonia radiata* is the major habitat-forming macroalga in Australia, providing key resources that underpin biodiversity and ecosystem functioning. In previous field surveys, we identified thallus bleaching as a potential disease phenotype in this kelp and showed marked differences in bacterial community structure associated with bleached and healthy kelp at several sites along East and West Australia. In other seaweed-pathogen systems, antibacterial compounds are critical in defending against infection. To test the hypothesis that potential pathogenic bacteria of *E. radiata* are inhibited by putative defence compounds of the kelp, we screened the effect of algal secondary metabolites to stimulate or inhibit growth and impede attachment of selected bacterial isolates. Observed biological activities of purified HPLC fractions included the stimulation and inhibition of bacterial growth, and the inhibition of bacterial attachment. These results suggest that low-molecular weight algal secondary metabolites affect epiphytic bacterial colonization. Further, the compounds isolated from kelp with inhibitory effect on bacterial growth and attachment, are different from other known brown algal bioactive compounds, such as fucoxanthin and oligomeric phloroglucinols.

PRINCIPAL COMPONENT ANALYSIS OF THE ANTARCTIC SPONGE *DENDRILLA MEMBRANOSA* SECONDARY METABOLOME

Christopher WITOWSKI[1,3], Alan MASCHEK[1], Charles AMSLER[2], James MCCLINTOCK[2], Bill BAKER[1,3]

[1]*University of South Florida, Tampa, Florida, USA*
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The sponge *Dendrilla membranosa* is common on the benthos around the Western Antarctic Peninsula (WAP) and is easily recognized by its yellow cactus-like bulbs. Chemical defenses are employed by *D. membranosa* and many other sessile Antarctic marine invertebrates to deter predation and fouling. Diterpenoids aplysulphurin, tetrahydroaplysulphurin, and membranolide have been reported as feeding deterrents against known sponge predators. Sampling was undertaken around Palmer Station, Antarctica, each containing deep and shallow sponges for a comparison. The research herein is to describe correlations between the depth, chemical profile, and the feeding deterrent capabilities of the Antarctic demosponge. Purified crude extracts were analysed via LC/QToF-MS to quantify feeding deterrent concentrations and subjected to Principalle Component Analysis (PCA) to determine metabolic variations between location and depth.

DISCOVERING THE HIDDEN SECONDARY METABOLOME OF *SALINISPORA ARENICOLA:* A STUDY OF INTRA-SPECIFIC DIVERSITY

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The intra-specific diversity of secondary metabolites can provide important information to assist in our understanding of species ecology and evolution but it has received little attention to date in marine chemical ecology. A typical approach has been to target a limited number of major compounds to define specific differences but this fails to reveal the complex nature of diversity. As an obligate marine Actinobacteria, the *Salinispora* species are known to produce a broad spectrum of secondary metabolites. However, the degree of metabolic diversity within the species remains unexplored. We investigated the intra-specific (bio)chemical diversity of the sponge-associated marine bacterium Salinispora arenicola, collected from the Great Barrier Reef (GBR) off the north east coast of Australia. The (bio)chemical profiles of *S. arenicola* (n=60), spread over 3500 km across the GBR were obtained by UHPLC-QTOF-MS. We used Principal Component Analysis, Orthogonal Partial Least Squares Discriminant Analysis and Hierarchical Clustering Analysis to examine the patterns of variation in chemical diversity of this species. We observed that the diversity of the *S. arenicola* secondary metabolome is significantly greater than had been previously noted by using targeted metabolic analysis. Additionally, we found a clear pattern of increasing chemical dissimilarity with increasing geographic separation. The high level of diversity suggests that a significant number of natural compounds in *S. arenicola* are yet to be discovered and characterised, some of which are likely to originate from PKS- and NRPS-type biosynthetic machinery. The isolation and chemical characterisation of compounds obtained from this study are ongoing research subjects.

UTP IS A MAJOR COMPONENT OF SOLUBLE SEX PHEROMONES IN A MARINE SHRIMP, *LYSMATA WURDEMANNI*

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Our previous study indicated that the major component of distance (soluble) sex pheromone of the peppermint shrimp *Lysmata wurdemanni* is a Uridine-5'-di-phosphate (UDP)-like chemical. Since UDP itself does not elicit pre-copulatory behaviour in the shrimps we hypothesize that the UDP-like chemical is the related nucleotide uridine-5'-tri-phosphate (UTP) that is released during the female molt. Here we analysed shrimp samples and used biological assays to show that a UTP-like chemical is the major component of the distance sex pheromones of the shrimp. We ran a series of bioassays to examine whether UDP, UTP or their mixtures elicit male mating behaviour in the peppermint shrimp *L. wurdemanni*. Molting water of female shrimp was collected to analyze using HPLC. Our results show that male *L. wurdemanni* responded to UTP (but not to UDP) in displaying a stereotype courtship behaviour (approach and follow) identical to the natural behaviour that female molting water elicits. The mixtures of UTP and UDP did not increase the male mating behaviour response. Minimum effective concentration of UTP to elicit the courtship behaviour in the male shrimp was between 10^{-6} and 10^{-7} M. HPLC analysis showed the existence of UTP in the molting water of female shrimp and part of UTP breaks down to UDP during sample preparation. Both bioassay and chemical analysis results suggest that UTP or a UTP-like chemical is a major component of the distance sex pheromone in *L. wurdemanni*. The major peak of chromatograms of *L. wurdemanni* pheromones previously identified is a metabolism product of UTP.

DECODING PEPTIDE PHEROMONES THAT ELICIT ATTRACTION, AGGRESSION, SPAWNING AND PATERNITY SUCCESS IN MOLLUSCS

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Researchers have discovered thousands of pheromones, however, only in recent years has there been progress in our understanding of mollusc pheromones. Molluscs represent the second largest animal phylum, inhabiting both land and aquatic environments. Our studies have aimed at decoding their pheromone system, as a means toward understanding molluscan behaviour. This multidisciplinary research has demonstrated that:

1. *Aplysia* (sea slug) mate attraction and resultant breeding aggregations is the result of a cocktail of water-borne peptide pheromones that are released during the process of egg-laying.

2. Male Longfin squid are typically not aggressive. However, they become extremely aggressive within spawning grounds upon contact with a peptide pheromone embedded within egg capsules.

3. Oysters spawn gametes into the surrounding seawater, relying on reciprocal spawning of the opposite sex for successful gamete fertilization. Male oysters have a peptide/protein on the surface of the sperm membranes that signal to others that it is time to spawn.

4. Helicid snails are hermaphrodites that can mate multiple times prior to egg deposition. Mating snails will plunge a 'love' dart into conspecifics that deliver with it a peptide pheromone that increases paternity success.

These investigations have demonstrated the importance of peptide pheromones in mollusc communication. We expect that with the expanding availability in animal genome data combined with advances in the efficiency of mass spectrometry-based identification of peptides within complex solutions, we are now at the cusp of breakthrough discoveries in this area.

DISRUPTING PATHOGENESIS: CHEMICAL INTERFERENCE OF QUORUM SENSING BY AUSTRALIAN ALCYONACEA

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Bacterial symbionts are integral to the health, development and resilience of eukaryotic host organisms. Colonisation of host tissues by either beneficial or pathogenic bacteria often involves processes that are regulated through the chemical signaling pathway known as Quorum Sensing. Consequently, interaction with Quorum Sensing pathways and manipulation of Quorum Sensing signal molecules can enable mediation of the types and numbers of bacteria that inhabit eukaryotic organisms. Alcyonacean soft corals are known for their high concentrations of bioactive secondary metabolites. Amongst the most biologically significant of these secondary metabolites are the cembrene diterpenes and their lactone containing derivatives the cembranolides. Structural similarities between cembranolides and bacterially produced Quorum Sensing signal molecules, namely the presence of lactone rings, suggested a role in mediating the microbial assemblages of soft corals. To investigate this interaction 11 cembrene molecules with different levels of similarity were isolated from Australian soft corals. Two bacterial biosensor strains (*Agrobacterium tumefaciens* A136 and *Chromobacterium violaceum* CV026) were used to detect Quorum Sensing mediation by these metabolites. Quorum Sensing activity was consistently linked with the presence of a lactone or furan ring. Further, the ring size and substitution had a direct impact on the form of Quorum Sensing interference (induction or inhibition) that was observed. The results of this study provide new insights into the role these metabolites have in their original biological system. Further investigation will provide an understanding of how this interaction contributes to the health and resilience of soft coral.

A UNIQUE HOST PLANT LOCATION STRATEGY OF AN AQUATIC BEETLE

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Aquatic plant-insect interactions remain poorly documented, especially for turbid freshwater ecosystems. Many Swiss lakes offer such habitats, some of which are inhabited by the leaf beetle *Macroplea appendiculata* (Panzer). This donaciid beetle is the only beetle known to complete its whole life cycle entirely under water, where it lives primarily on perfoliate pondweed (*Potamogeton perfoliatus* L.), with *Eurasian watermilfoil* (*Myriophyllum spicatum* L.) as an alternative host plant. Direct observations during diving trips, aquatic olfacometer bioassays, and stir bar sorptive extractions coupled with GC-MS analysis were used to understand how this tiny organism can localize and reach patchily distributed host plants and congeners in a harsh, often swirling environment. In olfactometer assays we observed that the aquatic beetle is strongly attracted to water extracts of pondweed, whereas neither mature males nor females seemed to produce any attractive cue. The analyses of stir bar sorptive extractions revealed that perfoliate pondweed releases one dominating compound, eucalyptol. Further assays confirm that this was the main attractant for the beetle, but we also observed attraction to phytol, a minor pondweed compound. This finding is rather surprising as both eucalyptol and phytol are poorly soluble in water, and frequently described as insect repellents in terrestrial ecosystems. We propose that eucalyptol and phytol normally have a defensive function against herbivores and pathogens, but that the highly specialized leaf beetle has evolved resistance against these compounds and uses them as cues for optimal host plant exploitation.

COMPARISON OF THE CHEMICAL COMMUNICATION ALLOWING HOST SELECTION IN TWO MARINE SYMBIOSES WHICH ASSOCIATE CRUSTACEANS WITH ECHINODERMS

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Symbiosis is defined as a sustainable interspecific relationship between two organisms from different species. Communication is needed to insure symbionts recognition and the longevity of the association. Most of the time, interaction between host and symbionts is allowed through the production and the detection of natural secondary metabolites. Two different crustacean-echinoderm symbiosis models were investigated in this study: the harlequin crabs *Lissocarcinus orbicularis* and the snapping shrimp *Synalpheus stimpsoni*, two ectocommensals found, respectively, on the body wall of different species of holothuroids and crinoids. Thanks to a multidisciplinary approach (behavioral, chemical and morphological characterization), we highlighted (i) the chemical origin of kairomones allowing the recognition between symbionts and their hosts, (ii) the minimal quantity of secondary metabolites needed to stimulate symbiont chemotaxy, (iii) the chemosensory organs involved in this recognition, and (iv) the morphology of these organs by electron microscopy. A comparison is made to show similarities and divergences between the two studied models. While *L. orbicularis* is only attracted by the saponins produced by its sea cucumber host, *S. stimpsoni* is attracted by potential quinones produced by host and non-host crinoid species. Shrimps are no longer attracted by their host when their antennules are removed while crabs are still able to detect sea cucumbers without antennae and antennules. Moreover, crabs need a higher concentration to realize chemotaxy. The differences existing between crabs and shrimps are finally discussed taking ecological factors in consideration (e.g. host size, ecological niche, symbionts motion behaviors…).

LINKING DMSP-METABOLISM AND ANTIMICROBIAL PRODUCTION IN CORAL-ASSOCIATED BACTERIA

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Bacterial communities associated with healthy corals produce antimicrobial compounds that inhibit the colonization and growth of invasive microbes and potential pathogens. To date, however, bacteria-derived antimicrobial molecules have not been identified in corals. Here we report the isolation of an antimicrobial compound produced by *Pseudovibrio sp*. P12, a common and abundant coral-associated bacterium. This strain was also capable of metabolising dimethylsulfoniopropionate (DMSP), a sulfur molecule produced in high concentrations by reef-building corals and playing a role in structuring their bacterial communities. Bioassay-guided fractionation coupled with nuclear magnetic resonance (NMR) and mass spectrometry (MS), identified the antimicrobial as tropodithietic acid (TDA), a sulfur-containing compound likely derived from DMSP catabolism. TDA was produced in large quantities *by Pseudovibrio sp*., and prevented the growth of two previously identified coral pathogens, *Vibrio coralliilyticus* and *V. owensii*, at very low concentrations (0.5 µg/mL) in agar diffusion assays. Genome sequencing of *Pseudovibrio sp*. P12 identified gene homologs likely involved in the metabolism of DMSP and production of TDA. These results provide additional evidence for the integral role of DMSP in structuring coral-associated bacterial communities and the importance of these DMSP-metabolising microbes contributing to coral disease prevention.

DIMETHYLSULFONIOPROPIONATE (DMSP) IS A STRONG FORAGING CUE IN THE MARINE MICROBIAL FOOD WEB

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Phytoplankton-produced dimethylsulphoniopropionate (DMSP) is a precursor of dimethylsulphide (DMS), which, as a source of cloud condensation nuclei, can influence climate. DMSP and DMS are also foraging cues for several groups of marine animals. However, the role of these chemicals in marine microbial food webs, where they are produced and cycled, remains ambiguous. We measured the chemotactic responses of functionally diverse groups of marine microbes to microscale pulses of DMSP. In the marine environment DMSP is released into the water column in point sources, following phytoplankton exudation or grazing and lysis events, or diffuses from the surfaces of benthic organisms, including corals. We created diffusing pulses of DMSP, simulating these environmental gradients, using a microfluidic device. The behavioural responses of several marine microorganisms, spanning multiple trophic levels, were assessed using video-microscopy. Several functionally diverse marine microbes, including phytoplankton heterotrophic bacteria, a bacterivore and a herbivore exhibited strong chemotaxis to pulses of DMSP. Responses involved marked changes in swimming behaviour and resulted in up to 82 % increases in DMSP exposure. These chemotactic responses were driven by divergent ecophysiological demands and ecological motivations. A second microfluidic device was employed as an in situ chemotaxis assay to directly examine the extent of chemotaxis to DMSP by natural microbial communities within the marine environment, and revealed high levels of chemotaxis towards DMSP several marine habitats. Chemotactic responses to DMSP can markedly increase exposure to this compound in the environment, which will influence microbial uptake rates, important trophic interactions and oceanic sulphur cycling.

ANTIOXIDANT DEFENCE CAPACITY OF ACROPORA CORALS IN A CHANGING CLIMATE

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Dimethylsulphoniopropriate (DMSP) is produced in high concentrations by *Symbiodinium* in Acropora. DMSP is the precursor to acrylate and dimethylsulfide, a climate-regulating compound, generating cloud-condensation nuclei and influencing coastal irradiance. It is speculated they play an antioxidant role in mitigating high levels of reactive oxygen species (ROS) produced during *Symbiodinium* photosynthesis. With predictions of increased stress to reefs from climate change, it is important to elucidate the role of these molecules in corals. Likewise, reduced forms of ubiquinone-10 (CoQ_{10}) and plastoquinone-9 (PQ_9) are powerful antioxidants for cellular protection in addition to their roles in electron transfer. Although damage resulting from excessive ROS generation is implicated in coral bleaching, little is known about them during the stress response. Quantification of DMSP, acrylate, CoQ_{10} and PQ_9 in Acropora using nuclear magnetic resonance spectroscopy and liquid chromatography-mass spectrometry over the diurnal cycle is discussed. The coral CoQ_{10} and *Symbiodinium* PQ_9 pools are maintained predominantly in their reduced/antioxidant forms throughout the diurnal cycle. The PQ_9 pool undergoes midday oxidation due to high irradiance-induced oxidative stress while the CoQ_{10} pool and concentrations of DMSP and acrylate did not vary. These results provided a critical baseline from which stress-induced changes in these antioxidants in the symbiosis could be monitored. Hyperthermal stress causes oxidative stress in both *Symbiodinium* and coral. In contrast to the diurnal cycle, the coral CoQ_{10} pool was significantly oxidised and increased DMSP levels with a concomitant decrease in acrylate was observed, suggesting these molecules play an important role in the coral's antioxidant defence capacity.

SYMPOSIUM TOPIC 3: RHIZOSPHERE ECOLOGY

Topic Coordinators:
Leslie Weston (Charles Sturt University, Australia) and **Ulrike Mathesius** (Australian National University, Australia)

PLANT ROOT EXUDATES AND THEIR ROLE AS PLANT PROTECTANTS IN THE RHIZOSPHERE

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Plant root exudates play important roles in the development of plant and microbial community structure through their involvement in complex rhizospheric interactions impacting establishment and growth. Root exudates contain a diverse array of primary and secondary products that impact the composition of the rhizosphere by acting as a carbon source or serving as chemical signals. Secondary products released by roots are now characterized using sensitive methods for detection in root exudates or in soils, through use of LC and GC coupled to mass spectrometry. Recent advancements have allowed determination of their specific role(s) in the rhizosphere. Exudates frequently contain allelochemicals which play important roles in stimulation or suppression of neighbours including seeds, insects, herbivores and microbial communities. Plant roots can release large quantities of novel allelochemicals which play important roles in successful invasion of noxious weeds, or interference in the growth of crop plants. These “novel weapons” are produced by diverse biosynthetic pathways, and genes regulating their biosynthesis are frequently activated in response to environmental stimuli, including temperature, water availability, and presence of pests, microbes and herbivores. Bioactive root exudates from crops such as sorghum, oats, canola, fine fescue, lucerne and selected invasive weeds are presented. Advanced nanotechnologies including metabolomics, proteomics coupled with transcriptomics, and localization studies provide evidence of where and how these compounds are produced in the root, and factors regulating their production and transport in the rhizosphere.

ORGANIC ANIONS IN THE RHIZOSPHERE: TRANSPORT MECHANISMS AND FUNCTIONS

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Organic anions such as malate and citrate are not only central to plant metabolism but also play roles in allowing plants to modify the environment immediately surrounding their roots (the rhizosphere). Proteins embedded within the plasma membrane enable the transport of organic anions from inside root cells where they are synthesized to the rhizosphere. Organic anions can protect roots from abiotic stress and are also thought to enable plants to acquire nutrients and to modify the composition of microbial populations in the rhizosphere. The most thoroughly characterized transporters involved in the efflux of organic anions from roots belong to the aluminium activated malate transporter (ALMT) and multidrug and toxic compound extrusion (MATE) families of membrane proteins. Members of both of these families of transporters have been implicated in aluminium (Al^{3+}) tolerance mechanisms of plants in a range of species. Acid soils are prevalent in many regions of the world where agriculture is practiced and it is primarily the Al^{3+} solubilized by the acidity that restricts plant productivity. This presentation will focus on TaALMT1 and TaMATE1B from wheat and discuss their roles in protecting plants from Al^{3+} toxicity. Although both transporters enable the efflux of organic acids, their substrate specificities and mode of action differ from one another. The cloning of the genes has thrown light on their evolution as well as provided tools for improving crop production on acid soils.

DETECTION AND TRANSFORMATION OF SECONDARY PLANT PRODUCTS IN SOILS USING LC AND GC/MS – A METABOLOMIC PERSPECTIVE

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Bioactive plant products released into soil from one plant and taken up by another may not be the same, as their structure may have been altered by microbial transformation in the soil environment. In the European Framework 5 project FATEALLCHEM “Fate and Toxicity of Allelochemicals in Relation to Environment and Consumer” (2001-2004) a major effort was initiated that focused on the transformation in soil of benzoxazinoids, important bioactive compounds from cereal crops. Analytical techniques based on LCMSMS and LCMS-QTRAP were developed and subsequently several research projects with a similar focus were initiated on legumes. A vast array of active metabolites of both cereal benzoxazinoids and legume flavonoids, formed in soil due to microbial activity, have been identified and will be reviewed in this presentation. Recently a metabolomic approach based on library identification was developed in our lab in order to suggest possible modes of action of bioactive plant products. With this approach primary metabolites are identified in target plants subjected to co-cultivation with putative donor plants that exude bioactive secondary plant products. The model system was developed with *Trifolium repens* and *Arabidopsis thaliana* and uses GC-TOF-MS analysis and BinBase library search for detection and identification of compounds. Observed decreases in *A. thaliana* of aromatic and branched-chain amino acids suggested a suppressant effect of bioactive substances excreted by *T. repens*. Perspectives in the use of an approach that combines targeted quantitative analysis with the untargeted metabolomics approach will be discussed.

ROLE OF FLAVONOIDS IN SYMBIOTIC AND PATHOGENIC ROOT-MICROBE INTERACTIONS

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Plants produce secondary metabolites called flavonoids that interact with rhizosphere micro-organisms in various ways. The biosynthesis of flavonoids emerges from a common precursor and branches into several major classes as defined by their chemical structures. We established a genetic based approach to either silence or overexpress the different branches of flavonoid biosynthesis in the model legume *Medicago truncatula*. We subjected these plant roots to symbiotic or pathogenic interactions with bacteria, fungi and oomycetes and identified phenotypic changes induced by either absence or overexpression of different classes of flavonoids. We have established that silencing of flavan-4-ols and flavonols significantly reduces the root growth when infected with *Rhizoctonia solani*. The silencing of flavonols also significantly reduced lengths of transgenic *M. truncatula* when subjected to infections with the pathogenic oomycete *Aphanomyces euteiches*. Symbiotic interactions between rhizobia and *M. truncatula* roots were also adversely affected by the absence of flavonols or the overexpression of isoflavonoids. A drawback of this genetic approach may be its inadvertent effects on fluxes of other metabolites. A mass spectral analysis will be conducted to conclusively identify changes in the biochemical construction of the transgenic plants. It is concluded that different classes of flavonoids have varying roles in root-microbe associations and there is a possibility of enhancing these interactions, particularly with symbionts through manipulation of flavonoid biosynthesis pathway.

THE MOMILACTONE-MEDIATED ALLELOPATHIC INTERACTION BETWEEN RICE AND BARNYARD GRASS

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When barnyard grass and rice seedlings were grown together with enough nutrient conditions, the growth of barnyard grass and rice was reduced to 28 % and 69 % that of control barnyard grass and rice, respectively. Therefore, the chemical-mediated allelopathic interaction between rice and barnyard grass was investigated. Allelopathic activity of rice was elevated by the presence of barnyard grass seedlings or barnyard grass root exudates. Barnyard grass seedlings or their root exudates increased potent rice allelochemical, momilactone B secretion level from rice. Thus, barnyard grass-induced rice allelopathy may be due to the enhancement of momilactone B secretion. On the other hand, allelopathic activity of barnyard grass was also elevated by the presence of rice seedlings or rice root exudates. A key compound, which increased the allelopathic activity of barnyard grass, in the rice root exudates was isolated as momilactone B. The result suggests that barnyard grass may elevate the allelopathic activity by sensing momilactone B in rice root exudates. Therefore, momilactone B may not only acts as a rice allelochemical but also have a function in rice-induced allelopathic activity of barnyard grass. Rice and barnyard grass may be “aware” of each other by detection of some key compounds in their root exudates, and this sensorial function may trigger a signal cascade resulting in increasing the allelopathy through increasing secretion of allelochemicals into the rhizosphere. During the evolutionary process, rice and barnyard grass may have developed the chemical cross talk to activate allelopathy by detection of certain key compounds.

INFLUENCE OF ROOT CHEMICAL ECOLOGICAL CHARACTERISTICS ON PLANT RESISTANCE TO HEAVY METAL STRESS

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Induced chemical defense in plants, the organic acids and other metabolites exuded from roots under environmental stress factors, are the frontier fields of plant chemical ecology in recent years. A hydroponic experiment was conducted to investigate the impacts of Lead Pb stress on root exudates and growth physiological characteristics by Papilionaceae species (*Arachis duranensis, Arachis hypogaea*) and Crassulaceae species (*Sedum telephium, Sedum lineare*). The metabolism capacity and stress resistance of wild species under lead acetate solution culture are significantly better than the cultivated species. Lead contamination promoted root exudation of organic acids and soluble carbohydrate in all experimental species, and the promotion in wild species achieved statistically significant difference. The organic acids secretion in *Sedum* species roots occurred within 30 min of lead exposure, while organic acids secretion in *Arachis* species roots was induced by at least 4 h. Therefore, the former was classified to be pattern I, and the latter pattern II. Novel protein synthesis was not involved in Pb induced oxalate secretion in *Sedum* species, but was essential in *Arachis* species. However anion channels were involved in organic acid secretion of both pattern plants. There is a direct link between the capacity of plant stress resistance to heavy metal and the intensity of the organic acids and other chemical ecological metabolites exuded from roots.

MICROBIAL DIVERSITY AND EXPLORATION OF THE RHIZOSPHERE - A NEW FRONTIER

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Soil health is of critical importance for nutritional, economic and social well-being, and the associated requirement for the delivery of ecosystem goods and services. Microbes, in tandem with plants, play a central role in delivering key rhizosphere services and recently there has been increasing attention at the global scale to understand the ecological rules which structure microbial diversity and its effects on soil ecosystem services. Here, we will discuss the emerging theories of microbial diversity structuring in the environment, its testing by large scale survey using molecular biological methods and the experimental determination of the effects of microbial diversity in key rhizosphere nutrient cycling processes. We will demonstrate that microbial diversity is not randomly distributed around the environment, but can be highly predictable and that key rhizosphere processes are highly dependent upon the diversity residing within the rhizosphere.

INTERACTION BETWEEN GLYPHOSATE AND RHIZOSPHERE MICROBIAL DIVERSITY

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Young Vine Decline has been a serious problem for many Australian new vineyard plantings in the past decade. Affected newly planted vines either die soon after planting, or they survive but suffer from poor vigor and low yields for many years. We have consistently isolated plant pathogenic fungi *Ilyonectria* spp. from the roots of the declining young grapevines and we have shown that these fungi originate from grapevine nursery soils during the propagation process. As many grape-growers have reported that the disease symptoms were more severe when their vineyards were treated with the herbicide glyphosate, we initiated pot experiments showing that under water stress, glyphosate applied to the soil increased the severity of the disease symptoms caused by *Ilyonectria*. Further pot experiments showed that when weed leaf blades were treated with glyphosate it was translocated to the grass roots and then moved to the roots of grapevines planted in the same pot, causing leaf distortion similar to that caused by soil glyphosate application. We are now investigating changes to the microbial diversity on the glyphosate affected grapevine roots.

SOIL-BORNE MYCORRHIZAL FUNGI-MEDIATED ABOVE-GROUND PLANT DEFENSE

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Soil-borne arbuscular mycorrhizal fungi (AMF) are the most important symbionts for the majority of terrestrial plant species. We investigated effects of AMF colonization in roots on above-ground plant resistance against pathogens and insect herbivores and the related mechanisms. Mycorrhizal colonization enhanced host plant defense against both foliar pathogens and leaf chewing insects. AMF inoculation led to more allelochemical accumulation, increased transcripts of defense-related genes, as well as elevated activities of the putative defensive enzymes in plant leaves upon enemy attack, suggesting that mycorrhizal infection induces systemic defense responses. Our results also indicate that mycorrhizal colonization could prime defense responses in tomato, and that the jasmonate pathway is involved in defense priming by AMF. Common mycorrhizal networks (CMNs) link many plants together. We demonstrated that CMNs mediated plant-plant underground communication between healthy plants and pathogen-infected tomato plants. Our study indicates that soil-borne mycorrhizal fungi play an important role in protecting host plants against multiple enemies.

EFFECTS OF A RHIZOSPHERIC BIOCONTROL FUNGUS ON PLANT GROWTH AND ABOVE-GROUND HERBIVORE DEFENCE

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Several species/strains of the fungus *Trichoderma* are known to promote plant health and are widely used as commercial biopesticides. Beneficial effects of *Trichoderma* are attributed to various mechanisms such as enhanced growth, mycoparasitism, increased stress tolerance and the induction of plant defences against pathogens. As with other rhizosphere-colonizing microbes, some *Trichoderma* strains have been found to elicit Induced Systemic Resistance (ISR) by jasmonic acid/ethylene (JA/ET)-dependent pathways and to prime the expression of defence genes. Despite such well-established effects on pathogens, surprisingly little is known about the influence of *Trichoderma* on plant defences against herbivorous insects. In our experiments we investigated whether supplementation of the established biocontrol agent *T. atroviride* LU132 to non-sterile soil affected the performance of spring oilseed rape (*Brassica napus* cv 'Ability'), the induction and/or priming of defence-related phytohormones, genes and secondary metabolites as well as the performance of *Plutella xylostella* caterpillars. Plants colonized by *T. atroviride* LU132 had significantly larger root and shoot biomasses than controls. JA levels were increased by *P. xylostella* but were independent of fungal treatment. Similarly, defence-related genes MYC2 and TPI were induced by herbivory but not primed/induced by the fungus. No effects were observed in the expressions of ACO and PDF1.2 and in the production of glucosinolates. On average, development of *P. xylostella* caterpillars was 12.6% ($p = 0.06$) faster on control plants but pupal weights did not differ between treatments. We conclude that *T. atroviride* LU132 has positive effects on plant growth but its effects on aboveground insect defences are not yet clear.

MELOLONTHA MELOLONTHA: A ROOT FEEDING PEST IN APPLE ORCHARDS

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The European cockchafer, *Melolontha melolontha* L., is a large scarab beetle whose larvae are serious pests in apple orchards. About 98% (egg, larval and pupal stages) of the life of *M. melolontha* is spent in the soil. During this period, the larvae feed and destroy the roots of their hosts. Although the adults feed on the leaves of *Quercus* spp., *Acer* spp., *Carpinus* spp., *Fagus* spp. and *Prunus* spp., the effect of their feeding on leaves is seldom dangerous to the host. The larvae are voracious pests that feed on the roots of many crops including potato, lettuce, strawberry, apple tree and even some forest trees. The effect of feeding on root could lead to the eventual death of the host. Using apples (M9 rootstocks), we investigated whether or not the feeding damage caused by *M. melolontha* on apple roots alter the release of volatile organic compounds and hence herbivore induced plant volatiles (HIPVs) from the roots. We also investigated if such damage on the roots will cause a systematic release of HIPVs on the leaves that could be priming neighbouring plants. Our investigations revealed an alteration in the constitutive volatile compounds profile of both the roots and leaves of apple plants that had their roots fed on by *M. melolontha*. Our results may contribute to the understanding of below ground insect-plant interactions and open a new way of biological control strategy of *M. melolontha* in apple orchards.

USING CHEMICAL ECOLOGY TO IMPROVE THE BIOLOGICAL CONTROL OF ROOT PESTS WITH ENTOMOPATHOGENIC NEMATODES

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Larvae of the beetle *Diabrotoca virgifera virgifera* (Western corn rootworm) cause tremendous damage to maize roots in the USA. Since its incidental introduction into the Balkan region in the 1990s it has rapidly spread and become a serious problem in Europe as well. We think we have revealed the primary reason why the insect is so successful on maize. In feeding assays the larvae preferred to feed on highly nutritious crown roots of maize plants. These valuable roots were found to be well-defended with toxic benzoxazinoids that normally deter herbivores from feeding on these roots. The rootworm larvae, however, are completely unaffected by the toxins and use them to identify crown roots, which are rich in sugars and amino acids. How then can we fight this pest? One solution may be entomopathogenic nematodes, tiny parasitic worms that kill the larvae within days. We discovered that the nematodes are attracted to sesquiterpene *E*-(β)-caryophyllene, a chemical signal that is specifically emitted from maize roots after rootworm attack. Using genetic transformation we restored caryophyllene emission in an American maize line that had lost the signal, resulting in enhanced protection by nematodes against rootworm damage. Yet, caryophyllene-producing roots were also found to attract the rootworm larvae themselves. Using our knowledge of the system, we are currently developing capsules of biodegradable polymers that can be planted in pest-infested fields. The capsule shells will contain attractants and feeding stimulants that will attract the pest larvae and induce them to feed on the deadly capsules.

SYMPOSIUM TOPIC 4: PLASTICITY OF CONSTITUTIVE PLANT DEFENCES: MICROBES TO CLIMATE

Topic Coordinators:

Daniel Ballhorn (Portland State University, USA) and **Ros Gleadow** (Monash University, Australia)

EFFECTS OF MICROBIAL PLANT SYMBIONTS ON INSECT HERBIVORES

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Legume-associated nitrogen-fixing bacteria play a key role for plant performance and productivity in natural and agricultural ecosystems. Although this plant-microbe mutualism has been intensely studied for decades, studies on effects of rhizobia colonization on higher trophic levels are scarce. Rhizobia have been demonstrated to influence plant-herbivore interactions by altering food plant quality through enhanced nitrogen content. However, whether or not nitrogen provided by the bacteria also affects plant defenses remains widely elusive so far. We investigated effects of rhizobia on cyanogenesis (a direct defense) and soluble proteins (a nutritive trait). Effects of rhizobia-mediated plant traits on insect herbivores were evaluated in feeding trials. Our study suggests that nitrogen provided by rhizobia is allocated to nitrogen-containing defense-associated cyanogenic glucosides, and thereby crucially determines the outcome of plant-herbivore interactions. Depending on leaf age, direct defense via cyanogenesis was enhanced by factor three to eight in rhizobia-colonized plants. Furthermore fitness relevant plant growth parameters were significantly enhanced in rhizobial plants, while soluble protein concentration was not affected. Our study supports the view that the fitness benefit of bacterial root symbioses includes plant defenses and thus extends beyond the mere promotion of plant growth. Due to the enhanced direct defense, we interpret rhizobia as integral part of the defense system in legumes. Since the associations between legumes and nitrogen-fixing rhizobia are ubiquitous in terrestrial ecosystems, improved knowledge on rhizobia-mediated effects on plant traits—and the resulting effects on higher trophic levels—is important to better understand the role of these microbes for ecosystem functioning.

DEPLOYING THE CHEMICAL DEFENCE COCKTAIL OF EUCALYPT SECRETORY CAVITIES

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One of the most distinctive features of eucalypts is the essential oil secretory cavities which occur at a particularly high density in leaves. In some leaves, the secretory cavities can occupy up to 25% of leaf volume and thus contribute significantly to defence against herbivores. We characterised the complex chemical cocktail found within the lumen of the secretory cavities of a range of eucalypts, mapped its organized spatial arrangement and showed that it can change in response to herbivore assault. Contrary to the long-held view that the secretory cavities simply contain a mixture of mono- and sesquiterpenes – essential oil – we found them to be rich in non-volatile compounds, especially monoterpene acid glucose esters. All of the characterized esters were found to be based on oleuropeic acid or methiafolic acid, or both, and many contained phenolic aglycones. All of the esters were shown to contain at least one α,β-unsaturated carbonyl, which affords them various biological activities. We found that the non-volatile component of the secretory cavities is spatially separate from the essential oil, partitioning to the periphery of the spherical lumen. We also found that the composition of the non-volatiles changes in the hours following leaf wounding. Our work shows that eucalypt secretory cavities are a dynamic, highly organized, chemically complex domain for the deployment of biologically active chemicals, which likely moderate interactions between leaves and herbivores.

ROOTS TO SHOOTS: ABOVE AND BELOW-GROUND INVESTMENT IN CYANOGENESIS IN CROP SPECIES SUBJECT TO ENVIRONMENTAL VARIATION

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Cyanogenesis – the release of toxic HCN from endogenous cyanide-containing compounds – is a constitutive N-based defence found in over 5% of all species and ~60% of crop species. Known to be an effective defence against generalist herbivores, cyanogenesis in crops poses health risks for humans and animals. Dhurrin, the cyanogenic glycoside in sorghum, can cause cyanide poisoning in cattle, while consumption of ineffectively processed cassava - a staple food for >850 million people in the developing world - can lead to permanent paralysis or death. Here we report results from several studies on these two important crop species investigating the effects of environmental variation on resource allocation to cyanogenesis in above and below-ground tissues. In cassava, we sought to quantify the effects of water stress and temperature on yield and cyanogenic glycoside concentrations. Foliar and tuber cyanogenic glycoside concentrations increased with drought, and decreased upon rewatering. Increases in tuber toxicity with drought and at lower temperatures point to trade-offs between growth and secondary metabolism. Greater complexity arises in the different responses of cyanogenesis in above and below-ground tissues. Similarly, in sorghum, root and shoot cyanogenic glycoside concentrations responded differently to variation in soil phosphorus supply. Critically, the responses of sorghum to phosphorus also depended on ontogeny, demonstrating the importance of ontogenetically controlled comparisons to detect true plasticity in defence traits in response to environmental variation. Results are discussed in relation to plant defence theories, the regulation of cyanogenic glycoside synthesis in plants, and possible non-defensive roles of these metabolites.

CHANGES IN DEFENCE CHEMISTRY OF PLANTS GROWN AT ELEVATED CO2 MAY REDUCE THEIR NUTRITIONAL VALUE

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Higher concentrations of atmospheric CO_2 generally boosts plant growth but increases in efficiency of photosynthesis is likely to also impact plant composition. About 5% of all plants produce cyanogenic glucosides that release toxic cyanide (HCNp) when the plant tissue is chewed. Pasture plants such as clover and sorghum and ~30 species of *Eucalyptus* are known to be cyanogenic. Important staples that are cyanogenic include cassava and taro. High HCNp can reduce attacks by pests but too much cyanide can cause neurological diseases or even death in grazing animals and humans. The ability to tolerate cyanide is enhanced when the diet is also high in methionine and cysteine. This paper reports the results of a series of elevated CO_2 experiments using cyanogenic plants. Plants grown in the elevated CO_2 had a higher biomass but also allocated more resources to defence. In all cases the concentration of foliar phenolics increased, but HCNp typically remained unchanged, but not always. Foliar N and protein decreased in all studies except one, leading to an overall but significant increase in HCNp relative to protein. A notable exception was forage sorghum, a C4 grass, which showed no change in the cyanide: protein ratio. A consequence of lower leaf protein means that grazing animals need to consume a greater biomass to meet their protein requirement, potentially also ingesting more phenolics and cyanogenic glucosides. The Monash Cyanogenesis Group are using a combination of plant breeding, agronomy and food processing methods to try to address this issue in agricultural plants. The probable decrease in nutritional value of trees such as *Eucalyptus* may mean that they will no longer be able to support arboreal herbivores such as koalas and possums in the future.

SYMPOSIUM TOPIC 5: CHEMICAL ECOLOGY OF POLLINATION

Topic Coordinators:
Rod Peakall (Australian National University, Australia), **Bjorn Bohman** (Australian National University, Australia) and **Anna-Karin Borg-Karlson** (Royal Institute of Technology, Sweden)

ADVANCES IN THE ELECTROANTENNOGRAM TECHNIQUE FOR USE IN CHEMICAL ECOLOGY RESEARCH

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The electroantennogram (EAG) technique is nearly 60 years old, and yet new ways are still being found to refine its use for addressing new questions in insect chemical ecology. The EAG continues to be effective in aiding in the isolation and identification of behavior-modifying chemicals such as pheromones and host plant attractants. Recent improvements in optimizing signal-to-noise ratios show promise in helping isolate behaviorally active trace compounds in airborne odor blends that would likely otherwise escape detection via coupled gas chromatophraphy/electroantennographic detection (GC/EAD). In the field, an EAG coupled to a sonic anemometer can provide evidence of "hits" of volatile compounds and identify the locations of the emission sources tens of meters downwind when insect pheromones are used as "taggants". Such experiments can help address questions concerning aerial transport of otherwise antennally undetectable plant volatiles to which even the conspecific antenna is too insensitive to record significant EAGs. There are many possible new ways to use the EAG technique in studying the ecology of pollination. Caution is advised, however, in using results from the GC/EAD to assume behavioral activity from positive EAD depolarizations alone. Sometimes EAG responses from GC/EAD recordings are over-interpreted, and EAG-responsive but behaviorally inactive compounds are incorrectly labeled as "components" of the attractant blend based solely on the GC/EAD results. Results of some newer studies using modifications of the EAG techniques that I will present may encourage members of the audience to be creative and try new EAG methods themselves for their own possible research advances.

THE KEY TO FLOWER OPENING: JASMONATE SIGNALING AND FLOWER DEVELOPMENT IN *NICOTIANA ATTENUATA*

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Numerous flowering plants evolved nutritious rewards or noticeable coloration to assure pollinator attraction, fertilization and hence, survival of their species. The expression of these floral traits has been shown to be tightly controlled by the combined action of jasmonic acid (JA) and its derivatives (jasmonates) with other phytohormones. For the success of its biological function, correct opening of the flower (anthesis) is crucial. However, little is known about the role of jasmonate signaling during anthesis. We used different *Nicotiana attenuata* transgenic lines impaired in jasmonate biosynthesis or perception to address this question. JA and JA-isoleucine-deficient plants showed a delay in flower elongation and aberrant corolla opening. Phytohormone profiling of WT corollas at different developmental stages revealed a pronounced jasmonate burst preceding the rapid corolla elongation and opening. Consistent with JA-Ile acting as central developmental cue for corolla opening, exogenous application of the JA-Ile precursor methyl jasmonate or a JA-Ile functional analog, coronatine (COR), restored corolla opening in jasmonate deficient lines. The COR treatment also partly rescued other flower alterations of transgenics such as floral volatile emission or nectar production. We further determined that the sensitivity of corolla tissues to COR as signal for flower opening was restricted to an early phase in corolla development. Altogether, our results suggest that a jasmonate burst, programmed early in corolla development, defines later steps including the flower opening. Ongoing metabolomic and transcriptomic studies of corolla limb tissue will provide further insights into how corolla primary metabolism, regulated by jasmonates, is driving floral opening.

CARRION MIMICRY OF THE WORLD'S LARGEST FLOWER, *RAFFLESIA CANTLEYI* (RAFFLESIACEAE)

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Most angiosperm plants associated with carrion flies as pollinators emit a foul-smelling stench and it has been shown in several studies that the chemical compounds emitted by the flowers are the same cues that normally guided the flies to locate carrion or dung. However, investigations of the chemical composition of the scent of sapromyiophilous flowers indicate that there may be a number of different chemical strategies including mimicry of carrion (characterized by high content of oligosulphides), mimicry of faeces (characterized by emission of p-cresol, phenol and skatole) and mimicry of urine (various acids). We investigated the floral scent composition of *Raffflesia cantleyi*, which is endemic in Peninsular Malaysia, via dynamic headspace and thermo-desorption/GC-MS. Eighteen compounds belonging to 4 main groups of chemicals were identified, ie aliphatic compounds (4), monoterpenes (5), sulphur containing compounds (2), and benzenoids (7). The scent of the floral bouquet of *Rafflesia* in full bloom was clearly dominated by the sulphur containing compounds (>85%), DMDS (dimethyl disulphide) and DMTS (dimethyl trisulphide), suggesting mimicry of carrion. Of all the insect visitors of *Rafflesia*, more than 80% were dipterans from 17 families. Of all the five species of calliphorid flies caught, only *Chrysomya chani* was found to bear the pollen of *R. cantleyi*. Laboratory wind tunnel assays using synthetic chemicals showed that both DMDS and DMTS are active and largely responsible in attracting *C. chani* to the flowers.

DO *PIERIS NAPI* HOSTPLANT FLOWERS MIMIC ANTIAPHRODISIAC TO AVOID EGGLAYING FEMALES?

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The aim of this study was to chemically compare the volatiles produced by the various host plants of egglaying and nectar feeding *Pieris* spp. Chemical investigation of the flower volatiles, using SPME-GC-MS, of the most preferred host plants of *Pieris spp*, revealed a number of aromatic compounds among them the characteristic antiaphrodisiac blends earlier identified in spermatophores from *Pieris napi, P.rapae* and *P. brassiace*. Whether the chemical mimesis reduces visits of egglaying females and thus protect the flowers from being eaten by butterfly caterpillars will be discussed.
Andersson et al. 2000. *Proc. Roy. Soc. B*, 267, 1271-1275.
Andersson et al. 2004. *Proc Biol Sci*. 271**,** 1765–1770.

SEX SMELLS: FLOWER-MIMICKING FUNGI EXPLOITING THE SAME HOST SPECIES ARE CHARACTERIZED BY DIFFERENT ODOUR BLENDS

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The pea rust *Uromyces pisi* [*sensu lato*] comprises a species complex that completes its sexual life cycle on Cypress spurge *(Euphorbia cyparissia*s L.). Plants infected by the species of this fungal complex all show the same phenotype and are morphologically indistinguishable. Infected spurge plants are generally inhibited from flowering; instead they form so-called pseudoflowers composed of yellow fleshy leaves, thus mimicking true flowers in color and shape. Furthermore, these pseudoflowers present scent and sugary nectar mixed with fungal gametes (spermatia) on their abaxial leaf surfaces and it has been demonstrated that nectar feeding insects are required to transfer spermatia – in analogy to pollen from flowers – from one mating type to another. We investigated the scents of three infected *E. cyparissias* populations near Würzburg, Germany by HS-SPME. Two scent types were recognizable to human noses: sweet-fruity-floral and spermous-musty. Fungal species identity was determined by sequencing the ITS region of the rDNA. Our results revealed that at least three different species (*U. pisi, U. striatus, U. punctatus*) colonized *E. cyparissias* in the Würzburg area. Phylogenetic analyses suggested that the taxonomic affiliation of the fungal parasite was responsible for the type of scent emitted by the pseudoflowers. Only *U. pisi* [*sensu stricto*]-infected plants emitted 1-pyrroline and were linked to the spermous-musty odour. This compound is associated with amino acid breakdown, bacterial fermentation or a fruit fly pheromone blend, for example. Further work needs to investigate whether these different odour blends attract a specific set of insect species that confer reproductive isolation.

THE EFFECT OF DIESEL EXHAUST POLLUTION ON THE ABILITY OF HONEY BEES TO DETECT AND RESPOND TO FLORAL VOLATILES

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In recent years the numbers of honey bees, *Apis mellifera*, and other pollinators have declined. The reduction in number of honey bee colonies is most likely caused by multiple stressors including pests, diseases, poor nutrition, habitat losses, environmental pollution, etc. The objective of our study is to investigate if diesel pollution is another contributor to this decline. We investigated if diesel exhaust alters floral volatile compounds using two different eight-compound blends, one representing oilseed rape and one composed of the most commonly occurring floral volatiles. Within 1 minute of exposure to diesel exhaust both blends were significantly altered, some compounds became undetectable, some were considerably reduced, and one was transformed into its *cis*-isomer. To investigate the impact of our findings on honey bees, we conducted a series of proboscis extension response assays and found that these alterations to the blends reduced the ability of honey bees to recognize them. Diesel exhaust is a complex mixture of toxic and reactive gases including carbon monoxide, nitrogen oxides (NO_2), and sulphur dioxide, any of which could be the cause of the changes in our floral blends. Additionally it contains particulate matter, which has the potential to act as a catalyst. We found that at concentrations of 10ppm NO alone reduces the amount of some floral volatiles significantly. The other exhaust gases and atmospheric ozone most likely also contribute to the changes in odour plumes, which could impact honey bee foraging and insect chemical communication in polluted environments.

EFFECTS OF A PLANT-ACCUMULATED POLLUTANT ON POLLINATOR-PLANT INTERACTIONS

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Selenium (Se) has contaminated soils and plants in many areas worldwide where pollination can be critical to the functioning of both agricultural and natural ecosystems, yet we know little about how pollutants can impact insect pollinators. The objectives of our research were to investigate the accumulation of Se in a common weed (radish, *Raphanus sativus* L.), and then determine the behaviorial, sublethal, and lethal effects on a common insect pollinator (*Apis mellifera* L.). Despite modest accumulation in the foiliage, the levels of Se in the pollen and nectar were quite high. Nonetheless, Se-treated plants remained attractive to honey bees and were not pollen limited. In laboratory studies, bees were fed the four common forms of Se found in plants (selenate, selenite, methylselenocysteine and seleno-DL-cystine). Inorganic forms of Se had significant sublethal consequences on the bee's gustatory behaviors. In toxicity bioassays, honey bee larvae were fed the same four forms of Se in artificial diet and survival was quantified. Inorganic forms of Se were most toxic to foragers, although all forms were toxic at high concentrations. Given that pollinators such as the honey bee do not avoid Se compounds in the plant tissues they are foraging on, and wild radish is prized by beekeepers as a transition plant that flowers when other species are not available, the potential exists for signifcant losses.
Hladun et al. 2013. *Environmental Pollution* 172, 70-75.

FLORAL SCENT AND FLOWER VISITORS IN A HYBRIDIZATION SYSTEM: A TEST OF THE HYBRID BRIDGE HYPOTHESIS

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The Hybrid Bridge Hypothesis (HBY) proposes that hybrid plant species might support more plant-associated animals compared to its parental species due to hybrids' intermediate phenotype. The cross between the central Chile asteraceaous shrubs *Haplopappus foliosus* and *H. chrysantemifolious* originates the fertile hybrid *H. decurrens*. Using this system, we measured flower fragrances and insect visitations to test the HBY in the field. Using dynamic headspace sampling followed by GC-MS analysis, we identified ten main volatile compounds in 112 samples, nine terpenoids and one aromatic compound. Nine of these were detected in the parental species *H. foliosus*. The parental specie *H. chrysantemifolius* had only five of these compounds, whereas the hybrid species (*H. decurrens*) had nine of these compounds. Moreover, we identified a total of 47 insect species in *H. foliosus*, while in *H. chrysantemifolius* and *H. decurrens* species richness equaled 30. The community of visitors of *H. foliosus* and *H. decurrens* has the highest degree of similarity (J=0.60), compared to the communities of *H. foliosus* and *H. chysantemifolius* (J=0.48), and *H. decurrens* and *H. chrysatemifolius* (J=0.42). Our results do not support the Hybrid Bridge Hypothesis. Instead, we suggest a relationship between floral fragrance chemical diversity and visitor species richness. Our findings stress the relevance of hybridization as a vehicle in the formation of new niches of diversification to flower foraging insects, and the value of plant fragrance in the conformation of the associated insect community. Research Funded by Proyecto Iniciación FONDECYT 11100130 and Proyecto Inserción CONICYT 79100013 to Villagra.

SYNOMONAL FRAGRANCE OF FRUIT FLY ORCHIDS, *BULBOPHYLLUM MACRANTHUM* AND *BU. PRAETERVISUM,* TO ATTRACT FRUIT FLIES AS POLLINATORS

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Several *Bulbophyllum* (Orchidaceae) species attract males of *Bactrocera* fruit flies (Tephritidae: Diptera) by emitting a specific floral fragrance during pollination in the rainforests of Southeast Asia. Flowers of two sympatric sibling species are morphologically very similar in Malaysia - *Bu. macranthum* and *Bu. praetervisum*, possess an elaborate mechanical structure comprising slippery lateral sepals with the highest synomone content, and a movable lip. While licking on the sepals, a fruit fly either directly falls or it slips on to the lip, which then tips it backwards. Either way, the fly lands abdomen first into the floral column cavity and the pollinia are stuck on the fly's abdomen and removed after much struggling. However, if the fly bears pollinia, the pollinia would stick to and ultimately be deposited on the stigma to complete pollination. The synomonal attractants in floral fragrance of both species were examined. Zingerone (ZN) was identified as the major component of *Bu. macranthum* originating from Malaysia and Thailand. This explains why the flower attracts both methyl eugenol (ME)-sensitive species (e.g. *Bactrocera dorsalis and B. umbrosa*) and raspberry ketone (RK)-sensitive species (e.g. *B. cucurbitae* and *B. tau*). Floral ZN is incorporated into the male rectal gland, and acts as a sex pheromone when emitted to attract conspecific female flies. In contrast, flowers of *Bu. praetervisum* exclusively produce RK; and selectively attract RK-sensitive fly species. The diversion of the floral synomone implies a possible fine-tuning of an adaptive mechanism of orchids to effective fruit fly species in the coevolutionary process between flowers and pollinators.

IS SEXUAL DECEPTION OPERATING AS THE POLLINATION SYSTEM IN THE NEW ZEALAND GREENHOOD ORCHIDS (*PTEROSTYLIS SPP*.)?

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Sexual deception is a species-specific pollination system, attracting male insects by plant emission of an attractive volatile mimic of the female insect pheromone. Previous studies in Australia suggest that *Pterostylis* species may be sexually deceptive, attracting small male fungus gnats (Diptera: Mycetophilidae). However, very few studies have been done on this genus in New Zealand, and no conclusive evidence has been provided for this pollination strategy in New Zealand or Australia. Knowledge of this reproductive strategy in *Pterostylis spp.* may explain niche partitioning enabling closely related species to co-exist. The aim of this study is to determine whether sexual deception is operating as a pollination system in sympatric *P. oliveri* and *P. irsoniana* growing in native beech forests in Arthurs Pass, New Zealand. Breeding system experiments suggest that the orchids are self compatible but exclusively dependent on pollinators. Male fungus gnats with pollen attached to their thoraxes were caught in traps adjacent to the *Pterostylis* flowers. According to DNA analysis, only *Mycetophila latifascia* males were found with pollen of *P. oliveri*, and only *Morganiella fusca* males with pollen of *P. irsoniana*, suggesting that sexual deception may be operating in these two species. Compounds of interest have been identified in the headspace volatiles of *P. oliveri* and *P. irsoniana* flowers. Further work is required to determine whether a putative pheromone mimic compound is present in the female *M. latifascia* sex pheromone, along with proof of the male response to the putative female pheromone.

SEXUALLY DECEPTIVE ORCHIDS: WHO NEEDS A PHEROMONE TO SEDUCE A WASP?

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Orchids are well known for their diverse pollination systems. One particularly unusual system is pollination by sexual deception, where the flower mimics the female's sex pheromone to attract male insects as pollinators. One of many Australian genera of orchids that employ sexual deception as a pollination strategy is the Western Australian endemic *Drakaea* (hammer orchid) that attracts specific male thynnine wasps as pollinators. Over the last few years we have conducted GC-EAD/MS analysis of flower extracts, SPME of sexually calling female wasps, and gland extracts from female wasps in order to discover the semiochemicals involved in the pollination of hammer orchids. During the process of identifying orchid semiochemicals, both the pheromone components and synthetic analogues have been prepared and subsequently tested with EAD and in field bioassays. The emerging evidence from our studies suggest that while the sharing of compounds between females and orchids is common, some orchids may be able to achieve pollinator attraction by using analogues of the pheromone. This finding was initially surprising given the extreme pollinator specificity that characterises this pollination system. However, it suggests evolutionary flexibility from the plant point of view that may in part account for the high diversity of sexually deceptive species among Australian orchids. The most recent results for several species of *Drakaea* will be presented and compared with the corresponding natural pheromones, providing new insights to the chemistry of sexually deceptive orchids.

THE SHARING OF POLLINATORS BY SYMPATRIC GENERA OF SEXUALLY DECEPTIVE ORCHIDS – THE IMPORTANCE OF CHEMISTRY FOR POLLINATOR BEHAVIOUR

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Pollinator sharing can offer powerful insights into the floral traits associated with the evolution of a pollination system and the consequences of floral differences for pollinator behaviour. Here we report the first known case of pollinator sharing between two sexually deceptive plant genera. *Drakea livida* and *Caladenia pectinata* (Orchidaceae) exhibit dramatic differences in floral display and the insectiform appearance of the labellum, yet both are pollinated by sexually attracted males of the thynnine wasp *Zaspilothynnis nigripes*. Floral dissections revealed that the semiochemicals used to attract the pollinator are released from the labellum in *D. livida* and the sepaline clubs in *C. pectinata*. Experiments revealed that the two species did not differ in their long-range attraction of pollinators. However, *Drakaea livida* was more efficient at converting pollinator attraction into potential pollen deposition, leading to higher fruit set. In *C. pectinata*, pollinators are frequently attracted to the sepaline clubs that are positioned well away from the pollen and stigma. Floral manipulations showed that sexual attraction and pollinator contact with the labellum increases when it is artificially made to be the source of the semiochemicals. Thus, interspecific differences in floral traits, including the site of semiochemical release, have important consequences for converting attraction into pollination. Recent work on the chemistry of the floral odour indicates that novel pyrazines are employed by *D. livida* to sexually attract its pollinator. Work is now proceeding to identify whether or not the same compounds are employed by *C. pectinata.*

SYMPOSIUM TOPIC 6: EVOLUTION OF CHEMICAL COMMUNICATION IN THE ERA OF GENOMICS AND TRANSCRIPTOMICS

Topic Coordinators:
Christer Löfstead (Lund University, Sweden) and **Astrid Groot** (Institute for Chemical Ecology, Germany)

PATTERNS EMERGING IN THE GENETIC BASIS OF MOTH SEXUAL COMMUNICATION

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Even though sexual selection is generally hypothesized to be an important factor in the speciation process, it is still unclear when and how divergence in sexual communication occurs. In moths, female signals and male response are governed by independently assorting genes, and it is still a mystery how moth signal and response may co-evolve. Our research focuses on the identification of these genes to gain insight into the underlying mechanism of divergence. Our main species of interest are the two closely related noctuid moths *Heliothis virescens* and *H. subflexa*, in which we are assessing the genetic basis of inter- and intraspecific variation. In our quantitative trait locus (QTL) analyses of inter- and intraspecific crosses, we found up to 12 QTL for interspecific pheromone variation and 1-3 QTL for intraspecific variation. Using RADtags, we identified homologous chromosomes of our interspecific and intraspecific genetic maps, and found overlapping genomic regions that are involved in the inter- to intra-specific variation. We obtained sequences for candidate genes involved in the pheromone biosynthetic pathway by generating a cDNA library of the pheromone glands. Recently, we also generated a large comparative RNA-Seq dataset, comparing the transcriptome of the glands to that of other tissues. We are currently assessing which genes map to the QTL regions and how these genes may be involved. So far, the main candidate genes do not map to the QTL regions, suggesting that trans-acting transcription factors are involved. Our results will be discussed in light of possible evolutionary forces shaping sexual attraction.

SEX PHEROMONE EVOLUTION IN NEW ZEALAND LEAFROLLER MOTHS: A STORY OF DIFFERENTIAL GENE REGULATION

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The nature of the molecular changes responsible for the evolution of new species remains a major question for evolutionary biology. The mate recognition systems used by many moths provide a unique opportunity to understand the molecular changes involved in producing and detecting new sex pheromones. We have generated transcriptome and genome databases from two sets of sibling species of New Zealand endemic leafroller moths that utilise distinct sex pheromone blends. Using these databases, candidate genes involved in pheromone biosynthesis and reception have been identified and functional work undertaken to test their roles and examine genetic differences between species. Seven desaturase genes have now been identified in species within the genera *Ctenopseustis* and *Planotortrix* using recombinant production in yeast, with activities ranging from the typical Δ9 desaturases through to a novel Δ5 desaturase. In both genera, differential regulation of the same Δ10 desaturase gene (*desat5*) within the pheromone gland is involved in producing the different sex pheromone blends. To investigate the molecular basis of the regulational change involving *desat5*, crosses between *P. octo* and *P. excessana* were conducted. The pattern of *desat5* expression in the progeny of all combinations of F_1 and F_2 crosses provides evidence for a major *trans*-acting repressor and also a required *cis*-regulatory sequence in explaining the differences in *desat5* expression and the resulting distinct sex pheromone blends.

THE EVOLUTION OF MULTI-COMPONENT SEX PHEROMONES IN MOTHS

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A substantial amount of pheromone research has focussed on the long-distance volatile sex pheromones produced by female moths. The chemical compositions of these pheromones have now been identified for several hundred species, providing us with a wealth of data that can be used to ask evolutionary questions in a comparative context. In approx. 30% species these pheromones consist of a single chemical component, but in all others the pheromone has been identified as a blend of several components (mean = 2.16 components, n = 570 species). Why do some species utilise single chemical compounds, whilst others have more complex formulations? I used a phylogenetic comparative approach to investigate the evolutionary history of multi-component sex pheromones. I found that there is very little phylogenetic signal in the 'complexity' (i.e. number of components) of pheromone blends, although closely-related species do tend to utilise chemical compounds with similar molecular weight. Greater complexity is not necessarily found in more species-rich clades in the phylogeny, but high levels of complexity tend to be found in species that have originated close to the tips of the phylogeny, suggesting that use of substantial numbers of components may represent a short-term evolutionary stage.

EVOLUTION OF GENETIC VARIANCE IN CUTICULAR HYDROCARBONS UNDER SELECTION

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Determining how the genetic variance evolves under selection in the presence of widespread pleiotropy is a fundamental issue that is ultimately related to the question of how genetic variance is maintained in populations. The study of sexually-selected traits is particularly useful here as male sexually-selected traits are often relatively easy to identify and selection generated by mate choice can be readily quantified. At the same time, sexually-selected traits are often postulated to be subject to natural selection, either through pleiotropic associations with condition and other fitness components, or as a consequence of selection on mate recognition generated by the presence of other species. In a series of experiments using *Drosophila serrata* as a model, we have investigated the relationship between selection and genetic variance in a multivariate set of contact pheromones. While directional sexual selection is associated with very low levels of genetic variance in natural populations of this species, the genetic variance in these traits can rapidly evolve, increasing dramatically as males are selected for higher attractiveness. However, it is the pleiotropic associations between the sexually-selected traits and other (unmeasured) traits under natural selection that have been revealed to play the key role in limiting the response to sexual selection, and the maintenance of multivariate genetic variance in traits under sexual selection.

FUNCTIONAL GENOMICS REVEALS MOLECULAR MECHANISMS UNDERLYING THE EVOLUTION OF SEX PHEROMONE DETECTION BY MOTHS

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Moth sex pheromone receptor genes are a conserved lineage of the odorant receptor (OR) family. They are expected to be subject to strong selective pressure because of their preeminent role in mating behavior. Male moths typically respond with high specificity to the sex pheromone blend produced by conspecific females. The molecular mechanisms that enable male moths to detect new pheromone blends when the communication channel shifts between closely related species has been a long standing question. Several closely related moth species in the genus *Ostrinia* use varying ratios of E11- and Z11-tetradecenyl acetate as their main sex pheromone (E11- and Z11-14:OAc). The Asian corn borer (ACB) has evolved to use E12- and Z12-tetradecenyl acetate (E12- and Z12-14:OAc), a unique sex pheromone in this genus. Using functional genomics we identified ACB OR3 and OR6 as having evolved specificity to its unique pheromones, compared to the European corn borer (ECB). By functionally expressing the receptors in *Xenopus* oocytes we determined that ACB OR3 and OR6 are more narrowly tuned to the E12- and Z12-14:OAc pheromones compared to ECB OR3 and OR6. Using a mutagenesis approach we identified specific amino acid polymorphisms that significantly reduce receptor responses to the E11- and Z11-14:OAc pheromones, making the ACB receptors more specific to the E12- and Z12-14:OAc pheromones. Inferences to the evolution of sex pheromone detection in the Lepidoptera will be discussed.

GENETIC ANALYSIS OF MALE PREFERENCE FOR FEMALE SEX PHEROMONES IN *OSTRINIA NUBILALIS*

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Lepidoptera possess a tremendous diversity of sex pheromones, but the evolution of novel pheromone systems presents a dilemma. Production of a new pheromone blend by females and preference for that blend by males must both be established, which is difficult to explain if male and female traits are controlled by different genes. The E- and Z-strains of the European Corn Borer *Ostrinia nubilalis* are a well-established model system for studying pheromone evolution. These differ in the proportions of E11- and Z11-tetradecenyl acetate produced by females and preferred by males. The gene responsible for the strain difference in female production is an autosomally encoded fatty-acyl reductase. The major gene for male behavioral pheromone preference has previously been shown to be sex-linked and mapped to one end of the Z chromosome. We performed a QTL (quantitative trait locus) analysis of male behavioral response using a repeated backcross design, and evaluated candidate loci for linkage to the trait. A cluster of 8 odorant receptor genes previously implicated in pheromone reception maps to the Z chromosome, but is at least 11 centimorgans away from the preference gene. The *acj6* gene responsible for a shift in pheromone preference in a mutant strain of silkworm *Bombyx mori* maps to the Z chromosome in that species, but not in *O. nubilalis*. Of 14 genes mapping near the preference trait, none have a known function relating to pheromones. QTL mapping has the potential to identify completely novel genetic mechanisms that may underly the evolution of pheromone communication systems.

SEX PHEROMONE EVOLUTION AND SPECIATION IN THE NEW ZEALAND ENDEMIC LEAFROLLER MOTHS OF THE GENERA *CTENOPSEUSTIS* AND *PLANOTORTRIX*: THE MALE'S STORY

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The major feature of mate recognition in moths is the ability of the male to distinguish between sex pheromone blends of their own and other species. While we are beginning to understand the molecular basis for differences between species of moths in their ability to produce distinct pheromone blends, little is known of how the males' pheromone reception system evolves to track any newly evolved pheromone blend, especially at the molecular level. To address this question we are studying sibling species pairs within the New Zealand endemic leafroller genera *Ctenopseustis* and *Planotortrix.* To identify putative pheromone receptors, preliminary genome assembles and antennal transcriptomes of *C. obliquana* and *P. octo* were searched and odorant receptor genes tested by quantitative RT-PCR for male-biased expression in their antennae. To date, five candidate pheromone receptors from *C. obliquana* and *C. herana* and three from *P. octo* and *P. excessana* have been identified. Functional assays reveal that OR07 from the *Ctenopseustis* species responds to (Z)-8-tetradecenyl acetate. Current work involves further functional testing of candidates in cell-based assays for the ability to detect sex pheromone components, and comparisons between species both in receptor sequence and gene expression levels, to identify the differences between species in the male's side of mate recognition.

IDENTIFICATION AND CHARACTERISATION OF PHEROMONE RECEPTORS FROM THE LIGHT BROWN APPLE MOTH (*EPIPHYAS POSTVITTANA)*

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Epiphyas postvittana is a common horticultural pest that causes damage in many important horticultural crops of Australia, New Zealand and most recently California, Hawaii and Europe. Control strategies include the use of insecticides, biological control agents and mating disruption. We aim to improve the efficacy of mating disruption by increasing our understanding of the insect's sex pheromone reception system. We bioinformatically identified 73 putative olfactory receptor (OR) genes from the genome and antennal transcriptome of *E. postvittana*. Some of these genes are part of clusters within the genome as determined by PCR from isolated BAC clones. Steady state transcript levels of OR genes were assessed in male and female antennae by counting reads in RNAseq data and by quantitative RT-PCR. Genes displaying male-biased expression patterns and all genes falling within the so-called 'pheromone receptor (PR)' clade were targeted initially for characterization studies. OR genes of interest were then expressed in HEK293 cells using an inducible expression system and tested for their ability to respond to a range of pheromone components. In total, eight ORs were found to group within the PR clade, some of which displayed male-biased expression patterns and others that did not. In addition, we found two receptors that did not reside in the PR clade, yet had significant male-biased expression. Functional characterization of these ORs revealed receptors with varying response profiles to *E. postvittana* pheromone components as well as to pheromone components of other moths.

DIFFERENTIAL EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF CANDIDATE PHEROMONE RECEPTORS IN THE HESSIAN FLY, *MAYETIOLA DESTRUCTOR* (DIPTERA, CECIDOMYIIDAE)

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Gall midges (Diptera: Cecidomyiidae) constitute a fascinating insect family in which the members display intricate relationships with their hosts. Most species are specialists and many are considered serious pests. One of the worst is the Hessian fly (*Mayetiola destructor*), which severely can reduce wheat production, especially in the US. Olfaction is crucial for host selection and reproduction; the latter evidenced by a female produced long-range sex pheromone. We have now annotated 122 odorant receptors (ORs) from the Hessian fly genome, corresponding to the first set of ORs identified in gall midges. In a sequence similarity dendrogram, the majority of the Hessian fly ORs had expanded in two major lineages that were completely devoid of ORs from other Diptera, i.e. the vinegar fly, *Drosophila melanogaster*, and the malaria mosquito, *Anopheles gambiae*. We further performed RNAseq on antennal transcriptomes and compared OR gene expression levels (read counts) between males and females. A large difference between sexes was apparent. Ten ORs confined to one of the most recent expansions were highly overexpressed in males, whereas most of the other ORs were overexpressed in females. Using heterologous expression in *Xenopus* oocytes we now test the hypothesis that these receptors detect the six identified compounds in the sex pheromone. This will be the first identification of receptors for a long-range multi-component sex pheromone in Diptera.

CASTE AND SOCIAL CONTEXT DEPENDENT PHEROMONE-RELATED GENE EXPRESSION IN THE MANDIBULAR GLANDS OF THE HONEYBEE

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Social context is often a primary regulator of social behavior, but genes that affect or are affected by it have rarely been investigated. In social insects, caste specific pheromones are key modulators of social behavior, e.g., in honeybees the queen mandibular gland (MG) pheromone mediates reproductive dominance, its absence prompting ovary activation and queen pheromone production in workers. It was therefore interesting to investigate the effect of caste and social environment on genome-wide expression patterns in the MG. We used microarrays to examine virgin and mated queens, queenright (QR) and queenless (QL) workers, with and without activated ovaries. Approximately 2554 transcripts were significantly differentially expressed among these groups, with caste (queen vs worker) and social context (QR vs QL workers) being the main regulators of gene expression patterns. Among these, 27 genes that are putatively involved in caste selective production of the fatty-acid derived MG pheromone were differentially expressed in queens and workers. For example, although stearic acid is a common precursor for these pheromones in queens and workers, its production and activation seems nonetheless to be controlled by caste specific genes. This emphasizes the occurrence of disparate pheromone biosynthetic pathways for queens and workers, adding another dimension regarding the regulation of these important pheromones. Gene ontology analysis also revealed genes of different functional categories whose expression was impacted by caste (oxidation-reduction activity, immune response, and several metabolic and biosynthetic processes) or by the social environment (protein catabolism), suggesting that the MG serve more than being a pheromone source in honeybee biology.

IMMUNOSTIMULATION MODULATE SOCIAL BEHAVIOR, CHEMICAL COMMUNICATION AND GENE EXPRESSION IN HONEY BEE WORKERS (*APIS MELLIFERA*).

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Social insects, such as honey bees, use molecular, physiological and behavioral responses to combat pathogens and parasites. The honey bee genome contains all of the canonical insect immune response pathways, and several studies have demonstrated that pathogens can activate expression of immune effectors. Honey bees also use behavioral responses, termed social immunity, to collectively defend their hives from pathogens and parasites. We tested the specificity of behavioral, chemical and genomic responses to immunostimulation by challenging workers with a panel of different immune stimulants (saline, Sephadex beads and gram-negative bacteria). While only bacteria-injected bees elicited altered behavioral responses from healthy nestmates compared to controls, all treatments resulted in significant changes in cuticular hydrocarbon profiles. Thus, cuticular hydrocarbons may enable workers to identify sick nestmates, and adjust their behavior in response. Moreover, immunostimulation caused significant changes in expression of hundreds of genes, the majority of which have not been identified as members of the canonical immune response pathways. Furthermore, several new candidate genes that may play a role in cuticular hydrocarbon biosynthesis were identified. Finally, there was considerable overlap with studies of genomic responses to immunostimulation in honey bees and other insects, suggesting that immune responses are conserved at the molecular level.

THE IMPACT OF SYMBIOTIC BACTERIA ON CHEMICAL COMMUNICATION IN *DROSOPHILA*

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Microbial symbionts are capable of interfering with their host's sexual behavior, particularly via sex pheromones, key players in chemical communication of mate choice. One such microbe is the ubiquitous alpha-proteobacterium *Wolbachia pipientis*, symbiont of many insects. These bacteria successfully manipulate many host traits, including mate selection, reproduction, and metabolic pathways. We have demonstrated recently that *Wolbachia* impact sexual behavior of *Drosophila paulistorum*, a classic speciation model system. The six *Wolbachia*-carrying semi species of *D. paulistorum* display strong female assortative mating behavior against each other. Females accept males from the same semi species (homogamic) and reject males from heterogamic ones. Upon reducing native *Wolbachia* titer in females, they lose the assortative mating phenotype and accept improper, heterogamic males randomly. Manipulation of male *Wolbachia* titer results in rejection of such males by wildtype homogamic females. Based on this finding, we hypothesize that these males differ significantly from their wildtype counterparts. Cuticular hydrocarbons (CHCs) are key players in chemical signaling between sexes, and we suggest that the characteristic CHC profile of males is alternated upon *Wolbachia* reduction. Here we report on alterations in CHC profiles of partially *Wolbachia*-depleted males compared to wildtype ones. Applying host transcriptomics, we identified, amongst others, desaturase genes (desats), key players in the pheromone synthesis pathway, as potential targets for *Wolbachia* to manipulate host pheromone production. Finally, we employed immunochemical methods to show co-localization of *Wolbachia* with organs relevant to pheromone signaling like, *e.g.*, fatbodies and oenocytes.

THE BUTTERFLY THAT SMELLS LIKE A MOTH: SHARING OF ANCESTRAL PATHWAYS FOR MALE COURTSHIP PHEROMONE BIOSYNTHESIS

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Satyrid tropical butterflies (Nymphalidae, Satyrinae) belong to a speciose genus that rely on male chemical cues for reproduction. Scents act as secondary sexual traits in the form of close-range pheromones released from specialized wing structures during courtship. Despite being taxonomically closely related, butterflies and moths have diverged early on in life history traits and mating strategies. The evolutionary origin and genetic bases of male butterfly pheromones has remained largely unexplored. We use the African squiting bush brown butterfly *Bicyclus anynana* as an emerging model and explore its male pheromone biosynthetic routes at biochemical, molecular and functional levels. We examine whether male butterflies have recruited novel gene functions and biosynthetic pathways to serve in pheromone scent production or use conserved parts of inherited genetic elements of female moth pheromone biosynthetic networks from ancient lepidopteran lineages. Our results evidence that the successful evolution of short-range pheromones in *B. anynana* is consistent with the conservation of *de novo* pathways once shared with common ancestors with moths over millions of years of divergent evolution in life history. As has been demonstrated in many moth species, we find that pheromone production is initiated *de novo* from palmitic acid. Our findings also evidence a combination of independent duplication, diversification, and divergent transcriptional regulation of biosynthetic gene functions in new production organs. Altogether, our genomic, phylogenetic and functional analyses support the inheritance of ancestral polygenic biosynthetic networks across Lepidopteran fatty-acid derived pheromones, combined with unique expression patterns of lineage-specific gene duplicates.

SYMPOSIUM TOPIC 7: THE CHEMICAL STIMULUS – ITS ANALYSIS AND SYNTHESIS

Topic Coordinators:

Stefan Schulz (Technische Universitat Braunschweig, Germany) and **Ales Svatos** (Institute for Chemical Ecology, Germany)

SYNTHETIC APPROACH AND STRUCTURAL IDENTIFICATION OF SIGILLIN, A COMPLEX POLYCHLORINATED NATURAL PRODUCT OF ECOLOGICAL IMPORTANCE FROM THE SNOW FLEA

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The snow flea, *Ceratophysella sigillata,* is a unique collembolan that is active in winter and can be found, sometimes in large quantities, on the surface of snow in the Swiss Alps. The snow flea is chemically defended and several arthropod predators avoid it as food source. During the investigation of a total extract by GC/MS a suite of unknown compounds were found, called sigillins. High-resolution mass-spectrometry revealed the presence of several chlorine atoms in the major compound. Microderivatization and GC/MS analysis showed the presence of a lactone group. Finally, small amounts were purified to obtain material for 1D- and 2D-NMR-spectroscopy. Due to the high chlorine content, the analysis led to two possible structures, with either a 6/6 or a 6/7 bicyclic ring system. Computer simulation of NMR- and IR-data using *ab-initio* calculations pointed to the 6/6 system as the most likely, and a putative structure was proposed including relative stereochemistry. Finally, after several purification steps, a single crystal was obtained which allowed the determination of the structure including the absolute configuration by X-ray analysis. The 6/6 system proved to be correct, but one stereogenic center was inverted compared to the model derived from NMR data and computer calculations. A synthetic approach to this class of molecules was developed starting from chloral.

IDENTIFICATION, TOTAL SYNTHESIS AND BIOSYNTHESIS OF STYLOPSAL, THE FEMALE SEX PHEROMONE OF *STYLOPS MUELLERI*

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The twisted-wing parasites (*Strepsiptera*) are a fascinating insect order, with all species being endoparasites of various insect *taxa*. They display an extreme sexual dimorphism: the wingless larviform female is embedded in the host's abdomen with the cephalothorax protruding from it, while the male is a winged free-living insect with a very short lifespan of only a few hours. Major aim of the investigation was the identification and the determination of the constitution and absolute configuration of the female sex pheromone of *Stylops muelleri*. The constitution of Stylopsal was determined by a combination of chromatographic electroantennographic and mass spectrometric techniques. Its absolute configuration was proved as (*3R,5R,9R*)-trimethyldodecanal by unified, but diastereodivergent total synthesis of four diastereomeric pheromone candidates and comparison with the pheromone. Analytical and synthetic investigations aimed at the biosynthesis show that the pheromone with its three stereocenters is biosynthesized entirely de novo by the otherwise full host-dependent female *Stylops muelleri.*

Cvacka et al. 2012. *J. Chem. Ecol. 38*, 1483-1491.
Lagoutte et al. 2013. *Chem. Eur. J. 19*, in press.

ENANTIOSELECTIVE SYNTHESES OF INSECT PHEROMONES WITH METHYL-BRANCHED SKELETONS VERIFIED BY CHIRAL HPLC ANALYSES

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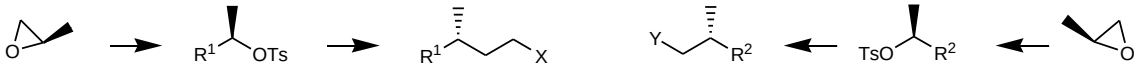
Many chiral insect pheromones with methyl-branched skeletons have been identified. Synthesis of optically pure stereoisomers is very important because the stereochemistry of natural pheromones can be elucidated by biological activity of the synthetic isomers. In addition to natural occurring chiral substances, commercialized chiral reagents are utilized as starting materials for stereospecific syntheses. Both of the enantiomers of propylene oxide (**1**) are inexpensive and can be easily converted into various chiral secondary alcohols by a Grignard reaction. Attacks of appropriate nucleophiles upon tosylates (**2a** and **2b**) derived from chiral secondary alcohols are expected to yield chiral building blocks (**3a** and **3b**) with a branched methyl group accompanied by inversion of the stereochemistry. The S_N2 reaction, however, has never been utilized for a pheromone synthesis because the complete conversion might be hard to monitor. Recently, we found that enantiomers of some methyl-branched compounds could be separated by chiral HPLC; thus the S_N2 reaction accomplished without racemization was verified. By a coupling reaction between **3a** and **3b**, we successfully synthesized some sex pheromones, such as 5,9-dimethylheptadecane of the leaf mining moth *Leucoptera scitella*) and 6,10,13-trimethyltetradecan-2-one of the stink bug *Pallantia macunaima*.

Tagri *et al. 2012. Tetrahedron: Asymmetry*, 23, 852–858.
Muraki *et al.2013. Eur. J. Org. Chem.*, 2009–2015.



(*R*)-**1** **2a** **3a** **3b** **2b** (*S*)-**1**

EUGLOSSA BEES: IDENTIFICATION AND SYNTHESIS OF COMPOUND FOUND IN HIND-LEG POUCHES

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Male orchid bees of the genus *Euglossa* collect volatile compounds from the environment in pouches on their hind legs, and these volatiles are a part of the hind-tibial perfume which may be used during courtship. Our group have previously identified (6R,10R)-6,10,14-trimethylpentadecan-2-one as an active component of the hind-leg pouches. When investigating the content of these pouches we recently found a new component which was purified with solid phase extraction (SPE) columns. Pure compound were isolated and subjected to GC-MS, GC-FTIR and NMR analysis for structural elucidation. Synthesis was used to confirm structure. Analysis of the hind leg pouches of two sibling species, *E. dilemma* and *E. virissima*, shows differences in content. *E.viridissima* male possess relatively large quantities of this compound but most male *E.dilemma* lack this compound. Compound purified from extract attracted *E .viridissima* males as could be expected since they collect this compound. This research was founded by European Union regional fund and Lennart Kennes minnesfond made it possible for me to go to this conference.

Eltz et al.2010. *J. Chem. Ecol.*, 36**,** 1322-1326.
Pokorny et al. 2013. *Oecologia*, 1-9.

EVOLUTION OF SYNTHESES OF THE IRREGULAR TERPENOID PHEROMONES OF MEALYBUGS

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Semiochemical syntheses proceed through two stages. The first stage consists of the development of syntheses of standards of completely known structure that can be used to zero in on and conclusively identify a tentatively identified new semiochemical, including all stereochemical details. As such, these syntheses are usually designed to be flexible so that they can produce multiple structures rather than a single structure, to provide standards of all structural possibilities. Having conclusively identified the basic structure of a bioactive compound, the second stage consists of developing syntheses that will produce a single compound in multigram quantities, and with high chemical and stereoisomeric purity. Furthermore, these second stage syntheses must be short and/or efficient in order to maximize the possibility of developing the semiochemical for practical use. Here, several case studies of first and second generation syntheses of mealybug pheromones will be described, showing the evolution from fast, flexible syntheses designed to produce milligram quantities for confirmation of structures, through to efficient scalable syntheses for commercialization of pheromones for use by growers, regulatory agencies, and other end users.

LASER ASSISTED MASS SPECTROMETRIC IMAGING OF PLANTS, INSECTS, AND BACTERIA SEMIOCHEMICALS

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[2]*Academy of Science of the Czech Republic, Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic*
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[4]*MPI for Chemical Ecology, Department of Entomology, Insect Symbiosis Research Group, Jena, Germany*
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Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) has recently found frequent application in mapping the distribution of small molecules in numerous biological samples or tissues. The data obtained using MSI have been used to follow or explain important biological processes or to rationalize previous biological observations. We developed and optimized protocols for sample preparation for MSI experiments either using laser desorption/ionization (LDI) of matrix-assisted desorption/ionization (MALDI) for ionization of metabolites from surfaces. In one case quantification of glucosinolates on *Arabidopsis thaliana* leaves was successfully implemented. Using the developed set of methods we can perform qualitative and quantitative MSI of surface-accruing metabolites ranging from neutral and polar lipids, phenolics, phytoallexins, sex pheromones and antibiotics. Obtained distribution maps were correlated with biological activity of visualized compounds and in many cases these MSI maps strongly support proposed biological hypotheses. Modern MSI methods are ready to contribute to surface chemistry analysis and addressing pending questions on spatial distributions of chemical signals in nature. Financial supports from Max Planck and Leibniz Societies are cordially acknowledged.

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Hölscher et al. 2009. *Plant J.* 60, 907-918.
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THE USE OF GC/FTIR AS AN IMPORTANT TOOL TO IDENTIFY SEMIOCHEMICALS

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Infrared spectroscopy (IR) is one of the most complete and important techniques in the structural elucidation of organic molecules. IR spectra provide information about functional groups and double bonds configuration, among others, acting as a complementary technique to mass spectrometry. The coupling of this technique to gas chromatography led to a powerful tool for identification of compounds, including those present in organic mixture in small quantities, such as the semiochemicals. In our research group, GC/FTIR has been a key technique for the correct elucidation of different classes of semiochemicals. The following examples will be discussed in this presentation: the sex pheromone of the Brazilian poplar moth *Condylorrhiza vestigialis*; the allomones produced by stink bugs; and the VOCs released by *Eucalyptus benthamii* after herbivory by *Thaumastocoris peregrinus*.

IDENTIFICATION OF THE SEX PHEROMONE OF THE CHILEAN FRUIT LEAFROLLER *PROEULIA AURARIA* (LEPIDOPTERA: TORTRICIDAE)

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[1]*Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*
[2]*Universidad de Chile, Santiago, Chile*
[3]*University of Lund, Lund, Sweden*

The Chilean fruit leafroller *Proeulia auraria* (Lepidoptera: Tortricidae) is a polyphagous insect native to Chile. It is distributed mainly in the central-southern region, and its host plants include economically important species, such as fruit trees, grapes, blueberries, and other crops. The aim of this work was to identify the sex pheromone produced by females, in order to provide information for the development of efficient lures for monitoring and/or control strategies. The calling behavior of virgin females was studied under natural light conditions. The majority of females initiated calling between 5 and 7 a.m. The stereotyped behavior includes activation of resting females, wing fanning, antennal movements, curving of the abdomen, and protrusion of the pheromone gland while lifting the wings. The biologically active compounds produced in the female sex pheromone gland were extracted with hexane and identified by means of gas chromatography (GC)-electroantennographic detection (EAD), GC-mass spectrometry, and comparison of analytical data of the natural compounds with those of authentic reference substances as tetradecyl acetate (14OAc), (*E*)-11-tetradecenyl acetate (E11-14OAc), (*Z*)-11-tetradecenyl acetate (Z11-14OAc), and (*E*)-11-tetradecenol (E11-14OH) in a relative ratio of 14:100:4:30. Field tests showed that all four compounds are behaviorally active. The most attractive blends contained the main compound E11-14OAc, 1% of the geometric isomer Z11-14OAc, and both of the other minor compounds. Addition of 4% of Z11-14OAc resulted in inhibition of attraction. Funding from Fondo Nacional de Desarrollo Científico y Tecnológico (grant nº 1110365) is acknowledged.

A NEW CLASS OF MEALYBUG PHEROMONES: A HEMITERPENE ESTER IN THE SEX PHEROMONE OF *CRISICOCCUS MATSUMOTOI*

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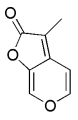
Mealybugs (Hemiptera: Pseudococcidae), which include several agricultural pests, are small sap feeders covered with a powdery wax. The conventional management of mealybugs depends on the regular application of insecticides, but the cryptic behavior, wax-coated body, and clumped spatial distribution pattern of these species render the use of many insecticides ineffective. It is therefore difficult to entirely suppress populations with chemicals. A more effective and more sustainable tool that can compensate for the limited efficacy of insecticides in a pest management program against mealybugs is required. Pheromone-based management techniques are a potential replacement for insecticides among a broad range of pest insects including mealybugs. Sex pheromones emitted by mealybug females facilitate copulation and reproduction by serving as a key navigation tool for males, which are winged but fragile and short-lived. Although the structures of the hitherto known mealybug pheromones vary among species, they have a common structural motif; they are carboxylic esters of monoterpene alcohols with irregular non-head-to-tail linkages. However, in the present study, we isolated from the Matsumoto mealybug, *Crisicoccus matsumotoi* (Siraiwa), a pheromone with a completely different structure. Using gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy, we identified the pheromone as 3-methyl-3-butenyl 5-methylhexanoate. Its attractiveness to males was confirmed in a series of field trapping experiments involving comparison between the isolated natural product and a synthetic sample. This is the first report of a hemiterpene mealybug pheromone.

DISCOVERY OF SMOKE-DERIVED BUTENOLIDES AND CYANOHYDRINS THAT PROMOTE SEED GERMINATION

Gavin FLEMATTI[1]**,** Adrian SCAFFIDI[1], Kingsley DIXON[2], Steven SMITH[1], Emilio GHISALBERTI[1]

[1]*University of Western Australia, Perth, Australia*
[2]*Kings Park and Botanic Garden, Perth, Australia*

The smoke derived from burning plant material has been known for some time to stimulate germination and landscape regeneration of ecosystems around the world. In 2004, we identified the key stimulant in smoke that is responsible for this effect as the butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (1), which is now known as karrikinolide. A number of related compounds have also been identified in smoke which has led to the collective name karrikins. However, not all smoke-responsive species respond to karrikins. The germination of Western Australia's floral emblem, the iconic red and green kangaroo paw, is stimulated by a different compound which we recently identified as the cyanohydrin, glyceronitrile (2). Glyceronitrile has been shown to act by releasing trace amounts of cyanide in aqueous solutions which is responsible for the stimulatory effect. Here I will present an overview of our recent work on karrikins and the role of cyanide as an ecologically relevant germination cue.



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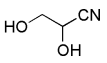

2

Flematti et al. 2004. *Science*, 305, 977
Flematti et al. 2011. *Nature Commun.* 2, 360.

METABOLOMICS REVEALS HERBIVORE AND PATHOGEN-INDUCED METABOLITES OF RESISTANCE AND SUSCEPTIBILITY IN MAIZE

Gaetan GLAUSER[1], Matthias ERB[2], Guillaume MARTI[3], Dirk BALMER[1], Brigitte MAUCH-MANI[1], Jean-Luc WOLFENDER[3], Ted C. J. TURLINGS[1]

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[2]*Max Planck Institute for Chemical Ecology, Jena, Germany*
[3]*University of Geneva, Geneva, Switzerland*

Traditionally, the study of chemicals involved in plant-host interactions is performed by targeted analytical approaches measuring only a limited number of selected compounds. Metabolomics, on the other hand, aims at the comprehensive analysis of metabolites in given organisms and represents an attractive alternative tool that may reveal as yet unknown metabolites involved in the interactions between plants and their aggressors. Progress in the field is closely related to advances in analytical chemistry techniques and among them, nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) are the methods of choice. However, it is increasingly evident that no single approach can embrace the immense complexity of plant metabolites. Here we propose a sequential approach that involves HRMS-based metabolomics followed by identification of detected markers using targeted NMR and/or HRMS experiments. The feasibility of this approach is illustrated using a biological study performed on maize leaves attacked by either chewing insects (*Spodoptera* spp.) or fungal pathogens (*Colletotrichum graminicola*). The results indicated that maize employs different chemical defenses depending on the aggressor type. While herbivory induced high amounts of benzoxazinone glucosides in maize leaves, infection by *C. graminicola* caused a strong accumulation of chlorogenic acids and flavonoids. Larval growth assays using selected induced compounds showed contrasting effects on herbivore and pathogen resistance. Hence, a metabolomic approach appears to have great potential to elucidate the specific biochemical strategies that plants employ against a vast array of antagonists.

CANNIBALISM AND NMR METABOLOMICS IN *HELICOVERPA ARMIGERA*

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[1]*Advance NMR Metabomics Research Team, Plant Science Center, RIKEN. Yokohama, Japan*
[2]*Graduate School of Nanobioscience, Yokohama City University. Japan*
[3]*Graduate School of Bioagricultural Sciences, Nagoya University. Japan*

Cannibalism is common in the larval stages of the lepidopteran *Helicoverpa armigera*, yet the consequences of this behavior on the metabolism of the larvae of this insect have not been evaluated before. Here we describe the differences in body weight and metabolic profile produced by cannibalism in 4th-instar larvae of *H. armigera*. Metabolic profiles of the larvae were obtained by one dimensional proton nuclear magnetic resonance (^{1}H-NMR). ^{1}H-NMR spectra were statistically processed using multivariate projection methods. Cannibal larvae were smaller than herbivore larvae (control). Metabolic profiles of cannibal *vs*. herbivore larvae formed two clearly separated clusters on principal component analysis; thus, cannibal and herbivore larvae had different chemical compositions. Further statistical analysis of the spectra showed a strong variation between cannibal and herbivore larvae associated with a spectral region (3.20 ppm) that matched signals corresponding to carnitine in our NMR spectral database. Carnitine facilitates transport of fatty acids and plays an important role in the development of many insects. To study carnitine metabolism in *H. armigera* larvae isotopic labeled carnitine (^{13}C-carnitine) was synthesized in the laboratory and fed to the larvae. The metabolic transformations occurring to ^{13}C-carnitine in the body of *H. armigera* larvae are currently being traced using ^{13}C-NMR and results will be presented during the conference.

SYMPOSIUM TOPIC 8: MICROBIAL-CHEMICAL ECOLOGICAL INTERACTIONS AMONG MICRO-ORGANISMS AND THEIR ENVIRONMENTS

Topic Coordinators:
Junwei (Jerry) Zhu (USDA, USA) and **Anna-Karin Borg-Karlson** (Royal Institute of Technology, Sweden)

DYNAMIC NATURE OF THE CHEMICAL ECOLOGY OF ALGAL-BACTERIAL INTERACTIONS

Roberto KOLTER[1]

[1]*Harvard Medical School, USA*

We have shown that there exists a dynamic association between the calcifying marine microalga *Emiliana huxleyi* and the marine bacterium *Phaeobacter gallaeciensis*. When both organisms encounter conditions propitious for growth, they produce compounds that aid in each other's growth. Upon aging, the alga produce chemical cues that induce the bacterium to switch from a mutualistic symbiont into a deadly pathogen due to the production of novel algacides, the roseobacticides. The chemical cues that are exchanged throughout this dynamic interaction are being analyzed during co-cultivation in the laboratory. These results will be discussed in the context of the importance of these interactions on geochemical cycles and global climate.

EFFECTS OF BACTERIA ON MOSQUITO BEHAVIOR

Coby SCHAL[1], Loganathan PONNUSAMY[1], Dawn WESSON[2], Charles APPERSON[1]

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[2]*Tulane University, New Orleans, LA, USA*

Bacteria perform multiple biological functions in water-filled, human-made containers that are critical to the life cycle of *Aedes* (*Stegomyia*) mosquitoes. Bacteria are an integral component of the diet of larvae mosquitoes. Bacteria metabolize organic detritus and in the process produce volatile and nonvolatile organic compounds that guide gravid mosquitoes to containers and stimulate them to lay eggs. This talk will review our progress on (a) volatile bacterial compounds that attract gravid females to oviposition sites; (b) involvement of bacteria in stimulating oviposition; and (c) demonstrating a role for bacteria in stimulating mosquito eggs to hatch. Progress on present efforts to develop a “lure and kill” strategy by combining bio-active bacteria with a lethal oviposition trap will be described.

MICROBIAL CHEMO- AND BIODIVERSITY RELATED TO THE PINE WEEVIL *HYLOBIUS ABIETIS* BEHAVIOUR

Muhammad AZEEM[1], Gunarathna Kuttuva RAJARAO[2], Olle TERENIUS[3], Göran NORDLANDER[3], **Anna-Karin BORG-KARLSON[1,4]**

[1]*KTH Royal Institute of Technology, School of Chemical Science and Engineering, Department of Chemistry, Ecological Chemistry Group, Stockholm, Sweden*
[2]*KTH Royal Institute of Technology, School of Biotechnology, Division of Environmental Microbiology, Stockholm, Sweden*
[3]*Swedish University of Agricultural Sciences, Department of Ecology, Uppsala, Sweden*
[4]*Tartu University, Institute of Technology, Division of Organic Chemistry, Tartu, Estonia*

The large pine weevil (*Hylobius abietis L.*) is a severe pest of conifer seedlings in reforestation areas of managed forests. Female weevils lay eggs in the bark or in the soil near root barks of freshly dead trees and cover them with their feces and chewed wood bark to protect them from conspecific predation. In order to identify the origin of the repellents/antifeedants acting on the pine weevils and to understand the mechanism behind frass deposition at egg laying site, microbes were isolated from the aseptically collected pine weevil frass. Their volatiles were collected by SPME - GC-MS after cultivating them on weevil frass broth. The influence of the major microbial volatiles on pine weevil behavior was tested using a multi-choice olfactometer. *Ewingella spp.*, *Penicillium spp., Ophiostoma spp.*, *Debaryomyces hansenii* and *Candida sequanensis* were the major microbes and styrene, 3-octanol, 1-octene-3-ol, 3-octanone, 3-methylanisole, methyl salicylate, 2-methoxyphenol and 2-methoxy-4-vinylphenol were the major volatiles of isolated microbes. In behavioral bioassay, methyl salicylate, 3-methylanisole and styrene significantly reduced the attraction of pine weevils to their host plant volatiles.

Borg-Karlson et al. 2006. *J. Chem Ecol*. 32, 943-957.
Azeem et al. 2013. *J. Chem Ecol*. 39, 120-128.

MICROBIAL CHEMOECOLOGY OF FLIES AND THEIR ENVIRONMENTS

Junwei Jerry ZHU[1]

[1]*University of Nebraska, USA*

Filth flies are among the most important insect pests in agricultural and urban settings. Their infestations have caused damage in animal production, food contamination and disease transmitting. The economic losses caused by those flies have been reported as up to several billions of dollar losses each year. The present presentation reports our recent findings on the development of filth fly control via manipulating microbial community in their larval development. Novel botanical-based antibacterial agents were identified from several plant essential oils, and were demonstrated as larval growth inhibitors and also used as oviposition deterrents. Studies were further carried out to understand which bacterial species or their complex community that is important for their fly larval growth, as well as how they affect infochemical interactions between filth flies and their host environments. The outcomes from these studies for further development in novel fly control strategy will also be discussed.

IS CHEMICAL ATTRACTION CAUSING YEASTS AND FLIES TO COEVOLVE?

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[2]*The New Zealand Institute for Plant and Food Research Ltd, Auckland, New Zealand*

Organisms do not just adapt to environments, they may also manipulate them. The yeast *Saccharomyces cerevisiae* engineers its ecosystem by fermenting sugars in fruit to produce a toxic cocktail of alcohol, heat, and carbon dioxide that sabotages microbial competitors. Fermentation by yeast also produces volatile compounds that impart flavour to ferment products such as wine, but the biological function of these compounds is unclear. We are investigating whether ecosystem engineering is a new and previously unstudied mechanism for the evolution of mutually beneficial relationships between species: Yeasts benefit from volatile production because attracting vectors allows them to 'hitch a ride' with flies and escape ephemeral fruits, and flies benefit from following yeast volatiles because they reproduce better in fermenting fruits. In laboratory and field experiments, we found population variance for attractiveness of *Saccharomyces cerevisiae* to female *Drosophila simulans*, and that attractive yeasts were more successfully dispersed than unattractive yeasts. Using Gas Chromatography-Mass Spectrometry we are currently identifying the classes of volatiles produced by yeast during fermentation to see which of those are especially attractive/repulsive to *Drosophila* species. Our results support the hypothesis that ecosystem engineering could drive mutualistic interspecies interactions.

NOVEL DEFENSE MECHANISMS OF ENDOPHYTIC FUNGI VIA THE PRODUCTION OF VOLATILE BIOCIDAL METABOLITES

Ross MANN[1,2], Scott MATTNER[1], Ian PORTER[1,2], Simone ROCHFORT[1,2], German SPANGENBERG[1,2]

[1]*Latrobe University, Bundoora, Victoria, Australia*
[2]*Department of Environment and Primary Industries, AgriBio, Bundoora, Victoria, Australia*

Endophytic fungi reside within the tissues of living plants, and often protect their host from pests and pathogens in a mutualistic relationship. The secondary metabolism of endophytic fungi is often central to this defensive role. A unique feature of some endophytic fungi is the production of volatile metabolites, which can be biocidal against plant pathogens and pests. Our research aimed to identify Australian endophytic isolates with this capability. For example, isolates of a *Nodulisporium* species were collected from a range of native plant species and shown to produce a suite of volatile metabolites, which were biocidal against fungi, bacteria and insects. Profiling the volatile metabolome of key isolates identified 52 metabolites, of which 45 were mono- or sesqui- terpenes (or derivatives of). These terpenes included eucalyptol, *p*-cymene, terpine-4-ol, myrcene/pinene and *β*-elemene, all of which are known to be biocidal against insects and/or fungi. In addition, many of these terpenes have previously been reported as defense metabolites of plants (e.g. eucalyptol), but only very rarely reported from fungi (particularly monoterpenes). The unique ability of *Nodulisporium* sp. to produce terpenes was also evident in the genome of the endophyte, as it possessed a total of eight terpene synthases, the enzymes regulating the production of these metabolites. This number of terpene synthases is greater than in other fungal genome sequenced to date. This research provides new information about the metabolomic and genomic diversity of terpene production in endophytic fungi, and the mechanisms they utilize to protect themselves and their host.

ALLELOPATHY OF MARINE EPIPHYTIC BACTERIA: ASSESSMENT OF THE REALISTIC ECOLOGICAL ROLE OF BIOACTIVE BACTERIAL SECONDARY METABOLITES

Jan TEBBEN[1,2], Martina ERKEN[1,2], Nachshon SIBONI[3], Dianne TAPIOLAS[3], Cherie MOTTI[3], Tilmann HARDER[1,2]

[1]*University of New South Wales, Sydney, Australia*
[2]*Sydney Institute of Marine Science, Sydney, Australia*
[3]*Australian Institute of Marine Science, Townsville, Australia*

The marine environment is home to a diverse array of microorganisms, many of which have evolved stable associations with marine animals and plants. Bacteria associated with macroalgae have been shown to be rich in bioactive secondary metabolites that inhibit fouling of the host, deter grazing and inhibit competitive bacteria. Because these activities have been identified mainly in-vitro, the precise ecological role of these bacteria and their metabolites in-vivo remains speculative. Here we report the bioactive secondary metabolite profile of the bacterium *Pseudoalteromonas sp*. strain J010, isolated from the surface of the crustose coralline macroalga *Neogoniolithon fosliei*. The organic extract of this bacterium yielded a range of bioactive compounds, including five novel as well as five known korormicins, the novel bromopyrrole 4'-((3,4,5-tribromo-1H-pyrrol-2-yl)methyl)phenol, tetrabromopyrrole, and several known bromoalterochromides. The bioactivity spectrum of these compounds was evaluated in-vitro against common colonizers of macroalgae, such as bacteria, protozoa and invertebrate larvae. To assess the potential ecological role of this epiphytic bacterium, its natural abundance on the algal surface, as determined by qPCR, was correlated with experimental bacterial densities required to elicit the antagonistic effects in-vitro. Whilst highlighting the broad allelochemical potential of the epiphytic bacterium *Pseudoalteromonas sp*. strain J010, our results suggest that of the many in-vitro activities triggered by the above mentioned secondary metabolites, the antagonistic effects against eukaryotic propagules are unlikely to be ecologically relevant.

VOLATILE TERPENOID RESPONSE FROM METHYL JASMONATE TREATMENT OF FOUR CONIFER SPECIES

Lina LUNDBORG[1], Henrik NORDENHEM[2], Göran NORDLANDER[2], Luis SAMPEDRO[3], Rafael ZAS[3], Anna-Karin BORG-KARLSON[1,4]

[1]*Royal Institute of Technology, Stockholm, Sweden*
[2]*Swedish University of Agricultural Sciences, Uppsala, Sweden*
[3]*Misión Biológica de Galicia – CSIC Pontevedra, Spain*
[4]*Institute of Technology, Tartu University, Estonia*

The pine weevil *Hylobius abietis* (L.) is a pest to young conifers in Europe. The risk for this polyphagous phloem-chewer is most critical during the first three seasons after clear-cutting, and decreases after 4-5 years. The chemical protection of insecticides is to be phased out against ecological alternatives. Exogenous application of methyl jasmonate, a plant hormone involved in herbivore damage signaling and plant induced responses, is known to mimic insect herbivory and may increase conifer resistance. In a Swedish-Spanish twin experiment, methyl jasmonate was administered at the nursery to seedlings of four conifer species: *Pinus pinaster*, *P. radiata, P. sylvestris* and *Picea abies*. The one-year-old juveniles were introduced into an area of naturally occurring weevils to collect damage and mortality data, and at the same time, random seedlings were freeze stored in -80 °C for chemical analysis of resistant seedlings. The volatile terpenoid fraction was analyzed using two-dimension GC-MS with a chiral column in the second GC. In *Pinus sylvestris,* induction of 1,8-cineole and (-)-β-pinene was observed. In preliminary orientation tests, both tend to be repellent to the weevil. The chemical findings will be presented using multivariate statistical software Canoco.
Nordlander et al. 2009. *Agric. and Forest Entomology*, 11, 91-100.

DOES CHOICE OF FOOD AFFECT THE REPELLENCY OF PINE WEEVIL FECES?

Karolin AXELSSON[1], Anna-Karin BORG-KARLSON[1,3], Göran NORDLANDER[2], Henrik NORDENHEM[2]

[1]*Department of Chemistry, Royal Institute of Technology, Stockholm, Sweden*
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The large pine weevil (*Hylobius abietis*) feed on almost all coniferous and some deciduous trees. Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) are their preferred hosts but they also feed on birch (*Betula pendula*). During egg laying the female gnaws a cavity in a root or in soil were she deposits her eggs. The cavity is sealed with feces and plug of bark. The feces emit repellent and antifeedant substances possibly produced by microorganism protecting the eggs from conspecific predation. Three groups of female pine weevils were fed for 2 weeks on Scots pine, Norway spruce and birch respectively. Feces from each group were collected antiseptically and sterile water was added. Volatile emissions were analyzed with SPME-GC-MS and the changes in volatile profiles were followed over time. Repellent compounds as 2-methoxy phenol, phenol, phenyl ethyl alcohol, styrene and methyl salicylate are found in all groups; birch shows especially promising results with several of them as main peaks.

EFFICIENCY OF CONSTITUENTS FROM THAI *ALPINIA GALANGA* (L.) AGAINST PHYTOPATHOGENIC FUNGI AND STRUCTURE ACTIVITY RELATIONSHIP

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The management of disease caused by phytopathogenic fungi is important to prevent agricultural product loss. The essential oil from both fresh and dried rhizomes of *Alpinia galanga* (L.), (Zingiberaceae) exhibited interesting antimicrobial activities. The dichloromethane extract was isolated by flash silica gel column chromatography and tested for antiphytopathogenic activity against *Alternaria porri, Colletotrichum gloeosporioides, Fusarium oxysporum* and *Phytophthora parasitica*. The activity was evaluated using broth dilution method for the minimum inhibitory concentration (MIC). 1′-Acetoxychavicol acetate (ACA) was identified as a major component together with hydroxycinnamyl acetate and coumaryl diacetate. ACA was hydrolyzed and used as a starting material for the preparation of coumaryl ethyl ether, hydroxycinnamyl acetate and acetoxy cinnamic alcohol. Among investigated compounds, all phytopathogens were sensitive to ACA, especially *A. porri* and *P. parasitica* (MIC 15.6 μgmL^{-1} and 62.5 μgmL^{-1}) followed by hydroxycinnamyl acetate, coumaryl ethyl ether, acetoxy cinnamic alcohol and coumaryl diacetate. From antiphytopathogenic activity of related compounds, the 1′-acetoxyl group was found to be essential for their strong activity. Since ACA displays antiphytopathogenic activities, it may be used as a natural compound as fungicide to control phytopathogens.

VARIETAL DIFFERENCES IN RICE METABOLITES ELICTED BY INSECT GUT CONTENTS

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Plants respond to herbivore attack through changes in volatile and nonvolatile secondary metabolites as well as major plant nutrients. In addition to essential amino acids used for protein biosynthesis, plants also produce non-protein amino acids. Some non-protein amino acids have been reported to play roles in plant defense against herbivores. In this study, we analyzed amino acids of rice seedlings and compared the foliar response differences of two japonica varieties (Nipponbare and Ginbozum) and one indica variety (Kasalath) to the extracted gut contents of oriental armyworm, *Mythimna separata*. Plants were individually damaged with a needle at distal portion of rice leaves, and each damage site was treated by applying *M. separata* gut content extracts in sodium phosphate buffer (pH 8.0). Control plants were similarly wounded and treated with buffer only on the damaged sites. Two days after treatment, plants were harvested for amino acid analysis. Aliquots of 0.1 N HCl extracts were derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and analyzed by LC/MS. In Nipponbare, significant increases in γ-Aminobutyric acid (GABA) were associated with decreased levels of the GABA precursor glutamic acid following gut content elicitation. In contrast, no significant differences between treatment groups were detected in Kasalath and Ginbozu. These results highlight the importance of comparing multiple varieties when investigating insect-inducible metabolites in crop plants.

DEFENCES WITHIN PLANT TISSUE, ARE OFTEN NOT ENOUGH TO PREVENT INSECT BORNE DISEASES

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Plants are a very important source of food (and non-food products) for humans but they also play a key role in supporting a vast number of organisms including: viruses, fungi, invertebrates and vertebrates. Evolving under constant pressure, plants developed many defence mechanisms to prevent or minimise herbivore attack and disease occurrence. Plants have developed morphological barriers, making themselves physically difficult to be penetrated by invaders as well as equipping themselves with an array of chemical compounds (toxic and repellent secondary metabolites) that target herbivores directly or indirectly by signalling invasion to the beneficial organisms. Within the natural ecosystem, by utilizing all the available physiochemical mechanisms, plants can defend themselves and keep herbivore damage to a minimum. In an agricultural setting, plants including food crops grown in monoculture, are highly vulnerable to insects and pathogens and despite the use of chemicals (insecticides, fungicides) and resistant cultivars, pest and disease outbreaks are not uncommon. Sap-sucking insects, including aphids and leafhoppers, can cause significant damage to crops directly but above all indirectly, by vectoring plant pathogens, to both host and non-host plants. In our study, we investigated in detail feeding preferences and behaviour of aphids and leafhoppers on host and non-host plants in relation to persistent transmitted viruses. Insect host-plant determination, was evident soon after pathogen transmission. Our results highlight the importance of pre-phloem resistance to minimize the spreading of phloem-restricted viruses.

SYMPOSIUM TOPIC 9: MULTIMODAL COMMUNICATION: (INTEGRATION OF OLFACTION, TASTE, VISION, ACOUSTICS, MECHANORECEPTION)

Topic Coordinators:
Gerhard Gries (Simon Fraser University, Canada) and **John Hildebrand** (University of Arizona, USA)

SEABIRDS SMELL BIRDS: MULTIMODAL SENSING AND SIGNALING IN PROCELLARIIFORM SEABIRDS

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Procellariiform seabirds (such as petrels, albatrosses and shearwaters) routinely forage over thousands of kilometers of ocean and navigate back to breeding colonies on remote islands using sensory mechanisms that are not completely understood. Members of this order have among the largest olfactory bulbs of all extant birds, and olfaction is now known to be critical for behaviors ranging from foraging and navigation to individual recognition. Current investigations in my lab are focused on better understanding how Southern ocean albatross species use multimodal information to forage effectively over vast distances, and we are approaching this problem in a comparative context. In parallel, we have been using Leach's storm-petrels *(Oceanodroma leucorhoa)* as a model to investigate the chemical and genetic basis for individual odor recognition, including how birds integrate chemical with other sensory information in behaviors such as burrow recognition and mate choice. This talk will review some of our recent findings and highlight new directions for research in avian chemical ecology.

WE CAN SMELL AND HEAR A RAT

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Norway rats, *Rattus norvegicus*, are abundant in many ecosystems and cosmopolitan centres. Despite their ecological and economic importance, knowledge about their communication system, and potential exploitation of this knowledge for control of rats, remain fragmentary. We recorded sonic and ultrasonic vocalizations of rat pups, and in two-choice laboratory bioassays obtained positive phonotactic responses by adult male and female rats, and by sub-adult male rats, to playback recordings of rat pup sound. For field experiments on various potato farms in British Columbia, we built a battery-powered electronic sound device with circuit board and piezoelectric speaker, and wrote an algorithm to drive the production of "synthetic" rat pup sound, modulating its frequency (Hz), duration, and intensity, and altering the intermittent silence. Trap boxes fitted with food-baited snap traps, baited with rat urine and feces odor, and equipped with this sound device proved significantly more effective in capturing rats than trap boxes lacking the device. Moreover, trap boxes fitted with food-baited snap traps and equipped with the sound device captured significantly more rats when traps were also baited with rat urine and feces semiochemicals. Our data show that both semiochemical and sound signals are part of the complex communication system of Norway rats, and that both types of signals could be exploited for more effective control of rat populations.

DETECTION AND LEARNING OF FLORAL ELECTRIC FIELDS BY BUMBLEBEES

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Insect pollinators use several senses to forage, detecting floral cues such as color, shape, pattern and volatiles. We use operant conditioning to reveal an unappreciated sensory modality in bumblebees (*Bombus terrestris)*, detection of floral electric fields. These fields act as floral cues, which are affected by the visit of naturally charged bees. Like visual cues, floral electric fields exhibit variations in pattern and structure, which can be discriminated by bumblebees. We also show that such electric field information contributes to the complex, multimodal array of floral cues that together improve a pollinator's memory of floral rewards. Because floral electric fields can change within seconds, this sensory modality may facilitate rapid and dynamic communication between flowers and their pollinators.

MULTIPLE COMMUNICATION MODALITIES BY FIRE ANTS AS THEY "EAT, PREY, LOVE"

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The ability of worker ants to aggregate in large numbers at distant food sites is central to the fitness of queens. The process by which aggregation occurs is called recruitment. The benefit of recruitment is that prey too large for a single ant to handle can be captured and butchered on the spot by the masses and transported, piecemeal, back to the nest. Hence, as a social unit, ants function as a much larger predator. Here, using video-tapes and standard-play and frame-by-frame techniques to quantify recruitment, I report that ant scouts employed six discrete behaviors to inform nestmates of the location and quality of a food site. Scouts laid incoming chemical trails, waggled their heads, increased walking tempo, stroked nestmates with their antennae, advertised with a brief food display, and led groups of nestmates to the food site by laying outgoing chemical trails with a "follow-me" element. In turn, nestmates assessed the food sample with antennae, then responded to or resisted recruitment based on the quality of food advertised, their employment status and their level of hunger. In summary, recruitment was based on supply and demand decisions made by discriminating ants, face-to-face inside the nest, rather than on the trail or at the food site.

BIMODAL COMMUNICATION IN INSECTS: NEUROETHOLOGICAL ASPECTS OF INTEGRATION OF ACOUSTIC AND ODOR STIMULI

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While moving through the environment animals' senses are constantly bombarded by different signals to which they must respond adaptively. Therefore integration of sensory information with for instance experience and memory is essential in order to manage which signals to attend to. To a nocturnal insect, ultrasound may indicate a nearby bat predator, or perhaps courtship songs from a conspecific. How do moths, with their simply tone-deaf ears discriminate between such? In some communication systems in moth, acoustic signaling is used together with pheromone communication. We studied moths exposed to acoustic and pheromone stimuli, in moth behavioral assays and in neurophysiological set-ups. In behavioral experiments we found that simultaneous chemical and acoustic stimulation may act synergistically to enhance mating success. In neurophysiological experiments we show that stimulation of one sensory modality can modulate the response to information from another, suggesting that behavioral thresholds are dynamic and depend on the behavioral context. The findings are discussed in the context of multimodal sensory integration.

NEURAL PROCESSING OF BEHAVIORALLY SIGNIFICANT "ODOR OBJECTS" IN THE INSECT BRAIN

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We analyze the neurobiological mechanisms through which information about behaviorally significant olfactory stimuli is encoded, processed, and integrated with inputs of other modalities in insect brains and specifically how olfactory information ultimately initiates and controls natural behaviors. Our studies of the giant sphinx moth *Manduca* sexta have focused mainly on the antennal lobes (ALs), the primary olfactory centers in the brain. The ALs are characterized by glomeruli – condensed-neuropil structures in which primary-sensory and central neural elements interact through synaptic connections. We seek to understand how primary-sensory inputs from olfactory receptor cells are processed in glomeruli and represented in their outputs, how those outputs are further processed down-stream in the brain, and how the context represented through other sensory modalities influences that processing. Insights from the moth's sex-pheromonal communication system led to recent analysis of olfaction-dependent interactions with host plants. A multidisciplinary approach combining chemical characterization of floral scent, behavioral experiments in a laboratory wind tunnel, and electrophysiology has enabled us to determine how mixtures of volatiles, at natural concentrations, control flight and feeding behaviors and are encoded in the ALs. Evidence points to coincident firing of glomerular output neurons as a mechanism for neural coding of the context or significance of an odor. Gas chromatography coupled with multi-channel CNS recording has enabled identification, in complex floral mixtures, of key odorants to which AL neurons are particularly responsive. Mixtures containing only a few of those floral volatiles are as effective as the complete, natural floral mixture in modulating flight.

HONEYBEES LEARN ODOUR MIXTURES VIA A SELECTION OF KEY ODORANTS

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Pollinating insects such as honeybees have to detect, process and learn numerous floral scents from their environment on a daily basis. Floral scents are complex mixtures of different odorants. How the bee brain unravels the complex information contained in scent mixtures and reliably discriminates different floral scents is unclear. Here, we investigated learning of complex odour mixtures in honeybees using a simple olfactory conditioning procedure, the Proboscis-Extension-Reflex (PER) paradigm. Restrained honeybees were trained to three scent mixtures composed of 14 floral odorants each, and then tested with the individual odorants of each mixture. Bees did not respond to all odorants of a mixture equally: They responded well to a selection of key odorants, which were unique for each of the three scent mixtures. Bees showed less or very little response to the other odorants of the mixtures. The bees' response to mixtures composed of only the key odorants was as good as to the original mixtures of 14 odorants. A mixture composed of the non-key-odorants elicited a significantly lower response. Neither an odorant's volatility or molecular structure, nor learning efficiencies for individual odorants affected whether an odorant became a key odorant for a particular mixture. Odorant concentration had a positive effect, with odorants at high concentration likely to become key odorants. Our study suggests that the bee brain processes complex scent mixtures by predominantly learning information from selected key odorants. We propose that the 'selection' of key odorants occurs via inhibitory neural mechanisms in the glomeruli of the antennal lobes.

INTEGRATION OF OLFACTION AND TASTE INFORMATION FOR FEEDING PREFERENCE IN THE FLY

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Olfaction and taste are closely related to feeding behavior and food preference in animals. In the blowfly, *Phormia regina* a nectar-sucking insect, odor palatability is important in distinction between foods and poisons. Fly has two pairs of olfactory organs, antennae and maxillary palps. Here, we behaviorally proved that the maxillary palps introduced olfactory information of preferable food flavors. For example, odors of acetaldehyde or 1-octen-3-ol enhanced feeding sensitivity to sugar via maxillary palps not via antennae. Next, we traced the olfactory receptor neurons (ORNs) from the maxillary palps and the gustatory receptor neurons (GRNs) from a labellar taste sensillum by double staining. The maxillar ORNs branched into antennal lobe (AL; primary olfactory center) and subesophargeal ganglion (SEG; primary gustatory center), where the GRNs from a labellar taste sensillum project. The neuronal projections of those multimodal chemosensory receptors were very close to each other in SEG. Based on a fine structure exhibiting neighboring projections of ORNs and GRNs, we constructed 3D models, suggesting integration of preferable olfactory information with sugar taste information in SEG. The taste response to sucrose was not enhanced in the presence of those odors. However, our morphological data suggested that the sugar taste information could additionally be integrated with preferable odor information via maxillary palps' ORNs at SEG and sent to the higher brain, resulting in increase of feeding sensitivity to the flavored sugar. We also introduced a little about difference in food preference formation between sexes in *Drosophila*.

MECHANISMS OF UPWIND ORIENTATION BY SHORT AND INTERMITTENT FLIGHT IN POTATO MOTHS, *PHTHORIMAEA OPERCULELLA*, INDUCED BY SEX ATTRACTANT PHEROMONE

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Male moths often use wind cues to acquire directional information of conspecific females when tracking an odor plume of sex attractant pheromone. It is widely accepted that flying moths maintain upwind steering by perceiving wind-induced visual drift during flight, termed optomotor anemotaxis. However, males of potato moth, *Phthorimaea operculella,* exhibit a series of short and intermittent flights, or 'hops,' while going upwind. It is questionable whether they navigate by employing the same behavioral pattern or wind direction detection mechanisms as flying moths use. To analyze pheromone-mediated anemotaxis of potato moth males, their behavior was observed in a flat wind tunnel, in which a computer-regulated valve system introduces pheromone-permeated air as pulsed stimuli into the airflow. Path analyses revealed that males surged upwind when they encountered with a pheromone pulse and exhibited *casting*, which consisted of successive hops, after a loss of odor contact. These behavioral patterns produced similar paths to those of flying moths, though these were composed of walks and hops. When wings of males were ablated and deprived of flight control, they could still go upwind. Furthermore, males could adjust their course angle to the orthogonally switched wind direction while they were on the ground. Thus potato moths can detect wind direction and orient upwind on the ground, unlike flying moths, which navigate upwind while in flight. It is suggested that potato moths orient upwind not by optomotor cues, but by some other means such as mechanosensory cues as in 'aim-then-shoot' mechanisms expected in some dipteran species.

TEMPORAL AND CHEMICAL PARTITIONING OF A SHARED PHEROMONE AMONG FIVE CERAMBYCID SPECIES

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Attractant pheromones are highly conserved in the family Cerambycidae, and many species of beetles produce similar or even identical chemicals to attract mates. Among Nearctic species of the subfamily Cerambycinae, males of dozens of geographically and seasonally overlapping species produce the chemical (*R*)-3-hydroxyhexan-2-one ("3*R*") to attract conspecifics, but cross-attraction among species is rarely observed. In this study, we characterized the pheromones of five cerambycine species in central Illinois that share habitat and phenology, and also determined the time of day ("activity period") when synthetic pheromones elicited attraction. All five species produced 3R, but two species produced blends that included either nonan-2-one or 2-methylbutan-1-ol. Species that produced these secondary components overlapped in activity period, whereas those that produced only 3*R* were separated in activity across the day. Moreover, secondary components combined with 3*R* enhanced attraction of species that produced them, while simultaneously inhibiting attraction of species with similar activity periods. We propose that these complementary mechanisms of temporal isolation and species specificity of chemical signals are primary barriers to cross-attraction among these species. It is likely that analogous reproductive isolating mechanisms are employed by the many species of cerambycids with shared pheromone components.

ANTAGONISM BETWEEN HERBIVORE-INDUCED PLANT VOLATILES AND TRICHOMES AFFECTS TRITROPHIC INTERACTIONS

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To resist herbivore attack, plants adopt both chemical and physical means of defense. However, the relative impact and interaction between various defensive traits remain largely unknown. Here, we used tomato genotypes that are either normal in defensive traits expression, or that are impeded in the jasmonic acid (JA) defensive pathway for studying the interaction between the production of volatile organic compounds that serve as information cues for attracting herbivores as well as natural enemies of the herbivore, and the production of foliar trichomes as physical defense barriers. We found that the JA signaling *jai1* mutant plants with both reduced odors and trichome production received higher oviposition of adult leaminers and parasitism by the leafminer parasitoids than the JA biosynthesis *spr2* mutant plants with odor-deficiency only. Both leafminers and parasitoids prefer trichome-removed plants from either *spr2* or wild-type (WT) genotypes compared to trichome-intact genotypes. These consistent host acceptances by insects were therefore driven by trichomes rather than odors, confirming the predominant role of plant trichomes in restricting host selection. Although odors of WT plants were more attractive to adult insect herbivores, insects preferred trichome-free *jai1* plants for oviposition that add greater reproductive success. Overall, insect foraging success was severely decreased in trichome-rich plants, indicating an antagonism between plant odor emissions and trichome production on leaves. Our study thus suggests that insect behavior and tritrophic interactions can be manipulated via modified plant cuticular structures.

INTERPLANT COMMUNICATION OF TOMATO PLANTS THROUGH COMMON MYCORRHIZAL NETWORKS

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Many plants can ‘eavesdrop’ on their neighbours via herbivore-induced volatile signals and tailor their defences accordingly. Although there is increasing evidence of plant-plant communication, the majority of the studies conducted so far have focused on signals transferred from the sender to the receiver through induced volatiles. Communication via volatile signals, however, is subject to the vagaries of atmospheric conditions. Mycorrhizae are ubiquitous plant-fungus symbiosis. Common mycorrhizal networks (CMNs) link multiple plants together in ecosystems, and the ecological significance of plant-to-plant carbon and other nutrient movement through CMNs is well addressed. Here, we show that CMNs mediate plant-plant communication between healthy plants and pathogen-infected tomato plants (*Lycopersicon esculentum* Mill.). After establishment of CMNs with the arbuscular mycorrhizal fungus *Glomus mosseae* between tomato plants, inoculation of ‘donor’ plants with *Alternaria solani* led to increases in disease resistance and activities of the putative defensive enzymes in healthy neighbouring **‘**receiver**’** plants. The uninfected **‘**receiver**’** plants also activated six defence-related genes. This finding indicates that CMNs may function as a plant-plant underground communication conduit whereby disease resistance and induced defence response signals can be transferred between the healthy plants and pathogen-infected neighbouring plants, suggesting that plants can ‘eavesdrop’ on defence signals from the pathogen-challenged neighbours through CMNs to activate defences before being attacked themselves.

SYMPOSIUM TOPIC 10: NEW STUDIES IN CHEMICAL ECOLOGY INVOLVING VERTEBRATES AND OPPORTUNITIES FOR SOLVING PRACTICAL PROBLEMS OF LIVESTOCK PRODUCTION AND HUMAN HEALTH

Topic Coordinators:

John Pickett (Rothamsted Research, UK) and **Mike Birkett** (Rothamsted Research, UK)

OPPORTUNITIES FOR LIVESTOCK PRODUCTION, INCLUDING FISH FARMING, AND HUMAN HEALTH FROM NEW CONCEPTS IN INVERTEBRATE CHEMICAL ECOLOGY

John PICKETT[1], Michael BIRKETT[1]

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Chemical ecology offers new opportunities for control of arthropod ectoparasites causing nuisance or acting as pathogen vectors in livestock production and for human health. Since the review article Pickett *et al*. of 2010, in which the hypothesis was advanced that semiochemicals signalling inappropriate hosts could be used against vertebrate host location, new examples have been identified and further practical developments made. As well as the further elaboration and testing of the hypothesis, new practical approaches will be exemplified and the scene set for further exemplification during the course of the Symposium from other laboratories around the world, including Africa and Brazil.

Pickett et al. 2010. *J. Chem. Ecol.* 36**,**113-121.

CHEMICALLY-MEDIATED INTERACTIONS IN RIFT VALLEY FEVER VIRUS (RVFV) VECTOR-HOST SYSTEM

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Rift Valley fever (RVF), a mosquito-borne anthropozoonosis, is a major public health and veterinary problem in sub-Saharan Africa. Enzootic transmission of arboviral diseases especially RVF continues to occur at a low intensity among mosquito vectors, which may remain undetected by most monitoring programs unless very sensitive tools are employed to detect virus activity before an outbreak occurs. In order to circumvent this challenge, we exploit the cues used by adult RVF virus (RVFV) vectors for locating their hosts for a blood meal, as a means to improve trap captures to maximize virus detection probability during the Inter-epidemic period (IEP). In field bioassays, we found that skin odors from RVFV hosts including cow, donkey, goats, sheep and human contribute significantly to the attraction of RVFV mosquito vectors. Using electrophysiological and chemical analyses, we found that both primary and secondary mosquito vectors of the virus detect similar components in the skin odors of these hosts which were identified by mass spectrometry as heptanal, octanal, nonanal and decanal. In field trials, each of these compounds when combined with CO_2 increased captures of these mosquito vectors in a dose-dependent manner. Additionally, a blend formulated from optimal attractive dose of each of these compounds combined with CO_2 significantly increased trap captures compared to control traps baited with CO_2 alone. Our study demonstrates that RVFV hosts produce a common chemical signature attractive to mosquito vectors, and that this trapping system offers the potential for its use in surveillance programs for these vectors especially during the IEP.

COWS IN 'WATERBUCK CLOTHING' FOR PROTECTION AGAINST TSETSE FLIES AND ENHANCED PRODUCTIVITY

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In sub-Saharan Africa, the tsetse fly (Genus: *Glossina*) is the cyclical vector of trypanosomosis, a disease of livestock and humans, caused by unicellular parasites of the genus Trypanosoma. Whilst the human form of the disease appears to be on the decline, animal trypanosomosis is still widely reported and causes considerable losses in the livestock sector resulting in major impacts on agricultural production, livelihoods and food security. Repellents for the control/management of tsetse have been identified at *icipe* from synthetic sources and from natural blends of un-preferred animals (e.g. waterbuck), which are common in tsetse habitats but not fed upon. The synthetic tsetse repellent 2-methoxy-4-methylphenol *(icipe patent)* and the 5-constituent waterbuck repellent blend *(icipe patent)* are being validated in large-scale field trials. Prototype repellent collars have been developed for dispensing of the repellents on the host (*icipe patent*). Despite the prototype nature of the technology, these repellents provide substantial protection to cattle (disease incidence reduced by >90%) either in 'push' mode or when used in conjunction with baited traps in 'push-pull' strategy. Body weights of protected animals have increased significantly resulting in higher selling prices and more traction power (of the bulls) which in turn has brought more land under cultivation. Milk production has also gone up even though the lactating cows are native. Farmers can now graze their animals anywhere including in areas where tsetse are present. Most livestock keepers prefer the repellent technology due to its simplicity and mobility. Further refinement of the technology continues.

MALARIA-INDUCED CHANGES IN HOST ODORS: IMPLICATIONS FOR VECTOR TRANSMISSION AND DISEASE DIAGNOSIS

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Malaria remains among the deadliest diseases afflicting human populations. Efforts to control the spread of this disease would benefit greatly from an enhanced understanding of the ecological factors influencing vector transmission, as well as from novel diagnostic techniques capable of efficiently identifying otherwise asymptomatic carriers of infection under field conditions. We are investigating how malaria-induced changes in host odors influence the recruitment of mosquito vectors to infected individuals and the potential of these altered volatile signatures to serve as diagnostic biomarkers of infection. Here we present data from work on the malaria parasite *Plasmodium chabaudii* in a mouse model. We report that malaria infection induces characteristic changes in the odors of infected mice that alter mosquito attraction. In wind-tunnel assays, mosquitos exhibited reduced attraction to mice exhibiting acute malaria symptoms. But, once mice recovered from the acute phase of the disease, mosquitoes consistently preferred the odors of infected mice (vs. healthy controls) during a critical period that coincides with high levels of infectivity. Mosquitoes furthermore preferred odors of healthy mice that were manipulated to mimic malaria-induced changes in individual volatile compounds (vs. un-manipulated controls). Furthermore, dramatic differences in the volatile profiles of healthy and infected individuals were detectable over the entire course of infection, even when mice were otherwise asymptomatic. These results suggest that volatile cues may have significant potential as biomarkers of malaria infection and, more generally, that volatile profiling may prove a promising technique for the development of diagnostic assays for infectious diseases vectored by insects.

ACACIA PROANTHOCYANIDINS AS INHIBITORS OF RUMINAL METHANE PRODUCTION: STRUCTURE-FUNCTION RELATIONSHIPS

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Interactions between gastrointestinal microorganism and the host are mediated in part by chemical constituents of the host diet. Understanding how diet affects interactions between animals and gastrointestinal microbes could boost animal productivity and diminish production of the greenhouse gas methane, a byproduct of rumen fermentation. We evaluated nine warm-season perennial legumes using an *in vitro* gas production technique. Leaf tissue was incubated with bovine rumen fluid under anaerobic conditions for 48 h, yielding between 0-40 mg CH_4/g dry matter for the various plants. Proanthocyanidins (condensed tannins) were found in all the plants, but the level (acid butanol) and bioactivity (protein precipitable phenolics) did not correlate with methane production. We evaluated the subunit composition of proanthocyanidins in the plants by thiolysis, and found that tannin from one of the lowest methane-producing plants, *Acacia angustissima* var. *hirta,* was uniquely resistant to degradation. Electrospray ionization mass spectrometry showed that the *Acacia* proanthocyanidin was comprised predominantly of 5-deoxy flavan-3-ol subunits. The 5-deoxy subunit decreases reactivity of the interflavan bond, resulting in low product yield in thiolysis and acid butanol reactions. We speculate that low reactivity might lead to increased lifetime and therefore enhanced activity in the gastrointestinal tract, resulting in inhibition of methane-producing microbes. We are assessing stability of these tannins under simulated gastrointestinal conditions, and evaluating the structural details of the tannins from the other legume species. Understanding the relationship between structure and activity for proanthocyanidins provides new insights into gastrointestinal microbial ecology and substantiates the hypothesis that tannins exhibit structure-specific bioactivities.

PRESENCE OF NATURAL REPELLENTS IN THE ODOR OF BEAGLE DOGS AGAINST THE TICK *RHIPICEPHALUS SANGUINEUS* (ACARI: IXODIDAE)

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The cosmopolitan tick, *Rhipicephalus sanguineus,* can parasitise, and transmit diseases to, dogs and humans. Recently, using artificial infestations to compare the development of the tick in two breeds of dogs (English cocker spaniel and beagle), it was seen that ticks fed on beagles had their development affected, thus leading to the suggestion that these can be considered as resistant dogs. Behavior studies were conducted to evaluate if the tick could perceive these resistant animals. When allowed to choose between substances collected from dogs, more ticks were arrested by extracts from the cocker spaniels than from beagles. In an olfactometer, the ticks were not attracted to the odor of either breed. However, the odor of the beagle was apparently repellent. Using coupled high-resolution gas chromatography-mass spectrometry (GC-MS), a higher number of chemical compounds was observed in the beagle odor extract. Some compounds, such as 2-hexanone, nonane, undecane, decane and benzaldehyde were exclusively found in beagle extracts and were tested in a Petri dish test for repellence. Undecane was repellent in the first five minutes, whereas benzaldehyde and 2-hexanone were repellent 30 min after deployment. Decane and nonane were not repellent in any interval evaluated. As far as we know, this work describes for the first time, the presence of natural vertebrate-derived repellents modulating parasitism between a tick and its natural host.
Louly et al. 2009. *Int. J. Acarol*, 35, 25-32.

NEW CHEMICAL ECOLOGY BASED INTERVENTIONS FOR THE CONTROL OF ECTOPARASITES AFFECTING FARMED LIVESTOCK AND HUMAN HEALTH

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Ectoparasites (flies, ticks and mites) affect farmed livestock production and human health on a global scale, and with the growing problem of the development of insecticide and acaricide resistance, alternative sustainable interventions are required, including those based on deployment of semiochemicals. Novel repellents can be developed according to hypotheses relating to the evolution of repellency (Pickett et al., 2010), specifically natural products that interfere with host location. In addition, pheromone-based trapping systems can be deployed. Thus, aggregation pheromones for ectoparasites have been identified, with a view to their deployment in farmed environments and domestic households. The prospects for deployment of repellents and pheromones in novel semiochemical-based control strategies for ectoparasites will be discussed.
Pickett et al. 2010. *J. Chem. Ecol.*, 36, 113-121.

CHEMICAL COMMUNICATION IN FROGS

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Frogs are well known to communicate by acoustic and optical signals, but their chemical communication is less well explored. Most research of amphibian pheromones focuses on peptides and other water soluble compounds. In contrast, we will here report on the production of volatile pheromones used by mantelline frogs from Madagascar and hyperolids from central Africa. Male mantelline frogs possess femoral glands that are used to disseminate volatile compounds, mostly alcohols and macrolides. These glands contain species-specific mixtures of compounds. A detailed analysis of individual *Mantidactylus femoralis* shows also differences between different collection sites. The pheromones induce increased mobility and attract frogs to its source. Hyperolid frogs contain gular glands that are expanded during calling. The gland content is chemically diverse and includes macrolides, sesquiterpenes, and alcohols. The compounds seem to function as part of a trimodal communication system because during every call the brightly colored gland is exposed releasing the volatile chemicals. The biosynthesis of the involved pheromones was also investigated.
Poth et al.2012. *Angew. Chem. Int. Ed., 51*, 2187–2190.

THE TOXICITY OF POISON DART FROG ALKALOIDS AGAINST THE FIRE ANT (*SOLENOPSIS INVICTA*)

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Hundreds of alkaloids, representing over 20 structural classes, have been identified from the skin of neotropical poison frogs (*Dendrobatidae*). These alkaloids are derived from arthropod prey of the frogs, and are generally are believed to deter vertebrate predators. We developed a method to put individual red imported fire ant (*Solenopsis invicta*) worker cuticle in contact with discrete amounts of the above alkaloids. We then tested the worker ant response following contact with each of 20 poison dart frog alkaloids (12 structural classes). Individual ants forced to contact the dried residues of 13 compounds exhibited convulsions and/or reduced ability to move. The cutaneous concentrations of several compounds were estimated and our results indicate that some anuran skin compounds can function defensively as contact toxins against arthropod predators, such as the fire ant.

LOVE MAKES SCENTS: OLFACTORY COMMUNICATION AND MATING BEHAVIOUR IN THE ECHIDNA

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Chemical signals are fundamentally important for coordinating mating behaviour in a range of mammalian species, however due to their complexity these signals are often not well understood. Furthermore, only a few species have been studied in detail and hence we have a limited understanding of the evolution of mammalian (and vertebrate) chemical signals. The short-beaked echidna (*Tachyglossus aculeatus*) is the most common extant species of monotreme, and Australia's most widely distributed native mammal. Echidnas are usually solitary, but form mating aggregations during the breeding season. In colder parts of the echidna's range, mating immediately follows several months of hibernation and is characterised by intense male intra-sexual competition for access to females. In Tasmania, male echidnas may locate and mate with females which have not yet emerged from hibernation. Males appear to respond to pheromones or chemical attractants produced by females; however this has never been investigated in detail. We used a combination of gas chromatography-mass spectrometry, behaviour observations, physiology and genetics to describe variations and possible functions of chemical signals in the Tasmanian echidna. We collected samples of spur and cloaca gland secretions from 78 wild individuals over a three-year period, and found that echidna secretions are seasonally variable and chemically complex: 186 compounds were identified, including a novel vertebrate sesquiterpene (juvabione) and sterol (desmostanol). Chemical profiles also varied between samples from different secretory glands, sexes and individuals. Our results support the hypothesis that echidnas use chemical signals during the mating season, and provide new insights into the function and importance of chemical signals in mammals and other vertebrates.

IDENTIFICATION OF SCENT SIGNALS CONTRIBUTING TO SPECIES, SEX, AGE AND INDIVIDUAL RECOGNITION FROM ANAL SAC SECRETIONS AND FECES IN THE DOMESTIC CAT

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Many mammals use scent signals for species, sex, age, and individual recognition. Urine, feces, and anal sac secretions are rich sources of volatile odorants, some of which differ across species, sex, age, and individuals. These variable compounds are candidate scent signals, which can be identified using gas chromatography-mass spectroscopy (GC-MS). Nevertheless, little is known about the compounds that are scent signals in each species, because not all of the volatiles emitted contribute to the odor. Therefore, it is essential to examine whether mammals can distinguish the variation of each candidate in the scent source using behavior bioassays. However, if our targets are companion and wild animals, and not laboratory rodents, we have to test candidates using limited numbers of both experimental animals and bioassays. Therefore, in addition to GC-MS, we tested whether an electric nose is useful to narrow candidate scent signals before performing bioassays. We examined animal behavior that domestic cats sniff the anuses of others. We first analyzed the chemical profiles of volatiles of anal sac secretions and feces using GC-MS. Then, we focused on free fatty acids and a mercaptan that vary across species, sex, age, or individuals, and examined them in omission experiments using an electric nose. Based on the GC-MS and electric nose analyses, we finally tested candidates in bioassays using cats, and found that the fatty acids were individual signals and the mercaptan was a species, sex and age signal. These studies will also contribute to identify scent signals in other mammals.

WHY DO LUMHOLTZ' TREE-KANGAROOS APPLY INEFFICIENT ANTI-PREDATORY STRATEGIES TOWARDS DOGS?

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The Lumholtz's tree-kangaroo is an endemic species in tropical rainforests of north-eastern Australia. It is classified as "near threatened" under the Queensland Nature Conservation (Wildlife) Regulation Act (2006). Besides habitat loss and collisions of tree-kangaroos with vehicles, tree-kangaroo populations are threatened by introduced predators. Wild and domestic dogs cause up to 10% of annual tree-kangaroo fatalities suggesting that tree-kangaroos may be unable to recognize them as predators or applying anti-predatory strategies that are inefficient towards these ground-based predators. Our study investigates the responses of tree-kangaroos to odour cues from an extant predator and from two novel predators and their ability to recognize them as predatory threats. Five captive tree-kangaroos were exposed to scat material from pythons (*Morelia amethistina*), from Dingos (*Canis lupus dingo*) and from Domestic Dogs (*Canis familiaris*). Subjects were also exposed to a non-predatory, novel odour (garlic). Duration and frequency of comfort, locomotor, chemosensory and stress behaviours were analysed. The study will help to understand the mechanisms of high tree-kangaroo mortalities due to dogs and may open up avenues for a possible anti-predation pre-release training of rehabilitated animals.

SYMPOSIUM TOPIC 11: INSECT (DROSOPHILA) NEUROETHOLOGY

Topic Coordinators:
Coral Warr (Monash University, Australia) and **Bill Hansson** (Max Planck Institute for Chemical Ecology, Germany)

GOOD AND BAD ODORS –CORRELATES OF VALENCE IN THE *DROSOPHILA* OLFACTORY SYSTEM

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How are attractive and repellent odors coded in the *Drosophila* peripheral and central nervous system? Does e.g. the glomerular topography of the insect antennal lobe have any significance? We know that male insects optimized to detect conspecific intersexual pheromones have a specific part of the antenna and the lobe devoted to this task, but we know very little regarding the importance of specific sensory neurons and antennal lobe regions beyond this. Besides sex attractants, brains have to decide whether and how to respond to detected stimuli based on, often, complex sensory input. The vinegar fly *Drosophila melanogaster* evaluates potential food sources largely based on olfactory cues. Here I will cover some of our recent investigations into coding of valence in the *Drosophila* brain, from sensory neurons to higher brain areas.

NATURALLY OCCURRING OLFACTORY RECEPTOR VARIANTS IN *DROSOPHILA*

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Insect olfactory receptor genes are large, rapidly evolving gene families that are of considerable interest for evolutionary studies as they determine the responses of sensory neurons which mediate critical behaviours and ecological adaptations. In *Drosophila melanogaster* most olfactory receptor neurons (ORNs) express members of the *Or* receptor gene family, and the *Or* endows the ORN with all its odour-specific response properties. High levels of variation in the amino acid sequence and odour response spectra of *Or* paralogues has hampered the identification of functionally important residues. Identifying naturally occurring variation in individual Or response properties, either within a single species, *D. melanogaster*, or across species in the Drosophila genus, provides opportunities to both undertake structure/function studies of *Or* genes and to understand the effect of Or/ORN functional variation on insect ecology. We are studying natural variation in Or response properties in natural populations of *D. melanogaster* from Australia and also within the *Drosophila* Genome Reference Panel strains. Identified Or variants that correlate with functional changes to ORN response properties are expressed in the empty neuron system to confirm causality. Detailed structure-function studies can then be performed to identify the causative amino acid changes, and olfactory behavior assays performed to determine the effect of ORN response changes on behaviour.
Mackay et al. 2012. *Nature* 482, 173-178.
Dobritsa et al. 2003. *Neuron* 37, 827-841.

THE IMPACT OF ENDOSYMBIOTIC *WOLBACHIA* ON DROSOPHILA OLFACTION, SEXUAL BEHAVIOR AND SPECIATION

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Maternally transmitted α-proteobacteria of the genus *Wolbachia* are common endosymbionts of most terrestrial arthropods; it has recently attracted much broader interest due to this microorganism's capacity to act as parasites by manipulating host reproduction in adaptive manners in germlines. Plus, because of potential boosting of host immunity against various insect pathogens, such as dengue virus and plasmodia by this remarkable obligatory endosymbionts against various insect pathogens, *Wolbachia* have become a promising tool for fighting insect-transmitted human diseases. Moreover, some *Wolbachia* infections also accumulate in fat bodies and neuronal tissues from where they presumably manipulate and orchestrate sexual behavior in an adaptive manner, by increasing host fitness and mating successes. In such, endosymbiontic *Wolbachia* can trigger sexual selection and thereby can foster incipient host speciation as by-products of their augmented mutation rates. Since neotropical *Drosophila* of *willistoni* group species serve as ancestral reservoirs of *Wolbachia* infection, *Wolbachia* have evolved intimate, fixed, obligate mutualistic functions by providing vital factors supporting host fitness and fecundity. Here we demonstrate impacts of this tightly coevolved symbiont on female mate recognition, since artificially *Wolbachia*-depleted females lose assortative mating behavior by accepting improper heterogamic males randomly. Contrary to the situation in *D. melanogaster* where the facultative endosymbiont occupies larval and adult brains globally, obligate mutualistic *Wolbachia* of neotropical *Drosophila* are restricted to well-defined brain regions associated with antennal lobes and mushroom bodies of *willistoni* group brains. Hence we discuss the role of symbiotic microorganisms in neuronal tissues on pheromone perception, signal interpretation, and mating behaviors.

NEURONAL MECHANISMS OF ODOR LEARNING IN *DROSOPHILA*: A THERMOGENETICALLY INDUCED MEMORY ENGRAM IN KENYON CELLS DRIVES LEARNED BEHAVIOR.

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Experience-dependent plasticity in odor-driven behavior is a property not only of higher vertebrates, but also of insects. A key question is where and how in the insect brain learned relevance of odor information is acquired and stored. *Drosophila melanogaster* represents a model organism for analyzing how neuronal circuits process odor information and mediate olfactory learning and memory. Fruit flies can be trained to avoid an odor that has been presented simultaneously with a punitive electric shock. In the mushroom bodies of the fly's brain odors are encoded as sparsely activated ensembles of Kenyon cells, and these neurons are required for associative odor learning and memory retrieval. It remains, however, unknown whether the mushroom body circuitry is indeed sufficient for odor learning. We show that associative learning can be artificially induced by thermogenetically activating random ensembles of Kenyon cells using a temperature-dependent cation channel (dTRPA1) in coincidence with a punitive stimulus. We find that the animals learn to adjust their behavior in a subsequent test situation and actively avoid reactivation of these Kenyon cells. Since the targeted, thermogenetic induction of learning bypasses sensory input, and mushroom body output is dispensable for learning, our data substantiate that associative learning can be allocated to the mushroom body. Learned avoidance behavior is driven by a reactivation of the trained Kenyon cell ensembles. Implications of these findings on odor processing and odor learning by insect brains in a neuroethological context will be discussed.
Heisenberg. 2003. *Nat Rev Neurosci*., 4, 266-275.

TASTE RECEPTORS AND FOOD PREFERENCES IN DROSOPHILA

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Animals rely on their taste systems to select appropriate foods for consumption. We use the model insect *Drosophila melanogaster* to understand the molecular and cellular mechanisms by which tastants are encoded by sensory neurons. One focus is on a large family of 68 Gustatory receptors (Grs), members of which are expressed in complex combinatorial patterns in sweet and bitter taste neurons. Gr proteins are novel chemosensory receptors found across all insect species, yet their functional properties are poorly understood. We have been analyzing functional properties of a highly conserved clade of eight Grs that include receptors that we and others have shown to be involved in sugar detection. More recently we found that Gr64e, a receptor in this clade, plays an essential role in feeding preference for beer and other yeast fermentation products. We linked this to a role for Gr64e in mediating neuronal and behavioral responses to glycerol, which is abundant in and around yeasts. A comparison of Gr64e function and glycerol sensitivity across *Drosophila* species suggests that Gr64e may contribute to specific evolutionary variations in appetitive selectivity. To extend these studies, we have developed a novel *in vivo* expression system to "decode" taste receptors and have found that individual Grs are determinants of sweet ligand specificity. Interestingly, we also found that sweet Grs are directly and selectively inhibited by bitter alkaloids suggesting combinatorial mechanisms for sweet and bitter detection. We are now poised to further investigate mechanisms of taste detection in *Drosophila* and other insects.

SUGAR AVERSION: A NEWLY-ACQUIRED ADAPTIVE CHANGE IN GUSTATORY RECEPTOR NEURONS IN MULTIPLE FIELD POPULATIONS OF THE GERMAN COCKROACH

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In response to the anthropogenic assault of toxic baits, populations of the German cockroach have rapidly evolved a novel adaptive behavior—a behavioral aversion of glucose, a phagostimulant component of baits, that lets cockroaches steer clear of the bait. We hypothesized that changes in the peripheral gustatory system are responsible for glucose aversion, and using behavioral and electrophysiological analyses we tested the sensitivities of gustatory receptor neurons (GRNs) in mouthpart sensilla of wild-type (WT) and glucose-averse (GA) cockroaches. In both strains, the phagostimulant D-fructose stimulated a sugar-GRN, whereas caffeine, a bitter deterrent compound, stimulated a bitter-GRN. D-glucose, like D-fructose, also simulated the sugar-GRN in WT cockroaches, but in GA cockroaches it stimulated both sugar- and bitter-GRNs. Similar changes in the GRN properties for D-glucose reception were observed in other field-collected GA cockroach populations. These results indicate that D-glucose is processed as both a phagostimulant and deterrent in GA cockroaches, and suggest that this newly ***acquired*** peripheral taste sensitivity underlies the GA trait. The rapid emergence of this novel and highly adaptive behavior underscores the plasticity of the sensory system to accommodate and adapt to rapid environmental change.

OLFACTORY MODULATION VIA BLEND DETECTION: INTRASENSILLAR INTERACTIONS OF GYPSY MOTH PHEROMONE

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The European Gypsy moth, *Lymantria dispar* is one of the world's most devastating forestry pests, and presently under Federal Domestic Quarantine in the United States and Canada. Gypsy moths share disparlure as a female sex pheromone component with the Nun moth, *Lymantria monacha*, which is sympatric through their European range. Species isolation is maintained, in part, via differential ratiometric production and attraction to (+/-) enantiomers: '+' disparlure ((+) D) being important for both species, and '-' disparlure ((-) D) being more prevalent (90% (-) D) in *L. monacha*. *Lymantria dispar* males are deterred by higher concentrations of (-) D in female *L. monacha* pheromone. In *L. dispar* there are two olfactory receptor neurons (ORNs) in every pheromone-sensitive sensillum; one neuron responds to (+) D and the other to (-) D. The objective of this study was to utilize responses of ORNs to ratiometric blends of disparlure enantiomers via single sensillum recording to examine interactions between ORNs at the sensillar level. Furthermore, the gap junction blocker heptanal was employed to evaluate possible ephaptic communication between ORNs. Inhibition, synergism and variations in threshold olfactory sensitivity to these odorants suggest significant signal modulation is occurring within the sensillum. This provides mechanistic evidence for early modification of odor coding in the olfactory system as affected by stimulus mixtures.

OLFACTORY RECEPTOR NEURONS FOR PHEROMONE AND PLANT VOLATILE COMPOUNDS IN THE CLOVER ROOT WEEVIL, *SITONA LEPIDUS*

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Antennal olfactory receptor neurons (ORNs) for pheromone-related and plant volatile compounds were identified and characterized in male and female clover root weevil, *Sitona lepidus* (Gyllenhal), using the single sensillum recording technique with five pheromone-related compounds, and 40 host and non-host plant volatile compounds. Overall, seven different types of olfactory sensilla containing specialized ORNs were identified in male *S. lepidus*, and seven different types in the females. Among them, three different types of sensilla in the males and two types in the females housed ORNs specialized for pheromone-related compounds. The ORNs in males were specialized for 4-methyl-3,5-heptanedione or one or more of four stereoisomers of (4*S*,5*S*)-5-hydroxy-4-methyl-3-heptanone. In contrast, female sensilla did not contain sensitive ORNs for 4-methyl-3,5-heptanedione while sensitive and specialised ORNs for the stereoisomers of (4*S*,5*S*)-5-hydroxy-4-methyl-3-heptanone were present in the female sensilla. In addition to the pheromone-related ORNs, four types of olfactory sensilla contained ORNs responsive to plant volatile compounds in male *S. lepidus*, and five types in females. Most of the ORNs identified in *S. lepidus* showed a high degree of specificity to specific volatile compounds, although some of the active compounds showed overlapping response spectra in the ORNs across different types of sensilla. The most active plant volatile compounds were the four green leaf volatile compounds (*E*2-hexenol, *Z*2-hexenol, *Z*3-hexenol and *E*2-hexenal), and two monoterpenols, (±)-linalool and (±)-α-terpineol, all eliciting strong responses from relatively large numbers of ORNs in male and female *S. lepidus*. Our study indicates that *S. lepidus* has a set of highly sensitive and selective ORNs for pheromone and plant volatile compounds.

WHOSE CUES? MIXED-SPECIES AGGREGATION IN LADYBIRDS: PHYSIOLOGY, BEHAVIOUR & CHEMISTRY

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The Tasmanian ladybirds *Harmonia conformis* and *Cleobora mellyi* are important predators in agriculture, horticulture and forestry when active, but can become serious nuisance pests forming large aggregations in some buildings during winter. The chemical basis for these aggregations was investigated to identify cues used in formation and persistence of ladybird aggregations, and to examine species, sex and seasonal diversity and similarity in hydrocarbon (HC) profiles between these two sympatric species. Reciprocal exposure bioassays between the two species were conducted with overwintering (winter) and active (summer) populations, and an allopatric active population of *H. conformis* (originating from Queensland). We measured "propensity to aggregate" in blank and previously occupied glass arenas, and linked this to physiological state (fat body size) and HC chemistry of cuticular and trail extracts. The two species consistently re-formed aggregations in winter when exposed to their own and each other's leg trails, but, unexpectedly, active *H. conformis* from both geographical regions also preferred surfaces previously occupied by both species in summer. Moreover, each species responded to each other's leg trails, but not their cuticular washes, suggesting leg trails are the bioactive cues used in aggregation formation. Numerous saturated, unsaturated and methyl-branched HCs were identified from both species with few being common to both and their production differing between overwintering and active individuals. Ongoing experiments are being conducted to determine how fat body accumulation and HC chemistry relate to aggregation behaviours in these species.

SECRETS IN THE DARK: SEXUAL COMMUNICATION VARIATION IN THE TWO *SPODOPTERA FRUGIPERDA* STRAINS

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The noctuid moth *Spodoptera frugiperda* (Lepidoptera: Noctuidae) consists of two morphologically identical but genetically and behaviorally different "host-strains": the corn-strain and the rice-strain. The two strains seem to be in the progress of sympatric speciation and so far three possible prezygotic isolation barriers have been described: 1. habitat isolation through differential choice of host plants, 2. behavioural isolation through differential timing of reproduction in the scotophase, and 3. behavioural isolation in terms of strain-specific female sex pheromone production. To assess whether strain-specific sexual communication is an important prezygotic mating barrier between the strains, we conducted female pheromone gland extractions and tested the male response to the strain-specific female pheromone in the wind tunnel and the field. Although we found consistent differences in the female pheromone production, males were not consistently attracted to females of their own strain in the wind tunnel and in a corn- and a grass habitat in Florida. Thus, differences in the sexual communication system of both strains are probably not strong enough to cause assortative mating in the field. When we tested the male response to synthetic pheromone blends in different regions in North America, the Caribbean and South America, we found that mainly corn-strain males, not rice-strain males, varied in their response between regions. Overall, strain-specific sexual communication seems to be the weakest of all prezygotic mating barriers that have been described so far, although it possibly interacts with other isolation barriers to drive divergence between the corn- and the rice-strain.

MANIPULATING ODOR CODING AND BEHAVIOR IN *DROSOPHILA MELANOGASTER*

Anandasankar RAY[1]

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The insect olfactory system uses sophisticated molecular and cellular mechanisms to detect and discriminate odorants in the environment. By uncovering attractive and repellent pathways we have been able to model olfactory behavior and design novel strategies to modify it with odors. Identification of these behavior-inducing pathways allow us to apply a powerful computational approach to screen ~0.5 million compounds to identify compounds that have electrophysiological activity with a high rate of success. From these ligands we find that inhibitory odorants of attractive pathways mask attraction, while excitatory odors can act as powerful lures. Odorants that cause prolonged activation “blind” the olfactory system and mask attraction. Conversely, excitatory ligands of avoidance pathways cause repellency, while inhibitors cause attraction. Our studies of “mask”, “push” and “pull” in *Drosophila melanogaster* reveal conserved principles in olfaction that can be used to manipulate the behaviors of insects that vector deadly human diseases and destroy agricultural plants.

SYMPOSIUM TOPIC 12: MOLECULAR MECHANISMS IN PERCEPTION OF SEMIOCHEMICALS

Topic Coordinators:
Judith Reinhard (University of Queensland, Australia) and **Charles Claudianos** (University of Queensland, Australia)

HONEYBEE OLFACTORY PLASTICITY: SCENT MEMORIES REGULATE OLFACTORY RECEPTOR EXPRESSION AND MODIFY ODOUR PREFERENCES

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Sensory systems plastically adapt to environmental conditions, inducing modifications in the way they process and respond to stimuli. The olfactory system is no exception, however little is known about the molecular mechanisms underlying olfactory plasticity. Honeybees are an ideal model system to investigate this question. They are relatively long-lived animals that encounter changing scent environments throughout their adult life. Here, we present evidence that olfactory experience triggers molecular changes in the sensory periphery, namely the expression of 7-transmembrane olfactory receptor proteins (ORs) on dendrites of olfactory neurons. The expression of six honeybee ORs that were shown to bind common floral odorants such as linalool and nerol, varies significantly with transition from hive nurse bee to outdoor foraging bees, and with exposure to different flowering plants in the four seasons. When bees were conditioned to these specific floral odorants, the respective ORs in the antennae were down-regulated. The physiological response of the corresponding olfactory neurons in the antennae was also reduced after scent conditioning. Importantly, OR down-regulation only occurred in context of scent learning; mere exposure to the same scents induced no changes in OR expression, suggesting that molecular mechanisms involved in memory formation also regulate OR expression. Our research demonstrates that the olfactory system of honeybees is highly plastic, constantly adapting via differential gene expression to scent experiences, which in turn affects odour preferences. We propose that this plasticity enables the olfactory system to be optimally tuned to process familiar odorants as well as detect novel ones.

MICRO-RNA REGULATION OF OLFACTORY LEARNING AND MEMORY IN HONEYBEES

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Honeybees forage in an ever-changing olfactory environment, which requires constant learning and re-learning of floral odours associated with food sources. Plastic olfactory memories hence are crucial to a honeybee's foraging success, however the molecular mechanisms regulating olfactory learning and memory are not well understood. Here, we investigated how learning and long-term memory formation affects gene expression in the honeybee brain using an olfactory conditioning paradigm (proboscis extension reflex, PER). A microarray gene expression analysis comparing groups of conditioned and control bees found 53 genes differentially expressed between these two groups. Most of these genes were down regulated in trained bees, with only a few non-coding RNAs upregulated. Many of the down-regulated genes were enriched with binding sites for microRNAs (miRNAs), that putatively dysregulate these genes during memory formation. Indeed, qRT-PCR analysis validated that seven miRNAs were upregulated in trained bees. Of these seven miRNAs, we further investigated miR-210, which is associated with foraging, miR-928 and miR-932, which are embedded in key neurological genes: a potassium channel *(eag)* and a synaptic adhesion molecule *neuroligin 2 (Nlg2)*, respectively. We suggest that 'modules of miRNAs may regulate synapse development during learning and memory processes'. To test this hypothesis we used small interference RNAs (cholesterol conjugated antagomirs) to inhibit miR210, miR928, and miR932. Inhibition of miR928 and miR932 impaired the formation of olfactory long-term memory while inhibition of miR210 had no effect. Our results show that miRNAs associated with key synaptic genes are involved in plastically regulating long-term memory formation in honeybees.

PHENOTYPIC PLASTICITY IN OLFACTORY RELATED GENE EXPRESSION OF LEAF CUTTING ANTS (*ATTA VOLLENWEIDERI*)

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Olfactory systems in insects show an enormous diversity as a result of adaptations to species-specific selective pressures. For example, sexual selection in many moth species led to extremely large glomeruli (MGs) in the antennal lobe (AL). In eusocial insects, where selection acts on the colony level, even within workers of leaf-cutting ant colonies at least three different AL phenotypes (concerning MGs and number of glomeruli) have been described. Using this developmentally induced plasticity of the olfactory system, we investigate the molecular basis of AL-phenotypes and correlate these with odor-guided behaviors. Based on antennal transcripts, we performed phenotype-specific microarrays. We identified members of different olfactory related gene families (IRs, GRs, ORs, SNMPs, CSPs, OBPs). Ants have an amazingly high number of odorant receptor (OR) genes, even compared to other Hymenoptera, and ant-specific expansions in several OR-subfamilies have been described. Across AL-phenotypes, several genes are differentially expressed: For example, two OR and four ionotropic receptor (IR) genes are highly expressed in males, and one OR gene is highly expressed in large workers. While the highly expressed OR genes are good candidates for sex and trail pheromone receptors, the role of the IRs is still unsolved. Phylogenetic analysis of the candidate pheromone receptor genes indicates common ancestry of the two pheromone systems. Funded by DFG: SPP 1392; KL 1327/2.
Kelber et al. 2010. *Developmental Neurobiology* 70, 222-234.
Zhou et al. 2012. *PloS Genetics* 8: e1002930.

CHARACTERISATION OF CARBOXYLESTERASES ASSOCIATED WITH CLEARANCE OF SEX PHEROMONES AND PLANT ODORANTS ON THE BODY SURFACE IN TWO *SPODOPTERA* SPECIES

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Carboxyl/cholinesterase (CCE) is a large gene family of diverse functions in neuro/developmental processes, dietary detoxification and insecticide resistance, but it has been rarely functionally characterized with respect to degradation of sex pheromones and general odorants in insects. In the present study, two putative odorant degrading enzymes (*SexiCXE13* and *SlituCXE13)* of CCE family, from male antennae of *Spodoptera exigua* and *S. litura,* were cloned and functionally characterized. Quantitative real time PCR measurements revealed that two genes expressed ubiquitoustly in all tested tissues, with higher levels in abdomen, wings, antennae and probocis. The esterases encoded by the two genes were detected by SDS-PAGE and determined by mass spectrometry analysis in moth integumental elution, indicating their presence on the body surface of the moths. Further enzymatic analysis showed that the two in vitro expressed enzymes, integumental elutions, as well as the crude antennal extracts could degrade both ester sex pheromones and plant volatiles with high activity. Our results suggest that the two CXEs may fuction as integumental esterase to decrease the background noise of ester sex pheromones and other odorants absorbed onto the body surface, thus to maintain the olfactory sensitivity in *S. exigua* and *S. litura*. The work was supported by a grant from the National Natural Science Foundation of China (30770278).

DECIPHERING THE MOLECULAR MECHANISMS OF CHEMOSENSATION IN A CROP PEST MOTH, THE NOCTUID *SPODOPTERA LITTORALIS*

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Nocturnal insects such as moths are good models to decipher the molecular mechanisms of olfaction since these organisms mainly use their sense of smell to understand their environment and to communicate with conspecifics. We have developed the cotton leafworm, *Spodoptera littoralis*, as a model to decipher the underlying molecular mechanisms. We first developed a transcriptomic approach on both adult and larvae antennae. Sanger and next generation sequencing strategies allowed annotating of more than 77000 expressed contigs. Among them, we described a 57 odorant-binding and chemosensory proteins, 17 ionotropic receptors and 47 candidate olfactory receptors (ORs). We also highlighted the presence in antennae of genes potentially involved in non-olfactory functions, such as genes encoding defense-related elements involved in xenobiotic and pathogen protection, hormone/biogenic amine receptors and circadian clock components. Comparison between adults and larvae revealed different but somewhat overlapping expression of the chemosensory genes in the different developmental stages. We are currently deorphanizing the SlitORs via expression in the *Drosophila* empty neuron system coupled with single neuron electrophysiological recordings, revealing the occurrence of both narrowly tuned and broadly tuned receptors. These results represent an important step in understanding the molecular determinants underlying the mechanisms of olfactory mediated behaviour in *S. littoralis* and pave the way for adressing their plasticity, since the chemosensory transcriptome we established can be used as a reference for digital gene-expression profiling.
Poivet et al. 2013. *PLoS ONE*. 8: e60263. Montagné et al. 2012. *Eur J Neurosci*. 36: 2588-96.

CHEMOSENSORY GENES FROM COTTON BOLLWORM *HELICOVERPA ARMIGERA*

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The chemosensory system is critical in guiding insect behaviours. *Helicoverpa armigera* (Hübner) is one of the most polyphagous pest species in the world, so studies on its chemosensory system may give insights into the molecular mechanisms that underlie its ability to exploit a wide variety of host crops and help develop new approaches to control it. We sequenced and assembled transcriptomes from larvae antennae, larvae mouthparts, adult heads, adult tarsi and female adult abdomen. We identified fifty-eight odorant receptors (ORs), nine gustatory receptors (GRs), thirty-eight odorant binding proteins (OBPs) and nineteen chemosensory proteins (CSPs). The expression pattern of these genes were also analysed among different tissues, ages and genders. Seven candidate pheromone receptor (PR) genes were analysed by calcium imaging analysis and HarmOR13 showed significant responses to the major sex pheromone component, (z)-11-hexadecenal. This study will improve our understanding of the insect chemosensory system and assist in the development of more environmentally friendly, pheromone based control strategies.

MOLECULAR CHARACTERISATION, EXPRESSION PATTERN, AND LIGAND-BINDING PROPERTIES OF THREE ODORANT BINDING PROTEIN GENES FROM *DENDROLIMUS TABULAEFORMIS*

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We identified and characterized three new OBPs, one pheromone binding protein (PBP1) and two general odor binding proteins (GOBPs), from the antennal cDNA of an important conifer pest, *Dendrolimus tabulaeformis* by reverse transcriptase-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends-PCR (RACE-PCR). The deduced amino acid sequences of DtabPBP1, DtabGOBP1, and DtabGOBP2 revealed mature proteins of 140, 147, and 140 amino acids, respectively. Each had six cysteine residues in conserved positions relative to other known OBPs. Amino-acid alignments indicated that the two GOBPs were more evolutionarily conserved than PBPs. Real-time PCR indicated that *DtabPBP1* was mainly expressed in the antennae of males; female antennae had only 1.09% of the expression in male antennae. Both *DtabGOBP1* and *DtabGOBP2* were much more strongly expressed in antennae, but were expressed at lower levels in other tissues. *DtabGOBP1* was most abundant in male antennae and *DtabGOBP2* in female antennae. All three OBPs exhibited high binding affinities with one of the pheromone component (5*Z*,7*E*)-5,7-dodecadien-1-yl propionate (*Z*5,*E*7-12:OPr), in spite of that they showed various binding infinities with other volatiles or pheromones.

IDENTIFICATION AND CHARACTERISATION OF PHEROMONE RECEPTORS AND BINDING PROTEINS IN THE DIAMONDBACK MOTH, *PLUTELLA XYLLOSTELLA*

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Moths depend on olfactory cues such as sex pheromones to find and recognize mating partners. Pheromone receptors (PRs) and Pheromone binding proteins (PBPs) are thought to be associated with olfactory signal transduction of pheromone compounds in peripheral olfactory reception. We identified and cloned three PBPs and six PRs from the antennae of *Plutella xyllotella*. The PxylPBP genes are not only expressed in chemosensory tissues, but all of the six candidate PR genes display male-biased expression, which is a typical characteristic of pheromone receptors. To understand the functions of PxylPBPs and PxylPRs, three PxylPBPs were expressed in *Escherichia coli* and the ligand-binding specificities of purified recombinant PBPs were investigated. Fluorescence binding assays indicate that three PxylPBPs not only robustly bound sex pheromone components but also significantly bound pheromone analogs, while weakly bound tested plant volatiles. Although pheromone analogs bound PBPs, they could not elicit moths' electrophysiological response. We further used *Xenopus* oocytes system to explore PxylPRs functions. PxylPR4 is defined as another pheromone receptor in addition to the previously characterized PxylPR1. In the study of interaction between PRs and PBPs, PxylPBPs could increase the sensitivity of the complex expressing oocyte cells to the ligand pheromone component while decreasing the sensitivity to pheromone analogs. We deduce that activating pheromone receptors in olfactory receptor neurons requires proper pheromone/PBP complex. If the chemical signal is not the pheromone component, but instead, a pheromone analog with a similar structure, the complex would have a decreased ability to activate downstream PRs.

LIGAND-BINDING OF THREE PHEROMONE-BINDING PROTEINS IN THE BEET ARMYWORM *SPODOPTERA EXIGUA*

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Moth pheromone-binding proteins (PBPs) play crucial roles in pheromone reception. In *Spodoptera exigua*, three SexigPBPs (PBP1-3) were cloned from the antenna, and are classified into three distinct sub-groups. Considering the sex pheromones of *S. exigua* have been identified as a four-component blend, it is hypothesized that each PBP may be tuned to a specific component (s) of the blend. To test this hypothesis, we carried out ligand-binding assays of three SexigPBPs to sex pheromones, pheromone analogs and plant volatiles. Our results show all three SexigPBPs can bind all tested sex pheromones, suggesting these SexigPBPs have no obvious discrimination among different pheromone components. However, SexigPBP1 exhibited much stronger binding affinities to sex pheromones and their analogs than the other two SexigPBPs (PBP1 >> PBP2 > PBP3), especially those ligands with a double bond in the 9th position. Plant volatiles proved to be poor ligands for all three SexigPBPs. In addition, binding of SexigPBP1 and SexigPBP2 to the main sex pheromone *Z*9,*E*12-14:Ac were strongly affected by pH, in contrast binding affinity of SexigPBP3 appeared to be only slightly affected. Similar results were also observed in its sibling species *S. litura*. Our results suggest that SexigPBP1 might play major roles in the process of female sex pheromone reception.

INVESTIGATING THE STRUCTURAL BASIS FOR ODORANT RECOGNITION IN AN ODORANT RECEPTOR FROM THE MALARIAL VECTOR, *ANOPHELES GAMBIAE*

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Insects detect semiochemicals and other chemicals using several families of chemosensory receptors, including the OR family of olfactory receptors. Insect ORs are heteromeric, ligand-gated ion channels composed of a subunit that binds odorants to confer specificity and a common subunit (Orco). The subunit stoichiometry is unknown. To identify structural features of the odorant-binding site, we examined two highly homologous *Anopheles gambiae* odorant binding subunits. Agam\Or13 and Agam\Or15, when expressed in *Xenopus* oocytes (each in combination with Agam\Orco) and assayed by two-electrode voltage clamp electrophysiology, displayed distinct odorant specificities. Each residue in the predicted transmembrane and extracellular domains of Agam\Or15 that differed from Agam\Or13 was mutated to the residue present in Agam\Or13 and the resulting panel of 32 mutated ORs was screened for relative responsiveness to acetophenone and 4-methylphenol. Mutation of alanine 195 to isoleucine was found to partially shift the odorant specificity of Agam\Or15 toward that of Agam\Or13. Twelve additional mutations were made at this position and concentration-response analyses were conducted for acetophenone, 4-methylphenol and six related chemical structures. Varying the residue at this position had a profound effect on the potency of some odorant ligands, but had little effect on the potency of other odorant structures. This result implicates residue 195, located near the interface of the predicted second extracellular loop and the fourth transmembrane domain, as playing a critical role in odorant specificity. We are currently examining the surrounding area to gain a better understanding of the role of this region in odorant recognition.

A CONSERVED ASPARTIC ACID REGULATES ACTIVATION OF THE INSECT ODORANT CO-RECEPTOR (ORCO)

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Insect odorant receptors function as non-selective cation channels and are composed of a tuning receptor and a conserved co-receptor (Orco). There is scarce information regarding the structure of odorant receptors and the mechanism facilitating channel opening. Conserved acidic amino acids are known to have a role in ion permeation and gating of cation channels. An Orco agonist, VUAA1 capable of activating both heteromeric and homomeric Orco channels was used to investigate the importance of conserved Asp residues at positions 357 and 466, in transmembrane domains 5 and 7, respectively, of Orco from *Drosophila melanogaster*. Wild-type and Orco substitution mutants were expressed in HEK cells. Channel activity was determined by Ca^{2+} influx and whole-cell patch clamp electrophysiology. Substitution of D466 with amino acids other than glutamic acid resulted in a substantial reduction of channel activity. The D466E Orco variant showed higher activity and ~2-fold increased sensitivity to VUAA1. Cation permeability of the D466E Orco mutant was unchanged relative to wild-type Orco. When D466E Orco was co-expressed with a tuning odorant receptor, the heteromeric complex showed increased sensitivity to odorant. Thus, the effect from D466E is not restricted to VUAA1 agonism or dependent on homomeric Orco assembly. We suggest the D466E mutant may have a conformational state common to the activation mechanism of both Orco and tuning receptor agonists. In summary, this work identified an amino acid position that is important for activation of insect odorant receptor channels.

INSECT ODORANT RECEPTOR STRUCTURE AND FUNCTION WITH REGARD TO THEIR APPLICATION IN OLFACTORY BIOSENSORS

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The fundamental unit of odour sensing in insects comprises a conserved co-receptor (Orco) and a tuning receptor that confers odour-based sensitivity and selectivity on the ion channel. Still, little is known concerning the structure and function of this novel receptor complex, especially the stoichiometry of the complex and the mechanism whereby odours influence channel opening. To facilitate such studies and eventually utilise these receptors in olfactory biosensors, recombinant production systems for the receptor complex in surrogate expression systems were explored. Through transient expression in insect cells we have demonstrated homo- and heteromeric interactions between *Drosophila melanogaster* Orco and OR22a using Fluorescence/Förster Resonance Energy Transfer (FRET). An interaction between a third integral membrane protein involved in olfaction, SNMP1, and a tuning receptor, but not Orco, was also observed. Recombinant expression of insect odorant receptors in Sf9 cells using baculovirus and in eukaryotic cell-free systems are also capable of producing receptor subunits, with the baculovirus system expressing large amounts of protein that can be detergent-solubilised and purified for subsequent structural studies. To date no functional receptor complexes or subunit interactions have been demonstrated using material from either of these systems. Human Embryonic Kidney (HEK293) cells, however, can be used to produce functional receptor complexes using an inducible expression system for both *Drosophila* and lepidopteran odorant receptors, as demonstrated through calcium flux assays. Subsequently, crude proteoliposomes from recombinant HEK293 membranes have been successfully fused to a supported lipid bilayer system and channel activity observed in response to a ligand.

SYMPOSIUM TOPIC 13: WHAT'S YOUR BIOASSAY? – NEW DIRECTIONS IN CHEMICAL ECOLOGY

Topic Coordinators:

Markus Knaden (Institute for Chemical Ecology, Germany) and **Ryohei Kanzaki** (University of Tokyo, Japan)

OLFACTORY DRIVEN FORAGING STRATEGIES OF THE DESERT ANT *CATAGLYPHIS FORTIS*

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The desert ant *Cataglyphis fortis* is equipped with sophisticated navigational skills for returning to its nest after foraging. The ant's primary means for long-distance navigation is path integration, which provides a continuous readout of the ant's approximate distance and direction from the nest. The nest finally is pinpointed by visual and olfactory landmarks. While homing in desert ants is well investigated, the ants' strategies to localize suitable food is not. Despite the few foraging ants in the salt pan, individual food items are picked up within a short time. We found that *Cataglyphis* pinpoints dead insects by following food-derived odour plumes and we identified a highly attractive key compound released in these plumes to which the ants are extremely sensitive. Ants easily detected tiny food items when passing them 4 m downwind. We furthermore show that, instead of searching for food randomly, the ants perform extensive crosswind walks to screen the habitat for food plumes. Taking into account the ants' walking speed of about 50 cm per second, and the functional reach of the food plume of about 4 m, a single forager screens 120 m^2 per minute for food. This screening efficiency might account for the fact that despite the small number of foraging ants, any available food item is localized and removed within a short time.

DEVELOPMENT OF A NOVEL CELL-BASED ODORANT SENSOR BASED ON INSECT ODORANT RECEPTORS

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Insects are equipped with sophisticated olfactory mechanisms. Recent chemical ecological researches have shown that insects use various odorant receptors (ORs), which are expressed in olfactory sensory cells, to sensitively detect environmental odorants. So far, more than 100 ORs have been functionally characterized from several insect species, and it has become clear that each of ORs can selectively detect various types of odorants. In contrast, existing odorant sensors, using metal-oxide semiconductors or quartz crystal microbalances, have deficits in sensitivity and selectivity, and their performances are much inferior to those of insects. To overcome these problems, ORs are focused for development of an innovative odorant sensing system. Here, we report a novel odorant sensor using living cells expressing insect ORs. We introduced insect ORs and the olfactory receptor co-receptor as well as a calcium indicator protein, GCaMP3, into *Spodoptera frugiperda* Sf21 cells to construct stable cell lines. When these cell lines were stimulated with a set of odorants in solution, intracellular calcium as monitored by fluorescence imaging showed sensitive responses in accordance to the ligand specificity of the expressed ORs. Even after culturing for 2 months, the cell lines responded to odorants at the same fluorescent intensity as they did before. By combining these cells with a microfluidic channel technology, we constructed a compact odorant sensor chip, and found that the chip can be used to detect odorants. These results represent a first step towards practical cell-based odorant sensor chips that detect various kinds of odorants with high sensitivity and selectivity.

NEW STRATEGIES FOR PHYTOPLASMA VECTOR CONTROL BY SEMIOCHEMICALS

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Phytoplasmas are worldwide responsible for more than 700 different plant diseases and have an important economic impact. Phytoplasma species belonging to the apple proliferation group are the economically most important fruit tree phytoplasmas and are widespread in the temperate regions of the world. Phloem feeding insects (Hemiptera: Psyllidae) were identified as vectors, mainly one species transmitting a specific phytoplasma. We studied the role of plant semiochemicals on vector behaviour and the influence of phytoplasma infections on volatile production of host plants. Volatile compounds from host plants under field and laboratory conditions were collected and analysed by gas chromatography/mass spectrometry. The olfactory preferences of psyllids for certain plants and volatile chemical compounds were investigated in Y-shaped olfactometer bioassays. Several compounds were tested in field traps in apples, stone fruit and pears in various locations. Host plants were selected based on their attractiveness to psyllids under field conditions. We found that all investigated psyllid species use chemical cues for the identification of their host plants during migration between different host plants. The production of plant volatiles is influenced by phytoplasma infections which indirectly influenced the behaviour of vector insects. For some species we have identified species-specific attractive compounds and also potential repellent chemicals. Attractive compounds could be used in traps as lures for monitoring and mass trapping purposes and combined with repellent compounds these chemicals could be used in push-and-pull strategies. We already started constructing traps and tested them in field experiments. These results are also presented and discussed.

SHEDDING LIGHT ON HOW PLANT CHEMICAL HETEROGENEITY AFFECTS INSECT MOVEMENT

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Insects interact with plants at a scale relative to their body size. Constructing bioassays to test chemical ecology at this fine scale presents a number of challenges. Our group is interested in the role that the plant environment plays in insect movement, particularly how neonates respond to the chemical changes induced in plants following herbivore feeding. We have designed bioassays to investigate the pattern of induced plant responses within single plants using transgenic plant constructs expressing firefly luciferase as a reporter of defensive gene expression. This technique allows real-time visualization of plant chemical responses through production of visible light in plants and we have used these bioassays to map chemical changes both across time in the same plant and at a fine scale within plants. These maps combined with insect behavioural bioassays in our insect-plant model, *Helicoverpa armigera* and *Arabidopsis thaliana,* demonstrated that heterogeneity of plant chemical responses plays a role in insect movement choices within plants. In this presentation we discuss various bioassays used to interrogate the role of variability in insect movement responses and how data from these bioassays have been incorporated into a comprehensive model of herbivore movement on plants.
Perkins et al. 2013. *Proc. R. Soc. B* 280, 20122646.

NEW TOOLS FOR AN OLD PUZZLE

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Fatal injuries or death of a companion is a sign of predator presence for many organisms. Early detection of cues that can indicate such injuries is advantageous for the recipients to avoid predation. Karl von Frisch reported such responses in freshwater fishes in the 1930s. He discovered that members of many Teleost fishes adopt species-typical, anti-predatorial defensive behaviors after smelling substances (Schreckstoff) released from an injured shoal member. We recently identified one component of Schreckstoff or the alarm substance in zebrafish – a chondroitin like molecule, by designing assays to quantify behavior, image calcium activity in neurons, and combine them with biochemical fractionation. Now, to identify the olfactory neurons and receptors responsive to this semiochemical, we have micro-fabricated a PDMS-based device using soft lithography techniques that allows interrogation of activity of individual olfactory neurons upon exposure to a stimulus. The device also allows sorting and retrieval of neurons of interest for further analyses such as transcriptional profiling. We expect that implementing these new tools and techniques will yield mechanistic insights into properties of the neurons and the circuitry that mediates a behaviour of innate fear in a vertebrate.

von Frisch. 1938. *Naturwissenschaften* 26, 601–606.
Mathuru *et al.* 2012*. Curr Biol* 22, 538–544.

A PLANT FACTORY FOR MOTH PHEROMONE PRODUCTION - PROOF OF PRINCIPLE

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We produced moth pheromone components in *Nicotiana benthamiana* by transient expression of genes coding for three to four consecutive biosynthetic steps. By optimization of the system, we then specifically produced multi-component sex pheromones for moths. Fatty alcohol fractions from the genetically modified plants were purified, acetylated with conventional methods, and mixed to mimic the respective sex pheromones of the two small ermine moths (*Yponomeuta evonymellus and Y. padellus*). In spite of the composition of the plant-derived mixtures loaded on rubber septa being far from optimized, these mixtures were as efficient and specific for trapping of male moths as conventionally produced synthetic pheromones. Our long-term vision is to design tailor-made production of any moth pheromone component in genetically modified plants. Such semi-synthetic preparation of sex pheromones will be a novel and cost-effective way of producing moderate to large quantities of pheromones with high purity and a minimum of nonhazardous waste.

TRITROPHIC INTERACTIONS IN *ANTHERAEA ASSAMENSIS- MACHILUS BOMBYCINA / LITSEA MONOPETALA AND EXORISTA SORBILLANS* COMPLEX

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The uzi fly (*Exorista sorbillans* Weidmann) is an endoparasite of silkworm larvae causing 10- 30% loss to the silkworm industry. We examined electroantennogram responses (EAG) of the Uzi fly to host plant and host related chemicals in order to identify compounds that could be used as behavior modifying chemicals. Over 50 chemicals were evaluated from which a general pattern based on the functional groups was observed with respect to the EAG responses of *E.sorbillans* - a) In general, compounds with odd number of carbon atoms elicited higher responses; b) the response profile of alcohols and acetates were similar and usually higher than corresponding aldehydes. Sensitivity to a wide range of test compounds in *E.sorbillans* as reflected in their EAG responses is probably due to their wide host range including several lepidopteran species feeding on different crops/trees suggesting that other crop plants (and consequently, plant volatiles) are important to females of *E.sorbillans*. GC-MS analysis of undamaged and damaged host plants reflect qualitative differences in the daytime and night time volatile profiles. Several low molecular weight compounds are released in plants damaged by larvae compared to undamaged plants which were characteristic in releasing green leaf volatiles primarily composed of C_6 alcohols, aldehydes and their corresponding acetates. In addition, larval regurgitant when applied to undamaged plants release the same volatile blend as that released from a damaged plant. HPLC analysis of the regurgitant reveals the presence of fatty acids - linolenic and linoleloyl acids linked to amides (FAA's) and were identified as - N-linolenoyl-L glutamine; N-linolenoyl-L-glutamic acid; N-linoleoyl glutamine. Further, treating the regurgitants with acetic anhydride for GC-MS analysis confirmed the linolenic acid linked fatty acid amides. Subsequently, GC-EAD studies were conducted to identify the specific volatiles in the HIPV blend which attract the uzi fly. The specific components which stimulate the antennal sensilla of Uzi fly have been identified and efforts are currently underway to conduct behavioral studies with the identified compounds.

"BEE-NOSE" FOR THE DETECTION OF DISEASES IN HUMANS AND PESTS IN AGRICULTURE

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Animal or plant pathogens or parasites can be revealed by eavesdropping, and the same applies for human diseases (e.g. tuberculosis). Chemical ecology tools have revealed changes in the pattern of volatile organic compounds released by individual plants, which could be used as biomarkers to assess plant "health". We tested whether classical conditioning of restrained or free-flying honey bees (*Apis mellifera*) enables their discrimination of healthy from non-healthy plants. Specific volatiles released by apple seedlings infested by the pest light-brown apple moth (*Epiphyas postvittana*) were used to train bees in the laboratory and open field. The ability of bees to detect infection of solaneceous species by a newly identified bacterium, *Candidatus* Liberibacter solanacearum, vectored by the potato/tomato psyllid (*Bactericera cockerelli*), was also explored. These examples provide proof-of-concept for further development of insects as ultra-sensitive live biodetectors. Bees are low cost and widely available in agriculture globally and deserve further attention as sensors because of their excellent receiver-operator curve.
Suckling, Sagar. 2011. *Tuberculosis* 91, 327-328.

WOLBACHIA ALTER INDIVIDUAL PERCEPTION AND SEXUAL SELECTION IN TERRESTRIAL ISOPODS

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From prokaryotes to vertebrates, the use of chemical signals is widespread. Gregariousness and mate recognition have been found in some terrestrial isopods (Crustaceans). However, the underlying mechanisms that have led to these behaviors are poorly understood. *Armadillidium vulgare*, was used for this study and the presence of *Wolbachia*, an intracellular bacteria symbiont, results in feminization of genetic males into physiological and functional females. Previous results revealed that males interact more with uninfected females than feminized males. We focused on the ability of individuals to perceive other conspecifics at short-distance. To investigate biological parameters involved in individual attractions we tested individuals according to gender, moulting stage and *Wolbachia* infection status. Tested individuals were placed in a choice chamber separated by a mesh covered by a perforated opaque paper preventing visual and physical interactions. Males and females spent significantly more time close to a conspecific of the opposite gender than with those of the same gender. Moreover, males were significantly more attracted by asymbiotic females compared to symbiotic females. Other behavioral tests revealed that asymbiotic females were significantly more attracted by male compared to female but symbiotic females weren't more attracted by male. Tested individuals perceived and used chemical cues for distance mate-finding as well as to discriminate infected female status. Our results provide clear evidence for chemical sex-recognition and preference to detect a female's reproductive status. The chemical signal of target individuals inform on the individual chemical pattern and led us to specific compounds involved in *A. vulgare* recognition cues.

FIRST IMPRESSIONS MATTER: EARLY QUALITY ASSESSMENT LESSENS PHEROMONE SPECIFICITY IN A MOTH

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Pheromone orientation in moths is an exemplar of olfactory acuity. To avoid heterospecific mating, males respond to female-produced blends with high specificity and temporal resolution. A finely tuned sensory to projection neuron network secures specificity, and this network is thought to assess pheromone quality continually during orientation. We tested whether male moths evaluate each pheromone encounter and surprisingly found that male European corn borer moths, *Ostrinia nubilalis*, instead generalize across successive encounters. Although initially highly ratio specific, once "locked on" to the pheromone plume the acceptable ratio can vary widely, and even unattractive blends can become attractive. The relaxation of specificity observed here may also help explain the substantial and unexpected rates of field hybridization between the Z-pheromone strain of *O. nubilalis* studied here, and the E-strain, whose ratio of Z11 and E11 is opposite. At high population densities, males that have first encountered filaments of pheromone from their own strain may stray into a plume from the other strain and hybridize.
Kárpáti et al. 2013. *Proc. Nat. Acad Sci. USA* 110:7377-7382.
www.pnas.org/cgi/doi/10.1073/pnas.1216145110

BLEND BLINDNESS DURING PHEROMONE ORIENTATION CAUSED BY DIFFERENTIAL SENSORY ADAPTATION IN THE EUROPEAN CORN BORER

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European corn borer males generalize pheromone quality after initial contact with a plume. The broadening of behavioral acceptance of pheromone blends in the European corn borer in wind-tunnel trials is correlated strongly with sensory response dynamics. Antennal neurons tuned to either (*Z*)-11-tetradecenyl acetate or (*E*)-11- tetradecenyl acetate adapt differentially when reiteratively stimulated with the natural blend (97:3) ratio, thereby changing the relative firing ratio of the two neurons. Rapid interception of pheromone filament may cause olfactory “Gestalts” to develop within seconds, and induce blend generalization. Generalization may also be important in responses to general odorants, as circuits underlying these display vast sensitivity differences, complex interactions, and temporal intricacies.

CHANGE IN A SELF-MAINTENANCE BEHAVIOR OF A SLUG SPECIES (*DEROCERAS RETICULATUM*) AS A RESPONSE TO PREDATION-THREAT CHEMICAL CUES FROM GROUND BEETLES IS LINKED WITH THEIR ECOLOGICAL RELEVANCE

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Evidences that terrestrial gastropods are able to detect chemical cues from their predators are both obvious and scarce, despite the relevance to enhance our knowledge in this area. Here, we studied the influence of cuticular extracts from three different predacious ground beetle species (*Carabus auratus*, *Carabus hispanus* and *Carabus coriaceus*) that experience different ecological conditions, and a neutral insect species (*Musca domestica*) on the shelter-seeking behavior of naive slugs (*Deroceras reticulatum*). Slugs, known to have a negative phototactic response, were spurred by light and had to make a choice between either a shelter treated with a cuticular extract or a control shelter treated with pure ethyl alcohol. Their behavioral responses were recorded for one hour allowing determination of their first shelter choice, their final position and to compare the percentage of time spent in the control shelters with the time spent in the treated shelters. The test proved to be very effective as slugs spent most of the time of the experiment into a shelter. The slugs spent significantly more time in the control shelter than in the shelter treated with *C. coriaceus* cuticular, whereas the other cuticular extracts had no significant effect on any of the behavioral measured items. Results suggest that slugs are innately able to discriminate different potential predators and adjust their behavioral response according to the relevance of the threat conveyed by their chemical cues.

SYMPOSIUM TOPIC 14: CHEMICAL ECOLOGY OF INVADING SPECIES

Topic Coordinators:
Zhang Zhen (Chinese Academy of Forestry, China) and **Eric Jang** (USDA, USA)

TRAPPING LITTLE FIRE ANT, *WASMANNIA AUROPUNCTATA* (ROGER): LONGEVITY AND TRAP MECHANISM

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Considered one of the worst invasive pest ants, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) has negatively impacted both biodiversity and agriculture. Its distribution is nearly pantropical, while greenhouse infestations have been reported as far north as Canada and the United Kingdom. Pacific islands have been particularly affected by the ant, with larger ecological and economic impacts than in other locations. Improved quarantine and prompt eradication of invasive populations are key to controlling the spread of *W. auropunctata*. A one-way trap for *W. auropunctata*, containing the alarm pheromone 2,5-dimethyl-3-(2-methylbutyl)pyrazine, has been successfully used to monitor little fire ant populations. These traps use perforated weedmat that ants can squeeze through in only one direction. Longevity trials of one-way pheromone traps gave unexpected results showing longer than expected attraction and capture of *W. auropunctata* (42 days with a 1 mg lure), and trap catches that appear to increase over the first ten days of trapping. These results prompted a field experiment addressing the mechanism of ant attraction and capture. Three hypotheses related to trap capture were tested: 1) ants establish a trail or other markers to the trap, 2) captured *W. auropunctata* attract other ants to the trap, 3) ants are slow to find traps (discovery lag). Results suggest that captured ants contribute to trap captures while a small trail effect may also influence catches. It is hoped that understanding the mechanisms by which ants are attracted and enter traps will lead to the design of better detection and control methods for invasive ants.

TRAIL PHEROMONE DISRUPTION IN ANTS: COMMUNICATION DISRUPTION CAN REDUCE FORAGING

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The exploitation of trail pheromones for invasive ants such as Argentine ants *Linepithema humule* or red imported fire ant *Solenopsis invicta* presents a set of challenges that are not yet fully understood. Overflooding with trail pheromone caused a shift in the track angles of trail-following ants in both species. However, redundancy in the use of cues, from chemical (chemotaxis) or physical (thigmotaxis) sources, means that the ants in pheromone-treated areas may forage less successfully, but still show some foraging success despite the treatment. We have demonstrated disruption of worker trail orientation in these species in the laboratory and of *Linepithema humule* in the field. The feasibility of using trail pheromones for disruption of ants has been demonstrated using micro-encapsulated sprays, ant sand, aerosols, and passive release devices, but the level of disruption required for control, and nest level-responses is not yet understood. The lack of commercial supply of high purity trail pheromone makes this technique impractical against *S. invicta* but prospects appear better against *L. humule.*

Suckling et al. 2008. *J. Chem. Ecol.* 34, 1602-1609.
Suckling et al. 2010. *J. Chem. Ecol.* 36, 122-128.
Suckling et al. 2011. *J. Chem. Ecol.* 37, 1143-1149.

ATTRACTIVENESS OF VOLATILES FROM NATURAL HONEYBEE HIVE PRODUCTS TO THE SMALL HIVE BEETLE *AETHINA TUMIDA* (COLEOPTERA: NITIDULIDAE)

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Small hive beetle *Aethina tumida* is a scavenger of European honeybees*, Apis mellifera*. In its native sub-Saharan Africa it is a minor pest infesting weak or stressed colonies. Since 1996 the beetle has become established and a major apiary pest in the USA and then Australia. The beetles feed on unprotected bee brood, eggs, honey and pollen and contaminate hive products with their waste. The beetles are associated with a yeast, *Kodamaea ohmeri*, that is believed to be primarily responsible for fermentation of hive products. The resulting fermented honey (or "slime") is rejected by honeybees and cannot be marketed by the beekeeper. Heavy infestations result in hive death, queens ceasing to lay eggs or bees absconding from hives. Between 2009 and 2011 recorded losses in Queensland alone were in excess of $8 million. The beetles are attracted to a range of hive odours, particularly that of adult worker bees and the odours of fermenting hive products. We used gas chromatography-mass spectrometry and choice-test behavioural assays to investigate changes in volatile profiles through time of hive products altered by the action of beetle larvae and *K. ohmeri,* and differences in attractiveness of these hive products to the beetle. Attractiveness of the slime increased as fermentation progressed, and volatile profiles became more complex. Slime remained extremely attractive for more than 30 days. These results have strong implications for the development of an out-of-hive attractant trap to assist in the management of this invasive pest.

HOW DOES THE BARK BEETLE, *DENDROCTONUS VALENS*, INITIATE AND CEASE THE AGGREGATION ATTACK?

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The red turpentine beetle (RTB) *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae), is an invasive tree-killing species native to North America. Among *D. valens* attacking phases, mass attack and switching are critical; this is when individuals are recruited in order to overcome host resistance and avoid over-competition. Little is known about *D. valens* pheromone biology and no aggregation and anti-aggregation pheromones have yet been identified. RTB attacking behavior and its chemical communication were studied in both the field and the laboratory. Analysis by GC-MS of volatiles collected from live beetles showed that female beetles produce frontalin and males do not. Olfactory assays in the laboratory showed that males were attracted to frontalin at a wide range of concentrations, whereas females were attracted to it at a narrow range of concentrations. Field trapping showed that frontalin added to 3-carene significantly attracted more beetles than 3-carene alone, showing the function of aggregation pheromone. However, increasing concentrations of frontalin significantly decreased the percentage of female beetles trapped, showing the function of sex pheromone. Moreover, after the females were joined by males, males were able to release *exo*-brevicomin. It was only produced by males in *D. valens* and bioassay showed that it was a highly efficient anti-aggregation pheromone. Further analysis showed that the drumming sound, released by both female and male bark beetles of *D. valens* when encountering each other in the gallery, triggered males to produce the anti-aggregation pheromone *exo*-brevicomin, which prevents from aggregating and so helps the species avoid attack, overcrowding, and maintain monogamy.

THE VARIANCE IN RELATIVE CONTENTS OF INDUCED VOLATILE MONOTERPENES OF *PINUS TABULAEFORMIS* BY AN INVASIVE BARK BEETLE, *DENDROCTONUS VALENS*

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The resistance of Chinese pine, *Pinus tabulaeformis* to an invasive bark beetle, *Dendroctonus valens* was investigated by analyzing beetle induced monoterpenes. The volatile profiles of control, mechanical damaged and beetle damaged Chinese pines were extracted with Porapak Q headspace absorption method for eleven consecutive phases. The chemical constituents of the volatile were analyzed by GC and GC-MS and their relative content of monoterpenes were determined by area normalization. Beetle damaged pines contained significantly higher percentages of (+)-α-pinene (more than 90%) and myrcene, but lower levels of (+)-3-carene, (-)-β-pinene, camphene and limonene, when compared to control and mechanical damaged trees. The abundance of (+)-3-carene (less than 4%) in beetle damaged pines was lower than that in control and mechanical damaged pines. However, the abundance of (+)-3-carene in mechanically damaged pines reached to the level of control pines gradually from the lower level. The abundance of (-)-β-pinene and limonene were similar in different pines, but there are significant differences among them after infested by different sexual beetles. Our results suggest that *D. valens* may use specific variations of composition of the monoterpenes as chemical cues for host tree selection and population aggregation.

EVIDENCE FOR MALE-PRODUCED ATTRACTANTS OF TEN SPECIES IN THE CERAMBYCID BEETLE GENUS *NEOCLYTUS*

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The cerambycid beetle genus *Neoclytus* is native to the Western hemisphere, where the larvae of most species feed in the heartwood of hardwood trees. Several *Neoclytus* species are pests in managed and natural forests, and one species, *Neoclytus a. acuminatus*, has invaded Europe and has been intercepted in quarantine facilities throughout the world. We report results of a multi-year field and laboratory study assessing variability in composition of male-produced volatile pheromones within the cerambycid genus *Neoclytus*. Analysis of headspace volatiles, GC-EAD, field bioassays and surveys suggest that twelve *Neoclytus* species produce and/or respond to compounds of the 2,3-hydroxy alkanone or 2,3-alkanediol structural motifs, consistent with other cerambycid species of the subfamily Cerambycinae and tribe Clytini. However, the composition of the attractant blend and the chain length of the major component varies, even among closely related species. Our research suggests that minor components of blends and stereochemistry of components affect the response of sympatric and synchronic congeners, further suggesting that the composition of these male-produced pheromone blends may act as species isolating mechanisms.

EXPLOITING THE SCENT OF FRUITS TO PROTECT CROPS AGAINST *DROSOPHILA SUZUKII*

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Drosophila suzukii is a new invasive pest insect in Europe and North America causing an increasing damage to soft-skinned fruit farms. Blueberries, cherries, raspberries and strawberries are among the major fruits affected by this pest. Unlike most other drosophila flies, *D. suzukii* attacks ripening fruits making it difficult to protect fruit crops with synthetic pesticides due to the closeness to harvesting time. Therefore, alternative behaviourally-based pest control strategies are critical. In order to develop attractive host-attractants for *D. suzukii*, we extracted juices from blueberry, cherry, raspberry and strawberry and tested their attractiveness to *D. suzukii* in laboratory olfactometer studies. The results showed that all of the fruit juices are attractive to *D. suzukii*; however, those from raspberry and strawberry were most attractive. The headspace volatiles from these juices were also tested in GC-EAD experiments using both male and female *D. suzukii* antennae and strong electrophysiological activities were observed. Once these EAG active volatile compounds from raspberry and strawberry are identified and their activity confirmed under field conditions, they could be used as baits for mass trapping or attract-and-kill strategies to control *D. suzukii* infestation in soft-skinned fruit farms.

FORMULATING VOLATILES FROM WINE AND VINEGAR TO ATTRACT THE INVASIVE PEST SPOTTED WING DROSOPHILA, *DROSOPHILA SUZUKII*

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Scentry Biologicals, Inc. has been researching and developing devices for insect monitoring and control for over 30 years. Invasive pest detection, delimitation, and control using semiochemical-based attractants form the foundation of pest management programs. We report a summary and an update of recent research conducted to monitor and control Spotted Wing Drosophila (*Drosophila suzukii*). The Spotted Wing Drosophila (SWD) is a vinegar fly of East Asian origin that can cause damage to many fruit crops of economic importance. SWD has been in the United States in Hawaii since the 1980s, and was detected in California in 2008; it subsequently spread through the West Coast and was detected in Florida, Utah, the Carolinas, Wisconsin and Michigan. Currently, detection of SWD requires the use of wet-traps baited with apple cider vinegar. The use of the liquid bait by field users is inconvenient and laborious, and a trapping system that utilizes an extended life lure in a dry trap is preferred. Recently, volatiles have been identified from apple cider vinegar and red wine, and combining the identified chemistries with acetic acid and ethanol provide favorable field results compared to wet traps baited with vinegar. These compounds of varying solubility were formulated into a polymeric all-in-one dispenser, and field tested to optimize attraction and field life. Trap design is also critical in the detection of SWD, efforts to develop and commercialize a complimentary dry-trap are also presented.

HONEYDEW-BASED SUPERLURE FOR THE INVASIVE SOCIAL WASP, *VESPULA VULGARIS,* IN NEW ZEALAND

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Vespula wasps have become extremely successful invaders of the beech forests of New Zealand. In the summer, in the honeydew laden beech forests of New Zealand, the biomass of *V. vulgaris* is greater than the combined biomass of rodents, birds, and stoats. Carbohydrate consumption by these wasps from the secretions of the beech scale insects *Ultracoelostoma spp.* greatly reduces the abundance of honeydew available for native wildlife during the summer months, impacting significantly on forest ecology and native biodiversity. The production of honeydew in the southern beech forests is around 4 tons dry weight/ha per year and wasps harvest 90% of this honeydew during the summer months. There is clearly a need for controlling invasive *Vespula vulgaris* and we have already reported two sets of good attractants from green-lipped mussel and fermented brown sugar, respectively, that were discovered in a research program aiming at the development of new tools for control of social wasps in threatened ecosystems. In this talk we present attractants isolated from the major carbohydrate source in the New Zealand southern beech forests; the honeydew, and the combination of all the recently discovered attractants into a superlure. Trap catches was compared with the best lures found in literature and we found that the superlure was approximately 10 times more effective as any of the commercial lures.

PHEROMONE MEDIATED MATING IN THE WESTERN BEAN CUTWORM, *LOXAGROTIS ALBICOSTA*

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The geographic range of the Western Bean Cutworm (WBC), a serious, univoltine, pest of beans and corn, had been limited to the western United States. However, in the last decade its range has expanded eastward and it is now common in the Great Lakes region of both Canada and the United States. The sex pheromone had been identified and while pheromone traps have proved useful in detecting the presence of the WBC there is no correlation between trap catch and subsequent infestations. As nothing was known about the pheromone mediated reproductive biology we undertook studies to examine (i) the effects of age, temperature and humidity on female calling behaviour, (ii) the effects of abiotic factors on the periodicity of male captures in pheromone traps, (iii) the effects of age on male mating success, and (iv) and male previous mating history on fecundity. The results of these experiments will be presented and discussed within the context of using of pheromone traps in a pest management programme for the WBC.

DEVELOPING A LURE FOR THE WHITE BUTTERFLY, *PIERIS BRASSICAE*

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Pieris rapae was used as a proxy organism for field trials aimed at developing an effective trap and lure for *Pieris brassicae,* which is a "new to New Zealand" organism that arrived in 2010 in the Nelson Port. Probable host plants for this organism include rare and endangered native brassicas such as Cook's scurvy grass, which has suffered substantial herbivory already. Both species have similar nectar hosts. Synthetic compounds were prepared as lures based on nectar host flowers, and trialled in a variety of sticky trap designs mimicking flowers. Odour blends were chemical mimics of Californian thistle, dandelion, and butterfly bush (*Buddleja*). Californian thistle blend was found to be most attractive; none of the other blends caught any *P. rapae.* Red and yellow admirals (*Vanessa gonerilla* and *Vanessa itea*) were attracted to *Buddleja* odours. Different combinations and concentrations of the major components of the thistle blend were field tested to search for a more effective combination of components. Different combinations of colour and odour altered catches significantly but flat circles with thistle odour were effective. Further field tests will be required to optimise the effectiveness of our trap, with a view to including it in the set of tools available for the current eradication attempt.

EVOLUTION IN THE INVASIVE WEED, *PASTINACA SATIVA*, AFTER REASSOCIATION WITH ITS SPECIALIST HERBIVORE, *DEPRESSARIA PASTINACELLA*

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Wild parsnips, *Pastinaca sativa*, in North America have been associated with a detrimental florivore, *Depressaria pastinacella*, the parsnip webworm, for over a century. In 2004, webworms were discovered in New Zealand (NZ), an area with parsnip populations that have been free from herbivory since the mid-nineteenth century. We predicted that reassociation with a specialist herbivore will result in rapid adaptive evolution in the weed. We set up reciprocal common gardens with wild parsnips originating in the US and in NZ. Seeds from NZ were collected over six years from a total of nine populations that either had never been infested, had been infested since 2004 or had been infested since 2006. Half the garden was sprayed with insecticide to measure the impact of herbivores. We measured amount of damage and reproductive fitness, as well as leaf and floral chemical traits. Our results indicate that webworms significantly reduce the fitness of the plants irrespective of their geographic home. Plants high in octyl butyrate and myristicin and low in octyl acetate are associated with lower insect damage and higher plant fitness. US parsnips have a chemical profile well-suited to webworm resistance and experience greater fitness than NZ plants in the presence of the webworm. However fitness in both gardens was strongly correlated to plant size. NZ populations have dramatically different chemical profiles but, after 6 years of webworm infestation, NZ plants have higher fitness overall in part due to their larger size. Tolerance to herbivory might be favored when resources are unlimited.

SYMPOSIUM TOPIC 15: APPLIED CHEMICAL ECOLOGY: NEWEST RESEARCH AND DEVELOPMENT

Topic Coordinators:
Alex IL'ichev (Department of Environment and Primary Industries, Australia)
and **Jerry Zhu** (USDA, USA)

TOWARDS SUSTAINABLE PHEROMONE-BASED IPM IN HORTICULTURE: FUTURE CHALLENGES

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Pheromone-mediated mating disruption (MD) has been successfully used for long-term, area-wide treatments within sustainable Integrated Pest Management (IPM) programs in Australian horticulture. Effective MD control of low-medium infestation of oriental fruit moth (OFM) and codling moth (CM) have been well documented for over 20 years in Australian orchards. The ability of OFM and CM to migrate between orchards and together damage the same fruit variety stimulated development of multi-species MD dispensers. The results of our long-term field trials demonstrated that dual-species OFM and CM dispenser was as effective as single-species dispensers applied individually by reducing moth numbers and fruit damage to a similar degree, and will be economically advantageous when control of multiple pests is required in the same variety. We compared the performance of standard MD dispensers applied at full recommended and reduced rates, and "low dose" MD dispensers applied at full recommended rate to understand the role of point sources density for successful MD control of CM and OFM. The release rates, longevity and emission characteristics of sex pheromone active ingredients from multi-species MD dispensers were also investigated. The challenges of simultaneous disruption of multiple species in stone and pome fruit orchards such as differences in flight and mating time, MD application time and point sources density, emission rate, longevity and compatibility of active ingredients will also be discussed. Semiochemical-based IPM and selective area-wide MD programs are the key elements in development of cost effective strategies for pest control while protecting the environment by reducing pesticide pressure in orchards.

IMPROVING THE EFFICACY AND ECONOMICS OF AEROSOL EMITTERS FOR CONTROL OF CODLING MOTH (CM), *CYDIA POMONELLA* L.

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High levels of orientational disruption have been achieved with deployment of as few as two aerosol-emitting devices per hectare, each releasing 336 mg of pheromone/day. Results like this are encouraging, but making the approach robust or consistently effective will arise through sorting out the mechanism by which disruption is achieved and improving the economics. It has been widely assumed, based on the pattern of moth captures in grid-trapping studies, that the pheromone plumes created by aerosol emitters camouflage lure-baited traps or desensitize males. Recent field studies in Michigan do not support camouflage or desensitization as the primary mechanism of disruption for aerosol emitters. A comparison of male CM response to pheromone baited traps after direct or indirect exposure to pheromone emitted from an aerosol device, hand-applied disruption formulation or no pheromone control were conducted in 4-ha field plots. Direct exposure to high concentrations of pheromone did not subsequently inhibit male CM's ability to locate pheromone-baited traps. Indeed, such exposures increased the propensity of males to search for and find traps. Males appeared to be attracted to the high-releasing aerosol emitters. The control achieved using aerosol emitters may be greatly improved if less costly units that release lower rates of pheromone were deployed at densities of 8-16 per hectare. Large-plot field trials conducted in Michigan apple orchards revealed that 2 and 4-fold reductions in the amount of pheromone released did not significantly diminish the performance of aerosol emitters. We currently are testing the effects of further reductions in pheromone requirements.

ACTIVE SPACE AND OPTIMAL TRAPPING DENSITY OF TERPINYL ACETATE BAITED TRAPS FOR MONITORING *GRAPHOLITA MOLESTA* (BUSCK) (LEPIDOPTERA: TORTRICIDAE)

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Oriental fruit moth (OFM) *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) is now widely spread throughout the world, including Australia. Pest management decisions based on first catch of OFM male moths in sex pheromone traps are not reliable in orchards treated with pheromone-mediated mating disruption (MD) due to competition between MD dispensers and pheromone traps. However, female moths are attracted to food traps and currently terpinyl acetate/brown sugar solution (TA) traps are commonly used in MD treated orchards in Australia. The number of traps/ha required to reliably monitor a moth population in an orchard depends on the active space of the trap, the behaviour of the target moth species, and spatial distribution of the moth population in the orchard. The active space of TA baited food traps was determined firstly by finding the distance at which traps in grids of different trapping densities began to interfere with each other due to overlap of the active space, and confirmed the following season using geostatistical analysis of an intensively trapped orchard. A Miller-de Lame plot derived from the grid-trapping data had an x-intercept of 38.38 traps/ha, indicating an active space radius of 12.1m. The geostatistical analysis estimated an active space radius of 10-15m and an optimal trapping density of 9 traps/ha. Current practice in Australian orchards is to use only 1-2 traps/ha which is, as these data indicate, inadequate for reliable estimation of the population mean and therefore risky in terms of IPM decision making based on threshold levels of moth populations.

PHEROMONE TRAP MONITORING FOR EFFICIENT INTEGRATED MANAGEMENT OF TORTRICID MOTHS IN APPLE ORCHARDS

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New EU regulations on integrated pest management, to be implemented in 2014, will require more thorough monitoring and forecasting with the objective to decrease the use of insecticides. The objective of this study was to develop a web based decision-support tool that will allow for integrated management of a complex of tortricid moth species in Swedish apple orchards. Seven species of tortricid moths considered key pests were monitored using pheromone traps for four seasons (2008-2011) in nine orchards. Trap catch data was used to study species composition, population size and emergence/flight curves of adult moths. Combination with temperature data allowed for modeling of egg and larval development. The growers' management strategies, as reflected in choice of insecticides and spray dates, were also recorded and a survey was sent to all Swedish apple growers asking for information on main challenges for integrated management and prioritized features of a web-based tool. Trap data showed a high degree of overlap of the flight curves of the different species, indicating that careful monitoring make it possible to target several species with one application and thus decrease the amount of insecticide used. The tool provides information on available insecticides as presented in product sheets, including which life stage they are active against. Moveable bars, whose width corresponds to the persistency of the product, allow growers to optimize choice of insecticide as well as timing of applications.

MATING DISRUPTION OF THE CARPENTER MOTH, COSSUS INSULARIS (STAUDINGER) (Cossid: Lepidoptera) WITH SYNTHETIC SEX PHEROMONE IN APPLE ORCHARDS

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Recently the larvae of the carpenter moth, *Cossus insularis* (Staudinger) (Lepidoptera: Cossidae) has been found to infest the main branches and trunks of Japanese pear (*Pyrus pyrifolia* var. culta) and apple (*Malus pumila*) in central and northern Japan. On both host trees, aggregations of larvae were observed to bore into woody stems, causing significant damage and frequent mortality. Because burrows are not exposed, insecticides are not a practical measure for control. We, therefore, tested mating disruption of *C. insularis* with a synthetic version of its sex pheromone, a mixture of (*E*)-3-tetradecenyl acetate and (*Z*)-3-tetradecenyl acetate, in apple orchards in the Fukushima prefecture and the Yamagata prefecture, Japan in 2012. Pheromone trap catches and percentage mating of tethered females and of females enclosed in a screened cage with a male were measured in both the pheromone-treated and untreated control orchards. The attraction of male adults to pheromone traps was completely disrupted, and the mating of the tethered females was completely inhibited by the treatment of synthetic pheromones. The percentage mating of females enclosed in a screened cage was significantly reduced in the pheromone-treated orchard in comparison with the untreated orchard. These results suggest that mating disruption with the synthetic sex pheromone is promising for the reduction of damage by *C. insularis* in apple orchards. Since *C. insularis* requires more than two years to complete a life cycle, we should monitor the damage continuously to confirm whether the mating disruption contributes to the reduction of the damage by *C. insularis*.

PHEROMONE CONCENTRATION IN ORCHARDS TREATED WITH AEROSOL DISPENSERS

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Pheromone-mediated mating disruption to control insect pests in agriculture and forestry has been used successfully for over 30 years. There are many different types of products available but passive low release rate hand-applied products deployed at 500 to 1,000 dispensers per hectare are most widely used. Recently, mechanical high release rate pheromone delivery systems (aerosol emitters) applied at 1.5 to 3.0 dispensers per hectare, have been introduced. There are a number of studies published describing in-field or in-orchard pheromone concentrations when low release rate hand applied dispensers have been deployed but there are few studies published describing pheromone concentrations in fields or orchards when aerosol emitters have been deployed. In order to understand pheromone concentration and distribution when aerosol emitters are deployed, we set up experiments in orchards. Pheromone collection devices emanating in a concentric ring were placed 25, 50, 100 meters away from an aerosol emitter. Weather data including wind speed, direction and temperatures were collected during the experimental period. The data clearly showed that pheromone concentration within the orchard was significantly influenced by wind direction and the orientation of the spray from the aerosol emitter. The results of this study should be taken into consideration when deploying aerosol dispensers in orchards.

PATTERNS OF BEHAVIOURAL RESPONSES IN A GENERALIST AND A SPECIALIST: LESSONS FOR ATTRACT-AND-KILL

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We compared olfactometer responses of a highly polyphagous moth (*Helicoverpa armigera*, for which we have a commercial attract-and-kill technology) with those of an oligophagous moth (*Plutella xylostella*, for which no similar technology yet exists). For *H. amigera*, 33 out of 38 plants were attractive, including many not suitable for larval development. Only 7 of 34 volatiles from these plants were attractive on their own, and the level of attraction was much lower than the best plants. However, 21 of 31 blends were attractive, and the best of these gave attraction comparable to the best plants. None of these blends mimicked real plants, and some of the best had few components in common. These results are not compatible with the dominant paradigm of ratio-specific attraction to volatiles, mimicking host plants. In field trials, *H. armigera* could be attracted and killed by many of these non-host blends when they were sprayed on foliage, but would not enter traps baited with them. This suggests a multi-modal response with generalized olfactory mechanisms important only in initial stages. For the brassica specialist *P. xylostella*, two host plants were attractive. Of 12 volatiles tested, only one (a brassica-specific compound) was attractive, but on its own it gave attraction comparable to the host plants. Blending it with other volatiles, including some present in host plants, reduced attractiveness. This suggests a unique volatile response, with perhaps a ratio-specific component to distinguish non-host plants. The implications for developing plant volatile-based attract-and-kill strategies for generalists vs specialists are discussed.

ATTRACT-AND-KILL USING A MOTH ATTRACTANT: POTENTIAL ROLE IN RESISTANCE MANAGEMENT IN TRANSGENIC COTTON

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The Australian cotton industry follows resistance management plans (RMPs) to reduce the risk of developing Bt resistance in *Helicoverpa* moths. We are investigating the potential of 'moth busting' using a plant volatile-based moth attractant (Magnet®) for attract-and-kill, targeting potentially resistant moths emerging in late season cotton. Following a pilot trial in the 2011/12 season, we conducted our first large-scale field trials in the 2012/13 season in the Upper Namoi region to determine, whether, on an area-wide basis, appropriately timed and placed applications of attract-and-kill can reduce the numbers of potentially resistant *Helicoverpa* moths emerging and surviving in Bt cotton in late summer/early autumn. The field trials involved treating about 1500 ha of transgenic (Bt) cotton in each of two locations. Each location had replicated fields of treated and untreated cotton, and fields of pigeon peas (required as refuge crops in RMPs) as well as other *Helicoverpa* host crops. Light trap and pheromone trap data demonstrated that catches of *Helicoverpa* moths in cotton were suppressed during and soon after Magnet® applications, and that there appeared to be no impact of the treatments on moth populations in the refuges. The host origins of moths killed by the attract-and-kill formulation are being determined by stable carbon isotope and lipid analysis, and will be compared with those of the general moth population obtained from light and pheromone traps. Bt resistance levels are being monitored and will be compared between treated and untreated regions within the study area, and with other cotton growing regions.

NATURE CONTROLLING ITSELF: USING LIVE FEMALES AS BAIT FOR THE MASS TRAPPING OF BAGWORM MOTHS (LEPIDOPTERA: PSYCHIDAE) IN OIL PALM, MALAYSIA

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The bagworms (Lepidoptera: Psychidae) are currently major and common insect pests infesting oil palm in Malaysia. Especially in the central and southern parts of Peninsular Malaysia, bagworms (i.e. *Metisa plana* Walker and *Pteroma pendula* Joannis) outbreaks are quite common and often recurring. High bagworm populations left unmonitored often caused major outbreaks, manifested eventually with severe defoliation and browning of the palm crown. The use of sticky traps baited with live receptive females as pheromone bait for mass trapping appeared promising. Male moths captured by the sticky traps had reduced the probability of mating and oviposition of the females. The following generation of larvae was lower in areas where trapping was conducted. The live receptive female was used to capture massive numbers of male moths in order to arrest the population, or to reduce the population, prior to other control options (i.e. spraying of *Bacillus thuringiensis*). The efficacy of trapping using live receptive females as the pheromone bait was assessed in oil palm areas with 6-16 year old palms in Perak, Peninsular Malaysia, infested with the bagworm, *Metisa plana*. The efficacy of trapping was calculated by recording the percentage of traps capturing more than 5 male moths over a period of 3 to 7 days. The percentage of the adult moths removed from the population via mass trapping was estimated by recording the presence of male adults per palm (based on the number of male pupae and sex ratio) and later calculated to an area (hectare) basis. The total number of moths captured by the traps was then compared to the estimated presence of the male adults / hectare in order to derive the percentage of adult moths removal. Depending on the receptivity and timing of using the live females as bait, the trapping efficacy ranged from 23.5 to 98.9% between a period of 3 to 7 days for each session of trapping. The mean sex ratio of females to males for each frond sampled was 1: 1.2. Based on the mean number of males sampled during pre-trapping, the estimated removal of adults per hectare via trapping can be up to 70%. The use of live females as bait for mass trapping therefore seems a viable option as it decreases the chances of mating and was manifested by the reduced percentage of female bags with hatched eggs. However, correct timing in collecting the receptive females remains a critical prerequisite for the efficacy of mass trapping.

THE OPTIMISATION AND DEVELOPMENT OF A PHEROMONE TRAP FOR THE BANANA SPOTTING BUG *AMBLEYPELTA LUTESCENS*

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Ambleypelta lutescens lutescens (Coreidae) is a serious pest of tropical and subtropical fruit and nuts in Australia. The pest is highly mobile, cryptic and difficult to monitor and control, which often results in crop damage and losses occurring before the pest is detected. This leads many growers to apply insecticides on a regular calendar based schedule. However, with the withdrawal of endosulfan, growers are now forced to spray with broad-spectrum insecticides, which can lead to flare of secondary pests, such as mites. There are few remaining registered insecticides available for *A. lutescens* control, and they are not always effective. In order to better target and reduce insecticide sprays, we aimed to develop a pheromone trap monitoring system. Based on the results of previous research on the *A. lutescens* aggregation pheromone, we further optimised the chemical components of the lure. Using field trials on different component combinations, we found a two-component lure to be most effective. Using field based dose-response trials we optimised the field release rates of the lure. Furthermore, we optimised an effective "sticky" panel system for trapping the pests. This pheromone trap was found to be an effective monitoring tool, and could result in growers adopting a monitoring system, rather than relying on calendar based spraying. Efficacy results, from field testing in different crop situations, are presented in an accompanying paper. With further research it may be possible the lure could be used as part of a field control tool.

FIELD TESTING AND EFFICACY OF AN AGGREGATION PHEROMONE LURE AND TRAP FOR THE BANANA-SPOTTING BUG *AMBLYPELTA LUTESCENS* IN AVOCADOS, PAPAYA AND CUSTARD APPLE.

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Amblypelta lutescens lutescens (banana-spotting bug) is a Coreid bug native to Australia with a wide host range of both native and introduced plants. It is a major pest of tropical and sub-tropical commercial crops. In previous work, a pheromone lure for *A. lutescens* was developed. This aggregation pheromone attracts male and female adults and nymphs. In this study, field trials were conducted to determine the potential of the pheromone trap as a monitoring tool or a control method. Trials were designed to test use patterns, traps densities and efficacy against standard field monitoring techniques. The attractancy range of the trap was unknown. Trials were conducted in commercial orchards of avocado, papaya and custard apple. The traps were spaced throughout the crop at different densities and damage assessments were made to compare the trap catches with the damage caused by *A. lutescens.* Correlations between trap density and numbers of *A. lutescens* trapped, varied depending on the different trials and crops. There was generally little difference in fruit/shoot damage levels between the treatments (either between trap densities or negative controls), suggesting that the pheromone trap may not be an outright control tool, in its current form. However, further analysis of data will be conducted and discussed. Through further analysis of the data, economic thresholds for monitoring could be determined. Further development of the trap design is required. We will discuss how the pheromone lure could be used for alternative control strategies.

OPTIMISING TRAPS AND LURES TO PROVE AREA FREEDOM FROM MEDITERRANEAN FRUIT FLIES

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To prove area freedom from Mediterranean fruit fly (MFF) *Ceratitis capitata* (Wiedemann) the Australian Quarantine Code of Practice (MFF-COP) requires weekly data from traps (0.4 - 1km apart) arrayed in passive static grids covering orchards and towns. The method is extensive but inefficient when numbers are low. The infrastructure is cumbersome and expensive to deploy and maintain. To improve efficiency and reduce costs, studies were carried out on lure attractancy, host phenology and trap placement over three seasons (2008 to 2010) in Pemberton, Manjimup, Donnybrook and Kununurra covering a wide range of conditions suitable for MFF. A "dynamic" system was developed and assessed against the MFF-COP for equivalence in four categories: 0, >0 ≤2; >2 ≤4 and >4 male flies / trap / fortnight (FTF). Results were consistent over all three seasons. The most attractive lure + killing agent combinations were: BioLure® (ammonium acetate, trimethylamine and putrescine) + 70% propylene glycol for females; and Capilure® (trimedlure + extenders) + 70% propylene glycol for males. Phenology based trap placement provided overwhelmingly superior data. The "dynamic" system required one-third to one-half the number of traps as the static MFF-COP system to provide equivalence for detecting non-breeding itinerant flies (<2 FTF) or an early breeding population (≥3 FTF). In the "dynamic" system, male or female flies signaled an incipient breeding population 3-10 weeks earlier (than the MFF-COP system) proving effectiveness at considerably lower cost while increasing the likelihood of eradication.

DEVELOPMENT OF A FEMALE LURE FOR QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* AND JARVIS'S FRUIT FLY, *BACTROCERA JARVISI* (TEPHRITIDAE)

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A fruit volatile blend combined with protein and ammonium bicarbonate in a wax matrix was developed as a female attractant and tested against two Australian pest fruit flies, *Bactrocera tryoni* and *Bactrocera jarvisi*. Two field experiments to evaluate this lure in a mango crop are discussed. In the first experiment, the lure was tested in Steiner and Probodelt (cone) traps using maldison dosed wicks as the toxicant and the attractancy was compared against protein baits (Mauri's yeast autolysate and a dried brewery yeast). The protein baits were sprayed onto sticky panels. The wax matrix lure in the cone trap attracted 36 gravid females and 46 non-gravid females (total 82 females), compared to 0 gravid and 75 non-gravid females in the Mauri's yeast treatment. The cone trap, which has a yellow base, performed better (82 females) than the Steiner trap (only 6 females trapped). In the second experiment the female lure was tested with different toxicants (maldison incorporated in lure, maldison wick, dichlorvos strip and dichlorvos wick). There appeared to be an interaction between the type of toxicant and how it was administered. Significantly less flies (7) were caught when maldison was incorporated in the wax matrix compared to a maldison wick (82), dichlorvos strip (40) and dichlorvos wick (16). However, significantly more female flies (39) were caught in the dichlorvos strip treatment compared to maldison wick (13), maldison incorporated (7), dichlorvos wick (15) and control (0). Further work is required to determine the best lure/toxicant combination.

BACTERIAL INFECTION OF TREES INDUCES RELEASE OF A VOLATILE THAT 'DECEPTIVELY' ATTRACTS INSECT VECTORS; CAN WE MANIPULATE THE ENVIRONMENT TO PREVENT VECTORS FROM HOMING IN ON INFECTED PLANTS?

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We experimentally demonstrate specific mechanisms through which a bacterial plant pathogen induces plant responses that modify behavior of its insect vector. *Candidatus Liberibacter asiaticus*, a fastidious, phloem-limited bacterium responsible for causing huanglongbing disease of citrus, induced release of a specific volatile chemical, methyl salicylate, which increased attractiveness of infected plants to its insect vector, *Diaphorina citri*, and caused vectors to initially prefer infected plants. However, the insect vectors subsequently dispersed to non-infected plants as their preferred location of prolonged settling because of likely sub-optimal nutritional content of infected plants. The duration of initial feeding on infected plants was sufficiently long for the vectors to acquire the pathogen before they dispersed to non-infected plants, suggesting that the bacterial pathogen manipulates behavior of its insect vector to promote its own proliferation. The behavior of psyllids in response to infected versus non-infected plants was not influenced by whether or not they were carriers of the pathogen and was similar under both light and dark conditions. Feeding on citrus by *D. citri* adults also induced the release of methyl salicylate, suggesting that it may be a cue advertising location of conspecifics on host plants. We are currently exploring whether masking the environment with large amounts of methyl salycilate may prevent vectors from homing in on bacterially infected trees analogously to the mating disruption technique. Preliminary evidence suggests that we may be able to reduce the vector's ability to discriminate between infected and uninfected trees, potentially reducing pathogen spread.

HEALTHY PLANT PROTECTION

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Secondary metabolites provide a tremendous potential for host plant resistance and natural crop protection. This is especially important due to the rapid spread of agricultural pests worldwide. At the same time international law regulations restrict the use of pesticides. Therefore, we developed the eco-metabolomic approach to identify candidate compounds related to host plant resistance using NMR (Nuclear Magnetic Resonance Spectroscopy). We classify resistant and susceptible plants using *in-vivo* bioassays. Subsequently, we compare their metabolomic profiles by multivariate statistics to identify metabolites involved in host plant resistance. The negative effect of the candidate compounds is validated with *in-vitro* bioassays. As a proof of principle we used western flower thrips (*Frankliniella occidentalis*) a key pest worldwide. We applied the approach in different host systems including Senecio as a wild plant, chrysanthemum as an ornamental and tomato and carrot as vegetables. In all systems the metabolomic profiles of resistant and susceptible plants were significantly different leading to a range of different metabolites involved in thrips resistance. The majority of these compounds were phenylpropanoids and flavanoids. Interestingly, these did not only show a negative effect on thrips, but as anti-oxidants, are also linked to positive human health effects such as prevention of cancer and cardio-vascular diseases. As such these compounds do not only contribute to sustainable pest management but also to human health improvement. Therefore, our approach is of great relevance for practical implementation in crop breeding programmes as expressed by the large interest of plant breeders.
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INTRODUCING THE LACEWING EGG CONCENTRATOR FOR *CHRYSOPERLA* SPP

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Chrysoperla lacewings (Neuroptera, Chrysopidae) have a wide occurrence worldwide and their artificially reared larvae are commercially available and are widely used in biological control. Resulting from our previous research efforts we recently discovered a multi-component synthetic attractant. Our newly discovered attractant was unique among lacewing attractants as it lured great numbers of females (to our knowledge no other effective female-attracting artificial lure is known from the literature). In the course of our tests we were surprised to observe that lacewings coming to the attractant would lay eggs on different parts of the trap. This brought up the idea that by the help of the new attractant one can concentrate eggs of lacewings (naturally occurring in the biotope) to a preferred place (for example, to the plant to be protected). In order to increase the number of eggs laid, other possible stimuli affecting egglaying were studied. We found that if the attractant is placed on a spiny, rough surface, the number of eggs laid significantly increased. Presence of eggs laid earlier on spiny surfaces with the new attractant did not decrease the number of eggs laid afterwards. Based on our discovery a lacewing egg concentrator device was developed. When the attractant dispenser was placed on overwintering boxes, 2-3 times more lacewings overwintered in boxes with lures than in ones without.

ATTRACTION OF *CHRYSOPERLA SINICA*, A NATURAL ENEMY BY SYNTHETIC ATTRACTANTS FOR CONTROLLING COLORADO POTATO BEETLE

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Colorado potato beetle, *Leptinotarsa decemlineata*, has become the economically most important insect defoliator of potatoes in northern Xinjiang Uygur autonomous region in China. To develop a new sustainable control technique, some synthetic herbivore-induced plant volatiles were field tested for attractivenesss to *Chrysoperla sinica*, a natural enemy in potato fields. Traps baited with a tertiary mixture of methyl salicylate, acetic acid and phenylacetaldehyde were most attractive. The tertiary lure captured the most number of *Chrysoperla sinica* at the dose of 900 μg when traps were placed 1.75 m above ground. There was no significant difference between the mean number of *Chrysoperla sinica* caught by dispensers 26 days old and dispensers replaced every week. The most number of *Chrysoperla sinica* eggs was observed 5 m around the dispensers. There were significantly higher densities of *Chrysoperla sinica* eggs and adults in an attractant-baited plot than in a non-attractant-baited plot. In addition, significantly fewer Colorado potato beetle were found on the potato plants in an attractant-baited plot than in a non-attractant-baited plot. The results show that synthetic attractant for *Chrysoperla sinica* has great potential in integrated management of the Colorado potato beetle.

ASSESSING THE IMPACT OF SEMIOCHEMICALS IN THE MANAGEMENT OF MAIZE STEM BORER (*BUSSEOLA FUSCA,* FULLER) IN NIGERIA

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Maize is a staple food crop for most households in Nigeria and is grown in almost all agro-ecological zones. Unfortunately maize yield is too low to keep pace with the rate of population growth, leading to serious food insecurity and poverty. The low yield is associated with incidence of stem borers. Selecting suitable biodiversity cropping systems such as intercropping has been reported to release semiochemicals that protect some crops against insect pests like stem borers. The impact of intercropping maize with bambara groundnut in the management of African maize stalk borer, (*Busseola fusca,* Fuller Lepidoptera:Noctuidae) were assessed in Nigeria during 2009/2010 farming seasons. The results showed that maize plots intercropped with bamabara groundnut significantly reduced the larval densities of stem borer on average of 64.3% and 43.1% compared to maize monocrop in both seasons respectively. Equally, the percentages of stem bored and yield losses due to stem borer infestations were on average of four and two folds lower in intercrop than in monocrop respectively for both seasons. A build up of natural enemies of the borer was also observed in these intercropping systems and resulted in the reduction of pest infestations. Intercropping system has the additional advantage of higher land productivity than the monocrop. In conclusion intercropping maize with bambara groundnut improved; biodiversity of the ecosystem, stem borer management, and increased maize yield.

THE DEPENDENCE OF SEX PHEROMONE PRODUCTION ON LARVAL- AND ADULT-ACQUIRED METABOLITES IN VIRGIN AND MATED FEMALE *HELIOTHIS VIRESCENS*

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Polyandrous female moths produce sex pheromone to attract mates throughout their adult life. Following an initial mating, egg maturation typically increases. This may be problematic for pheromone production, since both physiologies use common metabolites and, therefore, potentially compete against each other. In the polyandrous moth *Heliothis virescens* (Noctuidae), nectar feeding by adult females meets some of this demand. However, mated females typically produce less sex pheromone than do virgins, suggesting that metabolite demand for egg production 'costs' pheromone production. We used a tracer-tracee approach, feeding females U-^{13}C-glucose daily, to determine the contributions of larval and adult nutrients over time on pheromone and egg production, and to determine whether differences in pheromone production between virgin and mated females could be explained by demand of common metabolites for egg production. Over time (i.e., with increased adult feeding), pheromone production in virgins became increasingly dependent on glucose from adult feeding, and therefore less dependent on larval-acquired metabolites. By contrast, in mated females, pheromone production became decreasingly dependent on glucose from adult feeding and more dependent on fats from larval feeding. This occurred in spite of the fact that egg production in mated females became increasingly dependent over time on glucose from adult feeding. Thus, egg maturation in this species can 'cost' pheromone production. Overall, our data support a model in which larval-acquired metabolites are used to produce a basal level of pheromone for attracting mates, which can be enhanced to more attractive levels by adult-acquired metabolites.

CHEMICAL ECOLOGY OF AMBROSIA BEETLE *MEGAPLATYPUS MUTATUS*: FROM IDENTIFICATION OF PHEROMONES TO FORMULATION IN RESERVOIR AND MONOLITHIC DELIVERY SYSTEMS AND MONITORING AND MATING DISRUPTION TRIALS.

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Megaplatypus mutatus (Chapuis) (Coleoptera: Curculionidae: Platypodinae) is an ambrosia beetle native to South America, but has recently been introduced to Italy and represents a serious problem in commercial poplar and fruit tree plantations. Male *M. mutatus* emits a sex pheromone composed of (+)-6-methyl-5-hepten-2-ol [(+)-sulcatol], 6-methyl-5-hepten-2-one (sulcatone) and 3-pentanol.
We made reservoir and monolithic type dispensers for pheromones to be deployed in the field during the flying period. The release rates of the dispensers were measured in a wind tunnel at controlled temperature and wind speed. The polymeric reservoir-type dispensers had constant release rate (zero order kinetics) in the range of milligrams per day for periods of until 15-20 days. The monolithic dispensers were made with different mixtures of waxes and polymers with inert components. They followed first-order kinetics. We performed field trapping trials of *M. mutatus* in highly infested hazelnut and poplar plantations of Italy and Argentina. Different shapes and colour of traps were tested. Taking into account that the beetle is relatively immobile, that males are monogamous, and that the pheromones are of very low commercial cost, stable in field conditions and can be formulated in controlled released systems with relatively high release rates, we evaluated the potential management by disruption of communication. After the treatment, the number of galleries where mating took place was significantly higher in control than in treated areas, indicating that pheromone application had interfered with female behavior and male localization. As damage reduction was greater than 56% in both countries, these results show the potential for the strategy of pheromone-mediated mating disruption of *M. mutatus* in commercial poplar and hazelnut plantations. Also, our study provides the first evidence for successful pheromone-mediated mating disruption in a forest beetle.

Gonzalez Audino et al. 2005. *J Econ Entomol*.
Hernán Funes et al. 2011. *Ent Exp Appl*.

ESSENTIAL OILS AND THEIR COMPOSITIONS AS SPATIAL REPELLENTS FOR PESTIFEROUS SOCIAL WASPS

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Eusocial vespid wasps include several subfamilies, such as Polistinae, Vespinae, and Polybiinae from Vespidae (Hymenoptera: Vespidae), commonly referred to as paper wasps, yellowjackets, and hornets in North America. Our study objectives were to (1) field test potential repellency of common essential oils against several pestiferous social wasps (Hymenoptera: Vespidae) using attractant-baited traps, (2) identify vespid antennally-active compounds from the repellent essential oils and (3) determine potential repellency of these electroantennographic detection (EAD) active compounds in the field. Of twenty-one essential oils tested, seventeen showed significant repellency on yellowjackets [mainly *Vespula pensylvanica* (Saussure)] and paper wasps [mainly *Polistes dominulus* (Christ)]: clove, pennyroyal, lemongrass, ylang ylang, spearmint, wintergreen, sage, rosemary, lavender, geranium, patchouli, citronella, Roman chamomile, thyme, fennel seed, anise, and peppermint. Two essential oil mixtures: 3EO-mix (clove, geranium, and lemongrass) and 4EO-mix (clove, geranium, lemongrass, and rosemary) totally blocked the attraction of vespid workers. Twenty-nine vespid antennally-active compounds were identified from solid phase microextraction (SPME) samples of eleven strongly repellent essential oils using GC-EAD/MS techniques. Among the synthetic EAD-active compounds field tested, eugenol, *P/I*-menthone, pulegone, α/β-thujone, *l*-carvone, *E/Z*-citral, citronellal, methyl benzoate, benzyl acetate, methyl salicylate, and 3-octanol, showed a significant repellency on vespid workers. These compounds are likely responsible for the repellency of their corresponding essential oils. These repellent essential oils and their active compositions have great potential for efficient, environmentally sound semiochemical-based IPM of pestiferous vespid wasps.

Schneidmiller et al. 2012. U. S. Patent Application Pub. # 20120107428.

CONTROL OF *CARPOPHILUS SPP*. (COLEOPTERA: NITIDULIDAE) FROM BASIC SCIENCE TO THE DEVELOPMENT OF A GREEN METHOD OF CROP PROTECTION

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Carpophilus beetles of the genus *Carpophilus* are significant pests of a wide variety of fruits and grains and are also vectors of plant pathogens. The beetles are difficult to control with conventional insecticides because the beetle damage typically occurs just before harvest, when toxic residues must be avoided. These insects are generally attracted to aroma such as that from overripe or decomposing fruit. Initial research developed fermenting fruit and similar materials plus pheromone as trap baits to control beetles and, from the standpoint of efficacy, the method had good potential but the large volumes of fruit and juice in the traps made them labour intensive, and time consuming to service. For the technique to become acceptable to growers, an easy to use alternative for the fruit and juice was required. After a thorough analysis of the chemical constituents of ripening and decomposing stone fruits, a blend of six water soluble compounds were identified as a potential synthetic co-attractant. Further field trials resulted in development of a practical attract-and-kill product that has been shown to be at least as effective as insecticides for protecting stone fruit crops in Australia. This compact and user-friendly tool is now commercially available for Australian growers.

RESPONSES TO HOST PLANTS AND PHEROMONE COMPONENTS OF SHOOT AND FRUIT BORER, *CONOGETHES PUNCTIFERALIS* (GUENEE) (LEPIDOPTERA: CRAMBIDAE) INFESTING CASTOR AND CARDAMOM

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The shoot and fruit borer, *Conogethes punctiferalis* is a serious pest on several cultivated plants and the population consists of two types, *viz.,* stalk feeding type (SFT) and fruit feeding type (FFT). The SFT and FFT *Conogethes* larvae perceived their host plants from 1.2 to 2.0 cm distance depending on the age of the larva. The minimum distance for the host perception increased with the increase in the age and size of the larva. All the instars of both the types responded and oriented readily to the host stimulus. On reaching the host, larvae palpated and bored in to the shoot/ fruit. Orientation consisted of random and directed movements accompanied with raising and lowering the head. FFT larvae preferred castor and SFT, cardamom. The inactivation of sense organs involved in host perception was accomplished by cold (-5^{0}C), hot (35^{0}C) and acid treatment. EAG and gas chromatography linked electroantennogram and windtunnel bioassays showed major pheromone component to be E10:16Ald, but there were differences in minor components. Field trials with pheromone traps resulted in moth catches in castor fields compared with no moth catches in cardamom plantations with same pheromone blends. Experiments indicated that relative suitability of plants as hosts for *Conogethes* larval types depended on differences in the responses involved in the six different phases of their establishment on the plant. These observations have implications for managing *Conogethes* on different cultivated crops/ plants.

INVESTIGATION OF THE BEHAVIORAL RESPONSE OF GREEN LACEWINGS TO APHID SEX PHEROMONES AND OTHER GREEN LACEWING SPECIES: RESULTS FROM FIELD EXPERIMENTS

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Attracting predatory insects as beneficial organisms is a major approach within chemical ecology for the control of agricultural pests. Common green lacewings, *Chrysoperla carnea* complex, are known to be attracted to floral volatiles, whereas attraction of some *Chrysopa* species, e.g. *C. formosa*, to aphid sex pheromone components has also been reported. In our previous studies however, a combination of the two stimuli resulted in decreased attraction of common green lacewings. Since *Chrysopa* spp. are known to produce defensive compounds when disturbed, it seemed reasonable that the above effect was due to compounds released by trapped *Chrysopa* adults, instead of the aphid sex pheromone components themselves. However, in previous studies, neither dead *C. formosa* adults (bearing the specific *'Chrysopa* smell') nor synthetic skatole caused a similar decrease in attraction. Therefore, to test the direct effect of aphid sex pheromone components, an early-season experiment was set up, when *Chrysoperla* were active, but *Chrysopa* adults were not yet present. With the same purpose, an experiment was conducted with egg-laying sheets designed for common green lacewings. Furthermore the effect of live *C. formosa* adults on *C. carnea* complex catches was also tested. The aphid sex pheromone components showed a clear negative effect on *C. carnea* complex catches, both in the early-season and the egg-laying experiment. At the same time, the presence of live *C. formosa* adults did not show any influence on the catches. Although no unambigous explanation is available on this repellent-like effect of aphid sex pheromone components on common green lacewings, further studies are required for the better understanding of the possibly complex ecological background behind this phenomenon.

DEVELOPING NEW MEASURES FOR RICE PEST MANAGEMENT VIA CHEMICAL ELICITOR-BASED MANIPULATION OF VOLATILE SIGNALS

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Synthetic chemical elicitors of plant defense have been touted as a powerful means for sustainable crop protection. Yet, they have never been successfully applied to control insect pests in the field. We developed a high throughput chemical genetics screening system based on a herbivore-induced linalool synthase promoter fused to a GUS reporter construct to test synthetic compounds for their potential to induce rice defenses. We identified 2,4-dicholorophenoxyacetic acid (2,4-D), an auxin homologue and widely used herbicide in monocotyledonous crops, as a potent elicitor of rice defenses. Low doses of 2,4-D induced a strong defensive reaction upstream of the jasmonic acid and ethylene pathways, resulting in a marked increase of trypsin proteinase inhibitor activity and volatile production. Induced plants were more resistant to the striped stem borer *Chilo suppressalis*, but became highly attractive to the brown planthopper *Nilaparvata lugens* and its main egg parasitoid, *Anugrus nilapavatae*. In a field experiment, 2,4-D application turned rice plants into living traps for *N. lugens* by attracting parasitoids. Our findings demonstrate the potential of auxin homologues as defensive signals and show the potential of the herbicide to turn rice into a selective catch crop for an economically important pest.

GENERIC PHEROMONE LURES ATTRACT CERAMBYCID BEETLES IN AN ASIAN TROPICAL MONTANE RAIN FOREST

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We tested the attraction of cerambycid beetles to a library of known cerambycid pheromones in a tropical rain forest in southern China. From 28 May to 25 June 2010, 1,526 cerambycids representing 71 species were captured, with fourteen species accounting for 92% of the specimens. Test compounds with a 3-hydroxyalkan-2-one or 2,3-alkanediol motif attracted significant numbers of both sexes for eight species in the subfamily Cerambycinae, including species in the genera *Demonax*, *Rhaphuma*, and *Xylotrechus.* The cerambycine *Rhaphuma horsfieldi* (White) was the only species that was strongly attracted to more than one test compound, with significant attraction to both (2*R**,3*R**)-2,3-hexanedioland (2*R**,3*R**)-2,3-octanediol. Within the Lamiinae, males and females of four species, including *Acalolepta formosana* (Breuning), *Monochamus bimaculatus* Gahan, *Pharsalia subgemmata* (Thomson), *Pseudomacrochenus antennatus*(Gahan), and *Xenohammus bimaculatus* Schwarzer,were significantly attracted to 2-(undecyloxy)-ethanol. Unexpectedly, only males of *Megopis costipennis* White, subfamily Prioninae, were significantly attracted to (2*R**,3*S**)-2,3-octanediol, suggesting that this compound may be a sex pheromone component for this species. To date, 2,3-octanediols have only been reported as aggregation pheromone components for cerambycine species. Overall, our results support the hypothesis that many closely and even distantly related cerambycid species share pheromone components. Our results also suggested that traps baited with even a limited number of pheromones may be useful tools for surveying the cerambycid diversity of a particular habitat, as well as for detection and monitoring of specific species, particularly those that have the potential to be invasive pests.

SYMPOSIUM TOPIC 16: STUDENT TRAVEL AWARDS SYMPOSIUM (YOUNG CHEMICAL ECOLOGISTS)

Topic Coordinators:

Ring Cardé (University of California, USA), **Jocelyn Millar** (University of California, USA) and **Yongping Huang** (Chinese Academy of Science, China)

DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY OF METHYL-BRANCHED HYDROCARBONS ISOLATED FROM SEVERAL ORDERS OF INSECTS.

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Insect cuticular lipids are comprised of a complex mixture of methyl-branched hydrocarbons (MBCHs), *n*-hydrocarbons, long-chain olefins, and polar compounds as minor constituents. The primary function of the cuticular wax layer is to maintain water balance, but in many species, components of the cuticular lipids also serve as contact pheromones. The majority of insect-produced MBCHs are chiral, but despite the fact that they are ubiquitous components of insect cuticular lipids, no studies have attempted to determine whether they are biosynthesized enantiospecifically. This is mostly due to the minute optical rotations of MBCHs, which make enantiomeric analysis through polarimetry impractical due to the inadequate amounts (ng to µg) of hydrocarbons obtained from insects. Difficulties in isolation of individual MBCHs from the cuticular blend, and the lack of enantiopure MBCH standards, have also hindered the stereochemical analysis of insect-produced MBCHs. Here we report methods for the identification of the absolute stereochemistry of monomethyl-branched hydrocarbons isolated from several orders of insects. The MBCHs were isolated by initial fractionation of the cuticular extracts with $AgNO_3$ impregnated silica gel column chromatography into fractions containing saturated hydrocarbons, olefins, and polar compounds respectively. The *n*-hydrocarbons were separated from MBCHs by adsorption of the former into 5Å molecular sieves, and the enriched MBCHs were further fractionated with reverse-phase HPLC. The sign of the optical rotations of individual MBCHs were measured with an advanced laser polarimeter detector, which were then compared to the optical rotations of synthesized stereochemically pure MBCH standards to determine their absolute stereochemistries.

POSSIBLE ORIGINS OF A MALE MATING PHEROMONE IN THE SEA LAMPREY

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The diversity of sexual signals is perplexing, with many signals influencing decisions for which they possess little inherent information. In some cases, signals appearing to hold little inherent information have been hypothesized to evolve as a result of underlying biases in the behaviour and physiology of an organism. We provide evidence for biases in the origin of the male mating pheromone employed by sea lamprey (*Petromyzon marinus*), a bile alcohol known as 3 keto petromyzonol sulphate (3kPZS), by comparing the behaviour and physiology of sea lamprey to that of the more basal silver lamprey (*Ichthyomyzon unicuspis*). Silver lampreys do not use 3kPZS as a mating pheromone; males do not release large quantities into the water and females do not respond by displaying behaviours useful in reproduction. However, larval silver lampreys were found to release 3kPZS into the water at rates sufficient to function as a migratory cue, adult silver lampreys showed strong electrophysiological responses to 3kPZS, and reproductive females displayed migratory behaviours in response to 3kPZS. Although male silver lamprey do not release 3kPZS quantities sufficient for mating communication, the 3kPZS release appears to be slightly higher than that of a similar larval release migratory cue, petromyzonol sulphate (PZS). We propose that the male mating pheromone in sea lamprey arose as a result of 1) a cognitive bias, where female preference for 3kPZS evolved as a means to locate habitat conducive to high offspring survival and 2) a physiological bias, where males appear to be pre-adapted to release 3kPZS.

CROSS SPECIES SEX ATTRACTION AND REPRODUCTIVE ISOLATION BETWEEN *VESPULA VULGARIS* AND *V. GERMANICA*

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There have been anecdotes about the presence of wasps in New Zealand that appear to have a mix of markings similar to the common wasp, *Vespula vulgaris* (L.), and the German wasp, *Vespula germanica* (Fab.). We tested if it was likely for hybridization to occur between these two species of wasps. Firstly, we investigated whether gynes of *V. vulgaris* and *V. germanica* could attract males from both species in field and wind tunnel conditions. A mating trial was performed to test whether males from one species could mate with gynes from the other species. Our results showed that gynes attracted significant numbers of males of their respective species, but more than 10% of the males attracted to *V. germanica* gynes were *V. vulgaris* males with little cross attraction in the other direction (0.4%). In the wind tunnel, gynes from both species proved attractive to the males of the other species, but again not as attractive as to their own species. The mating studies showed that while the males were initially attracted to the other species, there were no successful cross-species mating attempts, suggesting the presence of a short range recognition pheromone or other cue preventing this. Hydrocarbon analysis on the gasters of *V. germanica* and *V. vulgaris* gynes showed that 17 compounds occurred in different proportions in gasters of *V. vulgaris* and *V. germanica* gynes which might contribute to the reproductive isolation between the two species.

STICKY HYDROCARBON FOOTPRINTS AND STINKY DEFENSIVE ALLOMONES FUNCTION AS AGGREGATION PHERMONES IN *HIPPODAMIA* CONVERGENS

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The convergent ladybird beetle (*Hippodamia convergens*) forms dense aggregations during winter diapause, the mechanisms of which are not well understood. We evaluated the role of three defensive allomones and cuticular hydrocarbon extracts in the formation of *H. convergens* aggregations. A 2-choice laboratory bioassay demonstrated that diapausing ladybirds preferred to walk up the branch of the Y-tube that had previously been exposed to conspecifics. Analysis of the surface of this pre-exposed branch revealed that walking ladybirds leave behind a blend of non-volatile hydrocarbon compounds. Applying an extract of these hydrocarbons to one branch of the Y-tube also yielded significant preference for the treated branch. As opposed to diapausing females, mated female ladybirds avoided the treated arm. This suggests that depending on the physiological context, these same hydrocarbon footprint pheromones could also function as an oviposition deterrent in much the same way as those laid by ladybird larvae. In a separate series of bioassays, diapausing *H. convergens* aggregated within the section of an arena exposed to either of two pyrazines, 2-isobutyl-3-methoxypyrazine and 2-sec-butyl-methoxypyrazine. Laydbirds also aggregated to a blend of three pyrazines at their natural ratio. During fall migration to overwintering sites, significantly more ladybirds aggregated in artificial hibernacula baited with pyrazines. These three pyrazines also function as warning odors that in conjunction with other aposematic displays contribute to the multi-modal, anti-predatory defense of coccinellid beetles and some other arthropods. Confirmation of the role of some pyrazines in *H. convergens* aggregations suggests that these defensive allomones have been co-opted for intraspecific communication.

ENANTIOSELECTIVE SYNTHESIS AND FIELD EVALUATION OF METHYL-BRANCHED SECONDARY ALCOHOLS, A NOVEL PHEROMONE COMPONENT OF A LICHEN MOTH AND ITS STRUCTURE-RELATED COMPOUNDS

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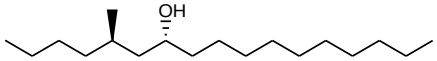
Females of the lichen moth, *Miltochrista calamina* (Arctiidae, Lithosiinae) produce 5-methylheptadecan-7-ol (**1**) as a sex pheromone. Of four synthetic stereoisomers prepared by separation from two synthetic diastereomer mixtures, males in fields were specifically attracted only by the (5*R*,7*R*)-isomer. To improve the synthesis, a new route for (5*R*,7*R*)-**1** starting from (*S*)-propylene oxide was achieved by utilizing the S_N2 reaction of an optically active secondary tosylate and the Jacobsen hydrolytic kinetic resolution of an epoxy intermediate as key steps. Chiral HPLC analysis of the target methyl-branched pheromone and the antipode synthesized from (*R*)-propylene oxide confirmed their high enantiomeric excess (> 99%). Based on the stereospecific synthesis, six homologues with the same configurations as (5*R*,7*R*)-**1** but a different alkyl chain(s) connected with the stereogenic centers were produced. Interestingly, attractive activity was observed for five compounds out of six, indicating that the males strictly distinguished the configurations of methyl and hydroxyl groups but inaccurately recognized the length of the two alkyl chains in the pheromone structure. Yamkawa *et al.* 2011. *Tetrahedron Lett.*, 52 , 5808.



(5*R*,7*R*)-**1**

HOST PLANT VOLATILES AND PREFERENCE IN A SPECIALIST AND GENERALIST PARASITOID: TO LEARN OR NOT TO LEARN?

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Herbivore feeding on plants induces the release of blends of volatile organic chemicals (VOCs). Parasitoids of these herbivores respond to VOCs and associative learning of these VOCs leads to improved foraging efficiency of host herbivores. We explored how parasitoids exploit learning of host herbivore-specific VOCs to perform better than naïve congeners, and investigated if preferences for host and/or non-host herbivore-induced plants varied over time. In olfactometer tests, experienced female *Cotesia glomerata* (a parasitoid of *Pieris rapae*) and *Diadegma semiclausum* (a parasitoid of *Plutella xylostella*) preferred plants which had been induced by their host herbivores over plants induced by non-hosts; naïve females did not discriminate between plants. Experienced females showed preferences for plants induced by both host and non-host herbivores over singly non-host-induced plants. This suggests that parasitoids may learn host-specific VOCs to locate hosts from within mixed infestations of host and non-host herbivores. However, experienced *C. glomerata* attraction to host-induced plants diminished by the second day after induction while experienced *D. semiclausum* attraction to host-induced plants lasted up to three days after induction. We hypothesize that it is beneficial for specialist parasitoids like *D. semiclausum* retain memory of host-specific VOCs to maximize foraging efficiency in heterogeneous environments, while generalist parasitoids like *C. glomerata* retain plasticity in the learning of host-specific VOCs to remain receptive to a broad range of hosts.

IDENTIFICATION AND VARIATIONS IN PHEROMONE-BINDING PROTEINS AMONG LEGUME POD BORER (*MARUCA VITRATA)* POPULATIONS FROM ASIA AND AFRICA

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Legume pod borer, *Maruca vitrata* (Lepidoptera: Pyralidae) can cause yield loss as high as 80% in food legumes in tropical Asia and Africa. Although growers primarily depend on pesticides to manage *M. vitrata*, control is difficult because the pest feeds concealed inside a webbed mass of leaves and flowers where pesticide sprays may not reach. Sex pheromones could be used as a monitoring and/or mass-trapping tool, but variations in the functional response of *M. vitrata* populations to sex pheromone blends have been observed. The current study was carried out to understand the differences in pheromone-binding proteins among *M. vitrata* male populations in Asia and Africa. A *de novo* transcriptome assembly that establishes a transcriptome without the aid of a reference genome was adopted to sequence the entire transcribed mRNAs in *M. vitrata* from Taiwan. The raw sequence data was assembled to compare with homologues in GenBank to detect *M. vitrata* pheromone-binding protein (MaviPBP). A 626 bp cDNA portion from *M. vitrata* was used to design the primer pair for PBP1, which amplified the full-length sequence of PBP1 with a partial signal peptide in *M. vitrata* populations from selected countries in Asia and Africa. A 742 bp cDNA portion from *M. vitrata* was used to design the primer for PBP2, which amplified the full-length sequence of PBP2. Both PBP1 and PBP2 sequences comprise three exons interspersed by two introns. Variations in PBP1 and PBP2 sequences among *M. vitrata* populations in Asia and Africa and their phylogenetic analysis will be discussed.

PROPIONATES AND ACETATES OF CHIRAL SECONDARY ALCOHOLS: NOVEL SEX PHEROMONE COMPONENTS PRODUCED BY A LICHEN MOTH *BARSINE EXPRESSA* (ARCTIIDAE: LITHOSIINAE)

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Females of a lichen moth, *Barsine expressa* (Arctiidae, Lithosiinae), which inhabit Iriomote Island in Japan, were captured by a black-light trap, and the pheromone gland extract was analyzed by GC-EAD and GC-MS. The females produced several EAG-active esters, and the mass spectrum of a major component indicated the mixture consists of propionates derived from C_{17}-saturated secondary alcohols, which were inseparable on the capillary GC column. In addition to these main components, the pheromone glands included two acetate derivatives of C_{17} alcohols, and other propionates of C_{16} and C_{15} alcohols. The crude extract was treated with K_2CO_3, and a 1:1 mixture of C_{17} alcohols with a C_6- or C_7-chain moiety was obtained. The two alcohols were uniformly converted into monodeuterated *n*-heptadecane by mesylation and succeeding $LiAlD_4$ reduction. This result revealed a straight-chain structure of the C_{17} alcohols with the acyl groups located at the 7- or 8-position. Field tests on Iriomote Island showed that the synthetic esters were behaviorally active. A 1:1 mixture of racemic 7-propioxyheptadecane and 8-propioxyheptadecane, which were prepared from the secondary alcohols synthesized by a Grignard coupling reaction, attracted male moths. Furthermore, propionates of the alcohols synthesized enantio-selectively by using a hydrolytic kinetic resolution with Jacobsen's catalyst were evaluated. Only the traps baited with a mixture of the two esters with the same *S* configuration significantly attracted *B. expressa* males. In the Tokyo area, the propionate mixture attracted a closely related species, *Barsine aberrans aberrans*.
Fujii et al. 2013. *J. Chem. Ecol.* 39, 28

PHEROMONE MASS TRAPPING FOR THE MANAGEMENT OF DISCRETE GENERATIONS OF THE LEPIDOPTERON PEST, *OPISINA ARENOSELLA* WALKER (LEPIDOPTERA: OECOPHORIDAE) IN COCONUT ECOSYSTEMS

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Opisina arenosella is a major defoliating coconut pest in India, Sri Lanka, Burma, Bangladesh and Thailand. The infested trees have a burnt-up appearance causing severe yield losses. The *O. arenosella* populations are spatially segregated with discrete generation cycles (5 generations/year) having the flight period of 35 to 40 days per generation. These interesting behavioural characteristics of pest make it susceptible for mass trapping. The sex pheromone released by the females was identified, artificially synthesized and field tested by the Bio-Control Research Laboratories (PCI), Bangalore. After standardisation of trap type, height of trap placement in the tree canopy and trap colour, an attempt was made to demonstrate the robustness of mass trapping technology. The study was conducted in 14.2 ha with 1700 infested coconut trees. Nearly 661 (I and II generation, respectively) and 836 (III generation) cross vane traps baited with lure (plastic vial with 100 mg synthetic sex pheromone ((Z,Z,Z)-3,6,9-tricosatriene) were uniformly installed in the study area. Totals of 73739, 52392 and 7953 moths were trapped in the I, II and III generations, respectively with the larval (2.97±0.63 (Mean±SD) larvae/leaflet before mass trapping) reduction of 34.27 (1.93±0.64 larvae/leaflet), 88.76 (0.12±0.37 larvae/leaflet) and 93.97 (0.09±0.03 larvae per leaf) percent in II, III and IV generations, respectively. Whereas in control plot (2.5 ha), the larval population was increased continuously up to III generation (1.83±0.22 to 5.27±2.12 larvae/leaflet in I and III generation, respectively) and reduced in the subsequent generations. Reduction in larval numbers was achieved in treated plot due to continuous trapping of male moths using the pheromone traps.

BEHAVIOURAL RESPONSES OF COFFEE WHITE STEM BORER (CWSB), *XYLOTRECHUS QUADRIPES* CHEVROLAT (COLEOPTERA: CERAMBYCIDAE) TO PLANT VOLATILES OF COFFEE

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Coffee white stem borer, *Xylotrechus quadripes* Chevrolat, preferentially attacks *Coffea arabica* while *Coffea robusta* is spared. Some preliminary studies indicated that there may be some volatiles in the bark of *C. arabica* that is attractive to the females for egg laying. To improve the efficiency of the sex pheromone trap in the field, an attempt was made to identify the plant volatiles and their synergistic effect with the pheromone. In two studies using electroantennogram (EAG), 30 combinations of CWSB pheromone ((S)-2-hydroxy-3-decanone) with six plant volatiles were presented to CWSB. In the first study, 30 test compounds along with standard pheromone were presented individually at one concentration to the CWSB virgin females. In the second study, the eight compounds that elicited higher responses in Study 1 were tested with virgin females, mated females and males of CWSB. Males and virgin females exhibited higher responses with the (E)-2-Hexenal and (E)-2-Hexenal:(S)-2-hydroxy-3-decanone (1:1), whereas there was no significant response by mated females. EAG results were confirmed in wind tunnel experiments. Virgin females responded more to these eight treatments compared to males and mated females. However, virgin females, mated females and males responded more to (E)-2-Hexenal: (S)-2-hydroxy-3-decanone (1:1) as 85%, 60% and 65%, respectively, in Wind tunnel. Field studies with the treatments of Study 2 are underway. Most of the test compounds elicited similar responses which suggest that several of the compounds may be used together by *X. quadripes* in habitat and/or host community location.

BIOSYNTHETIC PATHWAYS FOR C15-DIENAL SEX PHEROMONE IN *DOLBINA TANCREI*

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We surveyed the sex pheromones of fifteen hawk moths and found a novel sex pheromone component 9,11-pentadecadienal from *Dolbina tancrei*. Pheromone components with functional groups are most commonly made from even-carbon chain fatty acids such as C_{16} or C_{18} acids. Therefore odd-carbon chain pheromones may be synthesized by a unique biosynthetic pathway. Here we investigated the sex pheromone precursors of *D. tancrei* and propose a possible pathway. Common pheromone component in hawk moth such as 10,12-hexadecadienals are synthesized from C_{16} fatty acids through several modification steps. We postulated two pathways for the changes of *D. tancrei*; (1) C_{16} precursor conversion into C_{15} fatty acid, or (2) modification of the steps in biosynthesis of 10,12-hexadecadienal. To determine the precursors, we analyzed fatty acids in the pheromone gland extracts. Glycerolipids and free fatty acids from the extracts were converted to the corresponding methyl esters and analyzed by GC-MS and DMDS or MTAD derivatization. As a result, methyl hexadecanoate and methyl octadecanoate were the major components detected. In addition, methyl 7-hexadecenoate, methyl 9-hexadecenoate, methyl 11-hexadecenoate, methyl hexadecadienoate, methyl pentadecanoate, methyl 5-pendatecenoate and methyl pendadecadienoate were also detected. Although C_{15} fatty acids existed in the extracts, monoenyl C_{15} intermediates that can be pheromone precursors were not detected in the extracts. Moreover, the existence of 11-hexadecenoic and 10, 12-hexadecadienoic acids supports second postulated pathway. These results suggest that 9,11-pentadecadienal is biosynthesized from palmitic acid via 11-hexadecenoic and 10,12-hecadecadienoic acids rather than from C_{15} fatty acids, and predict a specific enzyme for 1C chain shortening.

ALLELIC SPECIFIC EXPRESSION IN RELATION TO *BOMBYX MORI* RESISTANCE TO Bt TOXIN Cry1Ab

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Allelic variation is an important genetic factor contributing to control gene expression by regulating allelic specific expression, resulting in quantitative trait variation. In contrast to induced mutations that eliminate or cause a large reduction of a functional gene product, allelic variation regulates gene expression and may be an underlying mechanism for developing resistance to Bt. First of all, we conducted insecticidal screening of 45 silkworm strains using Cry1Ab to separate resistance strains from susceptible stains. We found that P50 is a resistance strain and Dazao is a susceptible strain. Expression variation of 12 Bt resistance-related genes between two strains were revealed. Further, SNPs were detected based on ESTs database and were validated by allelic-specific PCR. Comparison of allelic specific expression between P50 and Dazao showed that the transcript levels of heterozygous genes containing two alleles rather than allelic imbalance expression contribute more to the resistance of P50 against Cry1Ab. Moreover, responses of allelic-specific expression in hybrid larvae to Cry1Ab were investigated. Our results demonstrated that *cis*-regulatory manner was involved in *ABC* and *APN3* allelic-specific expression in response to Cry1Ab.

NATURAL PRODUCTS AS ALTERNATIVE TO PYRETHROIDS CONTROL OF MALARIA VECTOR *ANOPHELES GAMBIAE*

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Laboratory and field studies have shown that pyrethroid-treated bednets reduce vector-human contact by disrupting mosquito behavior, thereby reducing the risk of malaria transmission. However, discovery of pyrethroid resistance in mosquito populations raises the issue of finding alternatives to these insecticides that will be viable malaria control options, especially in under-developed regions. The objectives of this study were to identify potential repellent and irritant compounds and to characterize their physiological mode of action. We first evaluated the repellent, irritant, and toxic effects of 20 plant extracts on *Anopheles gambiae* adults. The major compounds of the four most promising extracts were then evaluated singly on a pyrethroid-resistant and a susceptible strain of *An. gambiae*. This was accomplished via a high-throughput behavioral assay that permitted screening different concentrations of the compounds. DEET and permethrin were used as positive controls. Electroantennography was used to evaluate relative responses between different individual compounds and essential oils. Behavioral responses observed in bioassays with plant extracts were sometimes attributed to the effect of individual compounds in the extract. Generally, the behavioral response of *An. gambiae* was dose-dependent. In some cases, behavioral responses between irritant, repellent, or toxic effects were inconsistent, suggesting that the mechanisms mediating these behavioral effects may be different. The use of natural blends or single compounds derived from plant extracts for vector control will be discussed.

SYMPOSIUM TOPIC 17: CHEMICAL ECOLOGY (GENERAL TOPICS)

Topic Coordinators:
Myron Zalucki (University of Queensland, Australia) and **Alex IL'ichev** (Department of Environment and Primary Industries, Australia)

GUSTATORY RESPONSES OF *ANTHERAEA ASSAMENSIS* HELFER (LEPIDOPTERA: SATURNIIDAE) LARVAE

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The aim of this study was to identify and preliminary characterize physiologically sensory structures involved in feeding behavior of larvae of *Antheraea assamensis*, specifically for phagostimulants and deterrents. Electrophysiological characterisation of the medial and lateral sensilla styloconica reveal that they are sensitive to a wide group of chemical compounds including sugars and sugar alcohols, salts and deterrents. Of all the sugars tested, only sucrose and inositol evoked significant spike activity by sensilla on the galea and maxillary palpi of *A.assamensis.* Among the two galeal sensilla, sucrose sensitive cells were present on the lateral sensilla styloconica while inositol sensitive cells were present on the medial sensilla. Myo-inositol is one of the few nutrients that strongly stimulates the peripheral taste system of *A.assamensis*. Also, the inositol sensitive cell responds selectively to inositol while the sugar sensitive cell appears to respond selectively to sugars. Cells responding to sugars and inositol were also present on sensilla on the maxillary palpi. In addition, the medial sensillum on the galea as well as sensilla on the maxillary palpi contain cells that are sensitive to known deterrents such as caffeine and nicotine. In addition, morphological and electrophysiological characterisation of gustatory sensilla on the epipharynx of *A.assama* is a first report. Responses to the epipharyngeal sensilla were obtained with inositol, salicin and salts such as NaCl and KCl. Spike amplitudes suggest that there are 3 cells innervating each of these epipharyngeal sensilla, one of which responds to deterrents. Thus, feeding behavior of *A.assamensis* allows for the acceptance of different plants that is determined by the relative activity levels in the four chemosensory neurons in each of the sensilla styloconica present on the galea and the three neurons in the epipharynx.

ABNORMAL THICKENING OF THE PERITROPHIC MEMBRANE OF CATERPILLARS CAUSED BY CHITIN-BINDING DEFENSE PROTEIN MLX56 FROM THE LATEX OF MULBERRY TREES: A NOVEL MODE OF ACTION OF DEFENSE PROTEIN

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Plant latex plays important defensive roles against herbivorous insects. We recently identified a defense protein, MLX56, from the latex of mulberry trees (*Morus spp.*). MLX56 showed a strong growth inhibition against lepidopteran larvae even at 0.01 % concentration in artificial diets. The DNA sequence data showed that MLX56 has an extensin-like domain surrounded by two hevein-like chitin-binding domains. The modes of action of MLX56, however, remained unclear. Since MLX56 showed strong chitin-binding-activity and protease-resistance, we analyzed the effect of MLX56 on the peritrophic membrane (PM), a thin membrane that consists of chitin and proteins, in the midgut lumen of caterpillars. We observed abnormally thick gel-like membrane in the midgut of the Eri silkworms, *Samia ricini*, fed MLX56-containing diets. The thickness of the membrane reached 1/5 the diameter of midgut lumen, while the PM of the Eri silkworms fed the control diet was thin and soft. Biochemical analyses indicated that the major components of the thick membrane were MLX56 and chitin. When polyoxin AL, a chitin-synthesis-inhibitor was fed together with MLX56, the toxicity of MLX56 disappeared, while the PM became thinner and fragmented. These results suggested that MLX56 modified the PM into an abnormally thick membrane that inhibits the growth of caterpillars. Further modes of action of MLX56 will be discussed. Overall, MLX56 is a defense protein with novel modes of action never reported previously from other anti-herbivore defense proteins.

Konno. 2011. *Phytochemistry* 72, 1510-1530.
Wasano et al. 2009. *Phytochemistry* 70, 880-888.

ANALYSIS OF GLUCOSE CONJUGATES IN LEAF BEETLES AND THEIR HOST PLANTS

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During evolution, larvae of the leaf beetles Chrysomelina developed nine pairs of dorsal glands from which defensive secretions are released in case of disturbance. Precursors of the main compound in the secretions are either produced *de novo* or sequestered. *De novo* producers like the horseradish leaf beetle, *Phaedon cochleariae*, synthesize precursors for cyclic monoterpenes, such as chrysomelidial. An example for the sequestration strategy is the poplar leaf beetle *Chrysomela populi*, which transports the host plant specific glucoside salicin to its defensive glands and converts it to volatile salicylalhdehyde. Previous *in vitro* experiments performed on Chrysomelina larvae with selected thio-analog of O-*β*-D-glucosides suggested a model of a non-specific uptake of phenolic glucosides into the hemolymph of both sequestering and *de novo* producing beetles. However, identification of natural glucosides in hemolymph as well as diet of larvae is critical to complete the proposed model. Therefore, extracts of leaves and beetle hemolymph were subject to RP-HPLC-UV-APCI-MS. So far, up to 40 different glucosidic compounds could be detected in Salicaceae leaf samples while hemolymph samples show the defensive precursors as well as possible plant derived metabolites. These findings support the hypothesis of a non-specific uptake, indicating to a pool of glucose conjugates in the hemolymph, which could have favored the evolution of the sequestering strategy. Improvement of analysis and identification of the detected metabolites could promote understanding of the first steps in sequestration.

BEETLE JUICE STRATEGY: ABC TRANSPORTER FUNCTIONS AS A PACEMAKER FOR SEQUESTRATION OF PLANT GLUCOSIDES IN LEAF BEETLES

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Plant-herbivore interactions dominate the planet's terrestrial ecology. When it comes to host-plant specialization, insects in particular are among the most versatile evolutionary innovators, able to disarm and adapt to multiple chemical plant defenses. Sequestration is a widespread strategy to detoxify noxious metabolites, frequently for the insect's own benefit against predation. The leaf beetle larvae of the subtribe Chrysomelina possess a sophisticated network for sequestration of plant derived compounds from their hosts. Here we describe the ATP-binding cassette transporter *Cp*MRP identified in the poplar leaf beetle, *Chrysomela populi*, as the first example of a transport protein involved in the sequestration of phytochemicals in insects. *Cp*MRP is localized in the defensive glands from juveniles for shuttling of pre-filtered metabolites into defensive secretions. Silencing of *Cp*MRP *in vivo* creates a defenseless phenotype which indicates its key function also for the secretion itself. By using the *Xenopus laevis* oocyte expression system various plant glucosides could be verified via HPLC-MS to serve as substrates for *Cp*MRP *in vitro*. Our analyses of the transport activity suggest that involving a broad-spectrum carrier in the sequestration of phytochemicals provides insects with flexibility in competitive interactions in natural ecosystems. We anticipate that the molecular identification of key transporters will contribute to our understanding of plant-herbivore interactions and may influence future strategies in crop plant protection.
Discher et al. 2009. *ChemBioChem*, *10*, 2223-2229.

PHYLLOTRETA STRIOLATA FLEA BEETLES SELECTIVELY SEQUESTER AND HYDROLYZE GLUCOSINOLATES FROM THEIR HOST PLANTS

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Glucosinolates are characteristic secondary metabolites in plants of the order Capparales which form an activated defense system against herbivores together with the enzyme myrosinase. Tissue damage brings these two components together and leads to the formation of bioactive isothiocyanates which repel and/or harm non-adapted herbivores. Different strategies for detoxification of glucosinolates have been identified in Lepidoptera, Hemiptera, and Hymenoptera; however, how crucifer-feeding leaf beetles cope with this plant defense is not known. We focus on the striped flea beetle, *Phyllotreta striolata*, a devastating pest species of *Brassica* crops in Southeast Asia and North America**.** In previous studies we found that *P. striolata* adults are able to emit volatile alkenyl isothiocyanates. Therefore, we hypothesized that this specialist sequesters host plant glucosinolates and hydrolyzes them using a beetle myrosinase to release isothiocyanates. To test this, we performed feeding assays with host plants differing in their glucosinolate profile. The accumulation of plant glucosinolates in *P. striolata* adults was traced using HPLC. We found that the beetles sequestered alkenyl glucosinolates up to 1% of their body weight, but not aromatic glucosinolates. The presence of a beetle myrosinase was verified and the enzyme was partially purified. By combining proteomics and transcriptomics we were able to clone and heterologously express the gene responsible for the myrosinase activity in *P. striolata*. These results indicate that *P. striolata* efficiently prevent glucosinolate hydrolysis by the plant myrosinase during feeding while possibly using controlled release of isothiocyanates for their own defense.

ELUCIDATION OF ELICITORS IN *SOGATELLA FURCIFERA* (HORVÁTH) CAUSING THE JAPONICA RICE PLANT (*ORYZA SATIVA* L.) VARIETIES TO INDUCE PRODUCTION OF THE OVICIDAL SUBSTANCE AGAINST THE EGG OF *S. FURCIFERA*

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Some Japonica rice plant varieties show a resistance against whitebacked planthopper (*Sogatella furcifera*), which is characterized by formation of watery lesions and production of an ovicidal active compound, benzyl benzoate (BB), when she lays her eggs in the rice varieties. We have reported that the production of BB by the rice plant varieties is induced by elicitors in only female *S. furucifera* and that the elicitors are phospholipids and their structures are 1,2-dilinoleoyl-*sn*-glycero-3-phosphatidylcholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, 1-palmitoyl-2-oleoyl-*X*-glycero-3-phosphatidylethanolamine and 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine, respectively. In this paper, in addition to these structures, 1,2-dilinoleoyl-*sn*-glycero-3-phosphatidyethanolamine, 1-stearoyl-2-linoleoyl-*X*-glycero-3-phosphatidylethanolamine, and 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine and 1,2-dioleoyl-3-palmitoyl-*X*-glycerol and triolein were isolated and identified as active elicitors in female *S. furucifera*. Authentic fatty acid and fatty acid methyl ester also induced the production of BB as well as the phospholipids. These results clearly indicated that the active site causing the *Japonica* varieties to induce production of the BB is not phosphorus moieties but fatty acid moieties.

OSHI-WRKY TRANSCRIPTION FACTOR REGULATES HERBIVORE-INDUCED SIGNALING AND DEFENSE RESPONSES IN RICE

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Plant WRKY transcription factors play an important role in protecting plants from damage by a suite of biotic and abiotic stresses. However, little is known about the role of WRKYs in mediating plant defense against herbivores. To explore these issues, we cloned a WRKY gene of rice (*Oryza sativa*), *OsHI-WRKY*, whose expression levels were rapidly and strongly enhanced when plants were wounded, infested by rice brown planthopper (BPH) *Nilaparvata lugens* or rice striped stem borer (SSB) *Chilo suppressalis*, and treated with jasmonic acid (JA) or ethylene (ET). *OsHI-WRKY* interacted with *MPK3/6* in vitro and in vivo. Silencing and over-expression of *OsHI-WRKY* revealed that it negatively regulates the herbivore-induced levels of jasmonic acid (JA), ethylene and trypsin protease inhibitor (TrypPI). Consistent with these data, *OsHI-WRKY* increased susceptibility in rice to SSB. In contrast, *OsHI-WRKY* was found to mediate the resistance of rice to BPH, possibly by increasing salicylic acid (SA) and H_2O_2 accumulation. Taken together, these results indicate that *OsHI-WRKY* is an important transcription factor, which controls herbivore resistance in rice by regulating JA, SA, ethylene and H_2O_2 signaling pathways.

POTENCY OF "FRASS AND PLANT VOLATILE" TO ENHANCE THE BIO-EFFICACY OF *DIADEGMA SEMICLAUSUM* HELLEN. AGAINST DIAMOND BACK MOTH, *PLUTELLA XYLOSTELLA* L.

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The diamondback moth (DBM), *Plutella xylostella* is one of the most important pests of brassicas worldwide. Intensive insecticide applications has lead to resistance in *P. xylostella.* This is leading to interest in finding an alternative controlling method beside insecticides, such as the use of biological control (parasitoid and predator). *Diadegma semiclausum* has been used in an integrated pest management (IPM) program to control DBM in cabbage in Indonesia. *D. semiclausum* is highly attracted to the DBM larval frass and the green leaf volatiles of cruciferae family in a Y-tube olfactometer. The aim of this research was to investigate the role of frass and plant volatiles from cruciferaes as single volatile and mixtures on the rate of parasitism of *D. semiclausum*. The laboratory result indicated that orientation of *D. semiclausum* females toward frass was significantly greater than toward plant volatiles. The frass odor was more attractive to parasitoids than plant volatile. However, when the plant volatiles mixtures were used, parasitoids preferred the volatile mixtures of cauliflower and Chinese cabagge more than the mixtures of broccoli and cabbage. Plant volatiles from broccoli, Chinese cabbage, cabbage and cauliflower at 25-50% concentrations increased the rate of *D. semiclausum* parasitism up to 80-100%. *D. semiclausum* preferred frass at 0.5-2.5 g concentrations and 10^{-5}-10^{-1} with increased rate of parasitism 80–120% and 132-250%, respectively. The results demonstrated that the plant volatiles and frass of larva *P. xylostella* played an important role in the foraging behavior of *D. semiclausum*.

HERBIVORE-INDUCED LEUCINE-DERIVED NITRILE IN THE EVENING PRIMROSE ATTRACTS THE PREDATORY SHIELD BUG

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Plants emit a series of characteristic volatile blends when damaged by herbivores. These herbivore-induced volatiles are known to either attract natural enemies of the herbivores or induce defense responses of other plants in the vicinity, and thus the volatiles play an important role in plant defense against herbivores. We have found an unique herbivore-induced volatile, isovaleronitrile, together with its corresponding aldoxime and some terpenes from the leaves of the evening primrose, *Oenothera* sp. (Onagraceae), either infested by the leaf beetle, *Altica* sp. (Coleoptera: Chrysomelidae), or treated with a cellular signaling molecule, methyl jasmonate (MeJA). We have also determined that isovaleronitrile was synthesized from leucine in the *Oenothera* leaves. The predatory shield bug, *Zicrona caerulea* (Heteroptera: Pentatomidae) is often found on the *Oenothera* leaves infested by the leaf beetle around crop fields in Akita City, Japan. We hypothesized that *Z*. *caerulea* could utilize the herbivore-induced volatiles as a cue to explore its preys, and we investigated the attractant activities of the herbivore-induced volatiles, MeJA-induced volatiles and isovaleronitrile using a two-choice tests. *Zicrona caerulea* showed a significant preference for the volatiles from the *Oenothera* leaves infested by the beetle or treated with MeJA compared to those of intact leaves. Our behavioral assay also revealed that isovaleronitrile functions as an attractant for *Z*. *caerulea*. *Zicrona caerulea* is a potent natural enemy of a wide variety of insect pests including several lepidopteran larvae. Our findings could be helpful to understand the behavioral manipulation of *Z*. *caerulea* between crop fields and their surrounding areas.

EVIDENCE FOR AGGREGATION CONTROL BY CUTICULAR HYDROCARBONS IN THE EUROPEAN EARWIG, FORFICULA AURICULARIA

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The European earwig, *Forficula auricularia* is a pestiferous insect, that has been introduced to most temperate regions of the world. Despite several attempts, the aggregation pheromone components used by *F. auricularia* remain unresolved. Furthermore, earwig population studies have shown that earwig aggregation sizes (trap catches) begin to decline soon after the imaginal moult. We attempted to isolate the aggregation pheromone used by *F. auricularia* and develop a synthetic pheromone blend for use in agricultural and urban pest management. Laboratory and field-based bioassays showed that earwig-exposed substrates are attractive to conspecifics. Solvent washes of earwig cuticles yield a total of 51 saturated, unsaturated and methyl-branched hydrocarbons (HC). Analysis of earwig-exposed substrates in both laboratory and field scenarios using GC-MS yielded numerous HCs. Field-based bioassays assessing a blend of four unsaturated HCs elicited significant behavioural responses on several occasions. However, these responses were not consistently observed. Sequential sampling of cuticular HCs from earwigs while simultaneously monitoring field populations over a five month field season demonstrated the production of the cuticular HCs fluctuates over time in adults. Moreover, the unsaturated HCs that elicited behavioural responses were shown to decline over time with this decline significantly correlating with the decline in earwig trap catches. We provide first evidence that *F. auricularia* utilise a suite of unsaturated cuticular HCs to mediate earwig aggregations and that these HCs decline after the imaginal moult, which initiates dispersal in earwig field populations prior to over-wintering.

REVEALING THE LOVE DART ALLOHORMONE: THE MOLECULAR BASIS FOR PATERNITY SUCCESS IN HELICID SNAILS

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Helicid snails undergo a unique sexual ritual that involves the shooting of a mucus-coated calcareous "love dart" into their mating partners. While seemingly painful, it appears to be a key mechanism to transfer an allohormone that increases paternity success. Once in the haemolymph, this allohormone evokes contraction of the female reproductive organs, particularly the copulatory canal and bursa tract. These contractions close the entrance to the bursa tract, which facilitates sperm transport to the fertilization pouch. This study investigated the identity of the garden snail love dart allohormone. Mucous gland peptides were isolated and separated by RP-HPLC. Fractions showing contractile activity on the copulatory canal were further analysed by MALDI-TOF/TOF, revealing the allohormone sequence. Other proteins and peptides identified within the mucous gland include HSP70, bursicon, pedal peptide, FFamide and an insulin-related peptide. A synthetic love dart allohormone peptide was generated and tested for copulatory canal contractile activity (10^{-3}-10^{-12} M). The full-length allohormone gene was then identified by transcriptome analysis; consisting of a mature prepropeptide harboring a signal peptide that is subsequently cleaved to release the active allohormone LoDA. The spatial expression of the allohormone gene and protein was investigated using RT-PCR, *in situ* hybridisation and immunolocalisation. A comparative analysis was performed with the Mediterranean snail *Theba pisana*. This is the first identification of an allohormone in molluscs which has important consequences to understanding animal biology and evolution of reproductive competition. It may also lead to the development of novel contraceptives that could be used to eradicate pest molluscs.

COMPARISONS OF FAMILIAL AND GENDER SPECIFIC DIFFERENCES IN CUTICULAR HYDROCARBONS IN TWO SPECIES OF AUSTRALIAN PSYLLOID (HEMIPTERA: PSYLLOIDEA)

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Psyllids are diverse, abundant and functionally significant in Australian ecosystems. Despite being such an iconic faunal component, our biological knowledge is confined to taxonomic descriptions. *Ctenarytaina bipartita* (Psyllidae) is a newly described species host-specific for at least two species of *Eucalyptus* (Myrtaceae). *Aacanthocnema dobsoni* (Triozidae) is specific for only one host plant, namely *Allocasuarina verticillata* (Casuarinaceae). Both species are multivoltine; *A. dobsoni* occurs in very large numbers whereas populations of *C. bipartita* are limited by the availability of apical buds. Volatile sex pheromones are known from species of Psyllidae and both families are known to use vibrational signalling to attract mates, yet the possibility that cuticular hydrocarbons might provide close range/contact cues for mate recognition has not been addressed. We studied the cuticular hydrocarbons of these Australian psyllid species to determine whether they play a role in gender recognition. Using GC-MS and a solid sampler injector, cuticular hydrocarbons of individual males and females of each species were identified. Both species exhibited hydrocarbons ranging from C23-C33, but there was a clear higher abundance of hydrocarbons in *A. dobsoni* compared to *C. bipartita*. *A. dobsoni* males had a much richer hydrocarbon profile compared to females, dominated by a series of 2-Methylalkanes ($2MC_{25}$-$2MeC_{28}$). Females contained $3MeC_{27}$ and $5,9diMeC_{27}$ in much higher proportions. In contrast, only smaller differences between *C. bipartita* males and females were observed. Further differences between males and females (mated and virgin) will be presented and discussed regarding their possible role in multimodal signaling in psyllids.

ECO-PHYSIOLOGICAL CONSEQUENCES OF UV-B ON BRASSICACEAE - IMPACT ON THE CO-EVOLUTIONARY ARMS RACE BETWEEN PLANTS AND THEIR ENEMIES

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UV-B mediated changes to defense secondary metabolites in Brassicaceae against insects and pathogens were investigated. Ecologically relevant low to moderate UV-B doses (0.6 – 1.5 kJ m^{-2} d^{-1}) were applied to sprouts of the crop *Broccoli oleracea* var. *italica* (broccoli) and the model plant *Arabidopsis thaliana* and elicited especially the accumulation of 4-methoxy-indol-3-yl methyl glucosinolate and 4-methylsulfinylbutyl glucosinolate. Furthermore in *A. thaliana*, UV-B induced pronounced increases in levels of the phytoalexin camalexin. Also the aphid *Brevicoryne brassicae* induced a plant response similar to pathogens and especially camalexin and 4-methoxy-indol-3-yl methyl glucosinolate accumulated. Contrary to the observed accumulation of defense metabolites, pre-exposure of *A. thaliana* plants and broccoli sprouts to 1.1 kJ m^{-2} d^{-1} UV-B resulted in a higher susceptibility to pathogen infection with *Alternaria brassicicola* and *Botrytis cinerea*. In line with higher levels of 4-methoxy-indol-3-ylmethyl glucosinolate and camalexin, population growth of the specialist aphid *B. brassicae* was lower on plants pre-exposed to UV-B. The UV-B induced accumulation of camalexin in *A. thaliana* plants was about ten-fold higher in co-treatments with *B. brassicae* and *A. brassicicola* indicating a hypersensitive plant response. Transcriptional responses were compared to the accumulation of secondary metabolites in broccoli sprouts and *A. thaliana* and showed increased transcript abundance of genes associated with salicylate and jasmonic acid signaling pathways. Furthermore, the *Brassica* microarray data revealed a remarkable UV-B mediated induction of potential orthologs of camalexin biosynthetic genes from *A. thaliana*. In summary, UV-B mediated plant responses show some similarities with responses induced by biotroph pathogens and *B. brassicae.*

BIOACTIVE FRACTIONS CONTAINING MALE SEX PHEROMONE COMPONENTS IN THE PESTIFEROUS FRUIT FLY, *BACTROCERA CARAMBOLAE* AND MELON FLY, *B. CUCURBITAE*

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Carambola fruit fly (CFF), *Bactrocera carambolae*; and melon fly, *B. cucurbitae,* are important fruit pests. Male flies are known to be strongly attracted to two different groups of male attractants - CFF to methyl eugenol (ME), and melon fly to raspberry ketone (RK), which are being used in male annihilation and quarantine detection. After pharmacophagy, ME is biotransformed to *(E)*-coniferyl alcohol (CF), a booster component of male sex pheromone in CFF*;* whilst RK was sequestered unchanged as one of male sex pheromonal components in the melon fly. The objective of this work was to investigate the presence of either CF or RK in the haemolymph, responsible for transporting the sex pheromonal component to the rectal gland for temporary storage prior to emission, of male *B. carambolae* and *B. cucurbitae*. Initial gas chromatographic analyses revealed that CF and RK were detected in the haemolymph of ME-fed CFF and RK-fed *B. cucurbitae*, respectively. The quantity of CF and RK in the rectal gland rapidly increased over time from initial feeding to peak at 2- and 1-day post-feeding in CFF and melon fly, respectively. However, there was only a small and gradual change in the quantities of CF or RK in the haemolymph. Interestingly, column chromatographic separations followed by biodetection using live males revealed the presence of specific bioactive fractions that corresponded to different range of molecular masses in both the *Bactrocera* species. These results and their implications in relation to that known about the oriental fruit fly, *B. dorsalis* will be discussed.

RNA INTERFERENCE OF *P450 CYP6CM1* GENE HAS DIFFERENT EFFICACY IN TWO BIOTYPES OF *BEMISIA TABACI*

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Cytochrome P450 monooxygenses are believed to be responsible for detoxifying plant defensive metabolites and have been proven to be associated with high resistance to neonicotinoid class of insecticides in *Bemisia tabaci* B and Q biotypes. In this study, experiments on RNA interference (RNAi) of *P450 CYP6CM1* gene were conducted on *B. tabaci* B and Q biotypes with a goal to compare the RNAi effects on these two biotypes and thereby to provide basis for potential RNAi application in management of this pest. Double-stranded RNAs (dsRNA) of *P450 CYP6CM1* genes corresponding to *B. tabaci* B and Q biotypes were synthesized using specific primers and introduced into the insect body through feeding. The RNAi effects on the target gene expression and the efficacy of B and Q biotypes were compared, and responses of two biotypes, after fed with dsRNA, to plant metabolites, including nicotine and gossypol, and neonicotinoid insecticide imidacloprid, were investigated. The results showed that feeding dsRNA significantly silenced the target genes and led to the high mortality in both biotypes. The RNAi caused mortality in biotype B (up to 77.99%) was higher than that in biotype Q (up to 46.40%). Feeding dsRNA inhibited significantly ability in detoxifying plant metabolites and imidacloprid in both biotypes, more effective for biotype B than for biotype Q. In conclusion, interference of *P450 CYP6CM1* decreased gene expression, increased mortality, inhibited ability to detoxify exotic chemicals in both biotypes of *B. tabaci,* with better efficacy in biotype B than in biotype Q.

SEMIOCHEMICALS OF THREE *TOMICUS* SPECIES ATTACKING *PINUS YUNNANENSIS* FRANCH

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Tomicus yunnanensis, *T. minor* and *T. brevipilosus* are serous wood-boring forest pests and they colonize *Pinus yunnanensis* together in southwestern China. Through bolt bundle attraction test, analyzing of host volatiles and pheromones in hind gut of different attacking phases, EAG and field test, the effective attractants for three species were developed. The attractant for *T. minor* is obviously better than that developed in Europe. Compared to Sweden, the most attractive pheromone isomer is different in Yunnan China. The analyses show that 1 monoturpene and 3 pheromones were the common active semiochemicals for the three species but they were more attractive to *T. minor* than to the other two species. Another monoturpene was more attractive to male *T. brevipilosus*. Further researches are needed to verify the detail of difference of attractive semiochemicals among these three species and the role of semiochemicals in the coexistence and competition of the three species.

CHEMICALS IN RICE PLANT RELATING HOST SELECTION FOR THE GREEN RICE LEAFHOPPER, *NEPHOTETTIX NIGROPICTUS*

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The feeding physiology of the planthoppers and leafhoppers has been clarified to consist of two phases; probing and sucking. These processes are controlled by physical and chemical factors in rice plants. However the elucidation of “the chemicals” for probing by green leafhopper, *N. nigropictus,* is still underway. We attempted to confirm the chemical factor in rice plant related to probing behaviour by *N. nigropictus*. The adult females of *N. nigropictus* were fed on 2 g fresh rice plant of aqueous extract containing 2% sucrose through a parafilm membrane. The result indicates that the extract had highly probing stimulant activities for this insect species. Moreover, the aqueous extract was separated on an ODS middle column to get ODS water, 20%, 40% and 100% MeOH fractions. Of these four fractions, the ODS 40% fraction was the most active. Bioassays were further conducted on the individual compounds as well as their various combinations in the ODS 40% fraction. The highest probing response similar to that of ODS 40% fraction recovered only when at least four compounds were combined. The structures of four compounds, were determined as peak A (Isoorientin 2″-*O*-glucoside), peak 7 {Isoscoparin 2″-*O*-(6‴-(*E*)-feruloyl)glucoside}, peak 8-1 {Isoscoparin 2″-O-(6‴-p-(*E*)-coumaroyl)glucoside}, peak 8-2 {Isovitexin 2″-*O*-(6‴-(*E*)-feruloyl)glucoside} by using NMR and MS spectra, respectively.

MULTITROPHIC INTERACTIONS BETWEEN APHIDS, LADYBIRD BEETLES AND THEIR PARASITOIDS MEDIATED BY PHYTOCHEMICALS IN A NATURAL AND MANAGED PLANT SYSTEMS

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The study was carried out during 2010 and 2011 to examine multitrophic interactions between aphids, ladybird beetles and their parasitoids in a natural and managed plant systems. The host plants included calotropis (*Calotropis procera* L.), Safflower (*Carthomus tinctorius* Lin.), Mustard (*Brassica compastris* Lin.) and Barley (*Hordeum vulgare* Lin.). The results showed that natural mortality of ladybird beetle larvae collected from different hosts due to diseases and unknown reasons ranged between 25.42±11.34 to 30.00±11.92%. The larval mortality caused by larval parasitoid, *Homalotylus flaminus (*Hymenoptera: Encyrtidae) was observed from 5.0±2.16 to 7.50±2.50% in barley and calotropis, respectively. The ladybird beetle pupal mortality caused by pupal parasitoid, *Oomyzus scaposus* (Tilomson) (Hymenoptera: Eulophidae) ranged between 9.58±1.98 to 17.08% on safflower and barley, respectively. Only one adult parasitoid, *Dinocampus coccinellae* Schrank (Hymenoptera: Braconidae) was recorded on coccinellid larvae collected from safflower crop. The number of parasitoids emerging from single host larvae varied from 3.33±0.14 emerging from larvae collected on barley to 4.33±0.12 on safflower. The weight of coccinellid pupae collected from different host plants varied from 6.06±5.74 to 11.20±0.90 mg/pupa. The number of pupal parasitoids from each pupa also varied from 11.54±1.23 to 28.97±2.08 parasitoids per pupa recorded on calotropis and mustard plants, respectively. The population of *Minochilus sexmaculatus* was significantly higher on calotropis compared with other host plants. The population of *Coccinella septeumpunctata* was significantly higher on mustard. The highest population of *Coccinella undecipunctata* was recorded on safflower, while the highest population of *Brumus suturalis* was observed on barley.

RE-EVALUATION OF COMPOUNDS TOXIC TO THE MULTICOLORED ASIAN LADYBIRD BEETLE, IN THE COWPEA APHID INFESTING THE BLACK LOCUST

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When the cowpea aphid, *Aphis craccivora*, infests the black locust, *Robinia pseudoacacia*, it is toxic to the multicolored Asian ladybird beetle, *Harmonia axyridis*, whereas the same aphid species infesting the common vetch, *Vicia angustifolia*, is suitable prey for *H. axyridis* larvae. Although previous reports indicated that the toxicity of *A. craccivora* infesting *R. pseudoacacia* was due to canavanine and 2-aminoethanol, the toxicity of these compounds and their concentrations in the aphids remained unclear. In the present study, we measured the concentrations of cyanamide, canavanine, and 2-aminoethanol in *A. craccivora* infesting the two host plants. In the extracts of *A. craccivora* infesting either host plant, canavanine was undetectable and 2-aminoethanol was detected at concentrations of 3.0–4.0 μg/g fresh weight. Cyanamide was detected in the extract of *A. craccivora* infesting *R. pseudoacacia* (7.7 μg/g fresh weight) but not in that infesting *V. angustifolia*. To evaluate the toxicity of canavanine, 2-aminoethanol, and cyanamide to *H. axyridis* larvae, we performed a bioassay using artificial diet containing these compounds at various concentrations. Cyanamide exhibited 10–100 times stronger toxicity than canavanine and 2-aminoethanol. These results indicate that the toxicity of *A. craccivora* infesting *R. pseudoacacia* is due, at least in part, to cyanamide, which is present when the aphid consumes this host plant but absent when it infests *V. angustifolia*.

SEX PHEROMONE COMPONENTS AND FIELD TRAPPING TESTS OF A CLEARWING MOTH *GLOSSOSPHECIA ROMANOVI* (LEPIDOPTERA: SESIIDAE)

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A clearwing moth, *Glossosphecia* (formally *Toleria*) *romanovi* (Lepidoptera: Sesiidae), is a harmful pest of vine trees in Japan. The larvae of this species bore into the trunk of vine trees and cause serious damage. This species had not attracted much attention from the viticulturist; however, complaints about its damage were increasing in recent 10 years. Analysis of pheromone gland extracts using GC-EAD and GC-MS showed that the virgin females produced several compounds; such as (3*Z*,13*Z*)-3,13-octadecadien-1-ol (Z3,Z13-18:OH, **I**), the acetate derivative (Z3,Z13-18:OAc, **II**), and the geometrical isomer (E3,Z13-18:OH, **III**). A mixing ratio of **I**:**II**:**III** was about 100:5:1. In the field, the males were effectively attracted by lures baited with various mixtures of the two major components (**I** and **II**), but additional mixing of **III** significantly reduced the attraction. When the traps were placed at 0.5 - 4.0 m high from the ground, the males were effectively caught by the traps at the higher positions. While this species is univoltine, two different periods were recorded for the male attraction. The first period was the rainy season and the second period began after the rainy season. In the first period, small numbers of males were attracted to traps set at various environments including vineyard. On the contrary, large numbers of males were attracted to traps mainly set at coppices in the second period. These results suggest that the emergence in vineyard started from the end of May and the captured males mainly derived from coppices near vineyards.

ANT TRAIL PHEROMONE BIOSYNTHESIS IS TRIGGERED BY A NEUROPEPTIDE HORMONE

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Insect pheromones play a critical role in communication, and great progress has been made during the past half century. Neuropeptide regulation of pheromone biosynthesis is well understood for the sex pheromone of lepidopteran moths. However, over 22 years after the first pheromone biosynthesis activating neuropeptide (PBAN), was identified, extension of this pheromone biosynthesis mechanism to other insect groups (= Orders) or types of behavior has not happened. We report that PBAN stimulates trail pheromone biosynthesis in the fire ant, *Solenopsis invicta*. Biosynthesis of trail pheromone was induced by injection of PBAN peptide into worker ants. We identified, characterized and functionally expressed the G-protein-coupled PBAN receptor for the fire ant from the Dufour's gland, which is the source of the trail pheromone. RNAi knock down of PBAN or the PBAN receptor inhibited trail pheromone biosynthesis. Our research has extended the insect target, behavioral function, and pheromone class biosynthesized. We anticipate that this work will stimulate resurgence in research related to neuropeptide regulation of insect pheromone biosynthesis.

IDENTIFICATION OF LEAFHOPPER-INDUCED TEA PLANT VOLATILES AND THEIR ATTRACTION TO PARASITIC MYMARID WASPS

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In order to investigate potential effect of tea plant volatiles on the host finding behaviour of mymarid wasps (*Stethynium empoascae* Subba Rao and *Schizophragma parvula* Ogloblin), the egg parasitoids of the tea green leafhopper, volatiles from the fresh excised tea shoots (ETS), excised leafhopper-feeding damaged tea shoots (ELFDTS), excised tea tender stems (ETTS) and excised ovipositing-injured tea tender stems (EOITTS) were respectively collected. The volatile compounds from these samples were qualitatively and quantitatively analysed by gas chromatography-mass spectrometry (GC-MS), and were assayed in both lab and field on the mymarid wasps. Our GC-MS analyses detected 13 and 18 volatile compounds from ETS and ELFDTS, respectively. The content of (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate and (*Z*)-butanoic acid-3-hexenyl ester in ELFDTS samples seemed to be significantly higher than those in ETS samples; and following compounds, hexanal, (*Z*)-2-hexen-1-ol, acetic acid-hexyl ester, linalool oxide (*Z*)-furanoid, squalene, (*E*, *E*)-*α*-farnesene and (*E*)-*β*–myrcene, benzaldehyde and phenylethyl alcohol, were detected from ELFDTS, but not from the ETS. The volatiles from ETTS were similar to those from the ETS, and ovipositions on the tea stems by the leafhoppers resulted in the release of (*E*, *E*)-*α*-farnesene, benzaldehyde and (*Z*)-butanoic acid-3-hexenyl ester from the EOITTS. (*E*, *E*)-*α*-farnesene, (*E*)-*β*-farnesene, benzaldehyde, linalool, (*E*)-2-hexenal and methyl salicylate at the dosage of 10^{-4} g/mL, displayed significant attraction to the mymarid *St. empoascae*, with (*E*, *E*)-*α*-farnesene being strongest. These synthetic individual compounds, and their partial mixtures, also showed obvious attraction to the mymarid wasps, *St. empoascae* and *Sc. parvula* in the field trapping experiments.

CHARACTERISATION OF *APIS CERANA* AND *APIS MELLIFERA* EPICUTICULAR HYDROCARBONS AND THEIR ROLE IN *VARROA DESTRUCTOR* ORIENTATION BEHAVIOUR

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During the last three decades the parasitic mite *Varroa destructor* has become present world-wide, heavily affecting honey bee populations, honey production and beekeeping. In contrast, its original host the Asian bee *Apis cerana* has long coexisted with the mite, making it well adapted to the parasite. Previous studies demonstrated that Varroa mite uses the honeybee epicuticular hydrocarbon composition for a proper selection of the most suitable host stage to infest. During both phoretic and reproductive stages, Varroa mite discriminates between ages and tasks of the adult bees using host epi-cuticular hydrocarbon patterns, which enables it to choose the suitable host. However, studies on the epicuticular hydrocarbon profiles of *A. cerana* and its role in Varroa mite orientation behaviour has not been done yet. In this work, we aimed to characterize the epi-cuticular hydrocarbon patterns in the Asian honey bee *Apis cerana* and its role in Varroa mite behaviour. Firstly, adult honey bees of both species were collected in Chiang Mai, Thailand where the Asian honey bee is a native inhabitant and the European honey bees are commercially reared. Subsequently, the epicuticular hydrocarbons were extracted and characterized by gas chromatography - mass spectrometry (GC-MS). The results show differences among the profiles of both species which may highlight the diverse ecological aspects between parasitisation of *A. mellifera* and *A. cerana*.

INFLUENCE OF PLANT EXTRACTS ON DEVELOPMENT AND REPRODUCTIVE BIOACTIVITIES OF *DYSDERCUS KOENIGII*

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Bioefficacy of leaf extracts of *Lantana camara, Ocimum sanctum* and *Catharanthus roseus* in development and reproductive behaviour of *Dysdercus koenigii* was evaluated. Fifth instar nymphs of *Dysdercus* were treated with ethanol extract and hexane extract prepared from leaves of the plant tested. *Dysdercus* exhibited significant alteration in their survival, longevity, development and reproduction when treated with ethanol extract. A significantly higher mortality was observed in the nymphs and adults at higher dose of the extracts. When treated with lower concentrations *Dysdercus* exhibited delayed moulting, abnormal moulting, and formation of supranumery instars and adultoid. The adults emerged from the treated fifth instar nymph were characterized by wing deformities, malformed proboscis and shrunken body. The developmental aberrations were dose related and also the effects were more pronounced when the extracts were applied to newly emerged fifth instar. The ethanol extract of the plants also affected the reproductive bioactivities of *Dysdercus*. This resulted in reduction in the number of egg batches, number of eggs in each egg batch and total number of eggs laid by the treated female. More than 50% of eggs laid by treated females were sterile. The hexane extract of the plants impaired the mating behaviour of *Dysdercus*. Consequently, treated males showed decreased sexual activity and altered courtship behaviour. This resulted in delayed mating and decreased mating success. Possible roles of JH mimicking molecules and other phytochemicals present in *Lantana, Ocimum* and *Cathranthus* in defence mechanism against *Dysdercus* are discussed.

IDENTIFICATION OF ESTRUS-INDICATING CHEMO-SIGNALS IN WATER BUFFALO (*BUBALUS BUBALIS*): A NEW APPROACH IN DEVELOPING KIT TO ASCERTAIN ESTRUS

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The detection of estrus is one of the major problems in buffalo reproduction since it is a silent-heat animal in which there is no outwardly manifested sign of estrus. Realizing this problem, there are several attempts to devise methods to overcome estrus detection in buffaloes, but most of the methods developed have not yet been successful and reliable. Chemo-signals are among the reliable source for estrus detection in mammals; hence, there is search for chemo-signals that would be effective indicators of estrus. We analysed the chemical cues in urine, faeces and vaginal mucus during the estrus cycle in buffalo to explore estrus-specific volatile compounds adopting bull behaviour assay. The samples were collected at three phases of the estrus cycle (i.e., proestrus, estrus and postestrus) and subjected to gas chromatography–mass spectrometry analyses. In urine, 1-chlorooctane, 4-methylphenol and 9-octadecenoic acid; in faeces, 4-methyl phenol and trans-verbenol; and in vaginal fluid, 9-octadecenoic acid were found be estrus indicator. The presence of 4methyl phenol during estrus from the synchronized buffalo is noteworthy. Thus, 4-methylphenol emerges as a common metabolic product excreted for the purpose of chemical communication between buffaloes. Behavioral investigation showed that estrus-specific volatile compounds appear to be sex pheromones which initiate bull's reproductive behaviour. We conclude that the estrus indicating chemosignals in urine, faeces and vaginal mucus would be better estrus marker in buffalo. It is underway to use those chemical cues for developing estrus detection kit in order to enhance the success rate of conception through artificial insemination.

SYMPOSIUM TOPIC 18: INSECT SEMIOCHEMICAL AND PHEROMONE REGISTRATION: POLICY AND ISSUES (WORKSHOP)

Topic Coordinators:
David Williams (Department of Environment and Primary Industries, Australia) and **Max Suckling** (Institute of Plant and Food Research, New Zealand)

PHEROMONE REGISTRATION – WHY IS IT SO HARD?

David Maxwell SUCKLING[1]

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Insect pheromones have revolutionised surveillance and pest management of many moth species, and more than 1,500 moth sex pheromones are now known. Registration of straight chained lepidopteran sex pheromones remains surprisingly challenging in a number of jurisdictions, despite the lack of evidence of risk to humans or the environment, decades after these products began to be used for insect monitoring and control. In New Zealand, the use of group standards for products of similar risk to each other (cosmetics, paints etc.) is one strategy being employed to reduce the regulatory burden. Internationally, the development of more supportive regulations for registration and use of such low risk semiochemicals is essential to realise their tremendous potential to offer benefits to mankind. Boyd Wilson et al. 2012. *New Zealand Plant Protection* 65, 274-280.

REGULATION OF LOW RISK CHEMICALS – A CONUNDRUM FOR REGULATORY POLICY AND PRACTICE

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[1]*The Australian Pesticides and Veterinary Medicines Authority, Australia*
[2]*The Australian Government Department of Agriculture, Fisheries and Forestry, Australia*

Reducing the burden of regulation on low risk agricultural and veterinary chemicals (agvet) is a priority issue for the Australian Government Department of Agriculture, Fisheries and Forestry and the Australian Pesticides and Veterinary Medicines Authority, the national agency responsible for the regulation of agvet chemicals. However, determining, in a low cost manner, which chemicals can be considered low risk is a conundrum that needs to be resolved before regulation of a range of agvet chemicals like insect pheromones can be significantly simplified. Various alternative registration pathways exist to facilitate Australian registration of agvet chemical products of low regulatory concern. However, these require upfront investment in regulatory data and/or scientific argument, which to date has been largely beyond the resources of proponents of wider use of such chemicals. In conjunction with efforts to streamline the regulation of agvet chemicals in Australia, more work including greater international harmonisation is required regarding simplified registration of chemicals such as pheromones, if their regulation is to be streamlined. The Australian Government is responsible for the regulation of agvet chemicals up to the point of retail sale and States and Territories control their actual use. Australia is also an active participant in the Organisation for Economic Cooperation and Development (OECD) where there are significant efforts to find more effective approaches to regulating biological pesticides. Australia's regulatory policy settings include the ability to harmonise with international approaches where appropriate. The present paper will discuss some of the more recent OECD developments on the regulation of biological pesticides, including pheromones and semio-chemicals.

REGISTRATION OF PEST MANAGEMENT PRODUCTS THAT MANIPULATE INSECT BEHAVIOR

Agenor MAFRA-NETO[1]

[1]*ISCA Technologies, Inc., Riverside, California, USA*

ISCA Technologies' SPLAT (Specialized Pheromone & Lure Application Technology) is a biologically inert matrix for the sustained release of insect semiochemicals, phagostimulants, plant volatiles, biological control agents, insecticides and countless other compounds used for pest management in agricultural, urban and forest ecosystems. SPLAT formulations can be tailored to deliver species-specific control through a variety of mechanisms including attract & kill, mating disruption, repellency and mass trapping. The amorphous and flowable quality of the SPLAT matrix allows for flexible methods of application including disposable syringes, electric and pneumatic grease guns, tractor and gator-driven systems and aerial applications. Registration of ISCA Technologies' semiochemical products can be challenging depending on the country where they are being used. The industry would benefit tremendously from the harmonization of regulatory requirements for semiochemical-based pest management products on a global scale.

REGISTRATION OF A PLANT VOLATILE-BASED ATTRACT AND KILL FORMULATION FOR *HELICOVERPA* SPP. IN AUSTRALIA

Peter C. GREGG[1], Alice P. DEL SOCORRO[1], Anthony J. HAWES[2]

[1]*University of New England, Armidale, Australia*
[2]*AgBiTech Pty. Ltd., Richmond, Australia*

In 2009 we registered a formulation of plant volatile compounds, sucrose (feeding stimulant), and various excipients including antioxidants, thickeners, emulsifiers and dyes, for use against the adult stages of the noctuid moths *Helicoverpa armigera* and *H. punctigera* in cotton, corn and beans. We believe it is the world's first such product approved for application to crops. The volatiles are nature-equivalent, but synthetically produced. This meant it could not be registered as a biological product, but was potentially eligible under a category including "commonly used household or industrial chemicals with a history of safe use", for which the registration requirements were less stringent than for novel active ingredients. The Australian Pesticides and Veterinary Medicines Authority (APVMA) determined that, for plant volatiles, the primary criterion or a history of safe use was inclusion in the Generally Recognised as Safe (GRAS) list of the Flavour and Extract Manufacturer's Association of the USA (FEMA). Excipient ingredients had to be food-grade. Farmers can add insecticides (methomyl, thiodicarb or spinosad) to make the product lethal to the target insects. These insecticides were already approved as conventional larvicides, and were efficacious for moths at concentrations below those resulting in registered active ingredient loads in the relevant crops. The impact of these requirements on the commercialisation of the product, and implications for developing similar plant volatile blends for pest management, were reviewed in 2010 and published in Australian Journal of Entomology.

POSTER PRESENTATIONS

SYMPOSIUM TOPIC 1: INTERACTIONS BETWEEN PLANTS AND ANIMALS IN THE AUSTRALIAN BIOTA

Topic Coordinators:
William Foley (Australian National University, Australia) and **Ben Moore** (University of Western Sydney, Australia)

INTERACTIVE EFFECTS OF ELEVATED CO_2 AND TEMPERATURE EFFECTS ON PLANT-INSECT INTERACTIONS: COMPARISON BETWEEN TWO HOST PLANT SPECIES, *EUCALYPTUS TERETICORNIS* AND *EUCALYPTUS ROBUSTA*

Andrew GHERLENDA[1], Tony HAIGH[2], Ben MOORE[1], Scott JOHNSON[1], Markus RIEGLER[1]

[1]*Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, Australia*
[2]*University of Western Sydney, School of Science and Health, Penrith, Australia*

Elevated CO_2 is expected to reduce the palatability and quality of eucalypt leaves to herbivorous insects via the dilution of essential elements, such as nitrogen while increasing leaf defence compounds. Elevated temperature in combination with elevated CO_2 may ameliorate these foliar chemistry changes, and thus reduce the impact on insect performance. The *Eucalyptus* genus contains over 700 species with varying foliar chemistry traits that can display idiosyncratic responses to elevated temperature and CO_2 concentrations. This will affect insect herbivore performance and potentially shift insect-plant interactions differently between the two eucalypt species. The main objectives of this study were to assess how temperature and CO_2 concentrations alter foliar chemistry and how this may impact insect performance. The study used two *Eucalyptus* species with differing but overlapping home ranges: *Eucalyptus tereticornis*, with a wide latitudinal distribution, and *Eucalyptus robusta*, with a narrower distribution. Seed stock was obtained from one maternal tree in the field and grown in a glasshouse in a multi-factorial design (CO_2; 380 vs. 640 ppm, temperature; 26:18 vs. 30:22 day:night) for eight months. Larvae of *Paropsis atomaria*, a common eucalypt leaf beetle, were placed onto each tree and insect performance was scored for the entire larval development to adulthood. Foliar nitrogen, phosphorous, tannins and total non-structural carbohydrates of leaves were determined. The magnitude of foliar chemistry changes between the two species differed in response to elevated CO_2. Elevated temperature was found to ameliorate the effects of elevated CO_2. Similarly insect performance was significantly affected at elevated CO_2 while elevated temperature reduced the impact of elevated CO_2 on insect performance.

METABOLIC PROFILING IN *ECHIUM PLANTAGINEUM* (PATERSON'S CURSE) – A STUDY OF BIOACTIVE NAPHTHOQUINONES AND PYRROLIZIDINE ALKALOIDS ACROSS AUSTRALIA

Paul A. WESTON[1], **Leslie A. WESTON[1]**

[1]*Charles Sturt University, Graham Centre, Wagga Wagga NSW, Australia*

Paterson's curse is an invasive weed that has naturalized over southern Australia, causing $125 million in losses to livestock due to plant toxicity. Distinct populations of Paterson's curse (*Echium plantagineum*) found near roadsides across NSW and VIC were surveyed along 3 distinct longitudinal transects in July and November 2011 for presence of introduced biocontrol agents as well as secondary products involved in toxicity and defence. Sampling was conducted over 49 geographically distinct sites and biocontrol agents were identified. Survey results showed four biocontrol agents present, including a crown weevil, a root weevil, a leaf miner and a flea beetle. Infestation varied across location and clustered distributions of each species were noted across NSW and VIC, possibly due to climate or proximity to release sites. From each location, a composite sample of shoots and roots was collected. Root periderm extracts were analysed for naphthoquinone content spectrophotometrically and by LC-ESI/MS. Shoot extracts were subjected to SPE and LC-ESI/MS for determination of pyrrolizidine alkaloids (PAs) and related N-oxides (PANOs). Metabolic profiling of 14 possible PAs and PANOs showed their consistent appearance in all shoot extracts with leptanthine N-oxide, echimidine-N oxide and echiumine N-oxide predominant. Quantitative variation was noted over location and time of sampling. Root extracts contained shikonin, related naphthoquinones and two PANOs; content varied qualitatively and quantitatively with location and time of sampling. Additional studies to assess the relative roles of pyrrolizidine alkaloids and naphthoquinones in plant defence, herbivory and invasion of Paterson's curse across NSW and VIC are in progress.

SYMPOSIUM TOPIC 2: AQUATIC CHEMICAL ECOLOGY

Topic Coordinators:
Tilmann Harder (University of New South Wales, Australia) and **Justin Seymour** (University of Technology, Sydney, Australia)

CHEMICAL SEQUESTRATION AND RESILIENCE OF AN ANTARCTIC AMPHIPOD: THE FIRST OF ITS KIND

Jacqueline von SALM (FRIES)[1], Margaret AMSLER[2], Charles AMSLER[2], Craig AUMACK[2,3], James MCCLINTOCK[2], Ryan YOUNG[1], Bill BAKER[1]

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[2]*University of Alabama at Birmingham, Birmingham, Alabama, USA*
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The Antarctic benthic community is home to a plethora of macro and micro-organisms utilizing diverse chemistry to keep in constant communication and defend themselves from predation. Macroalgae such as *Plocamium cartilagineum* have evolved to produce secondary metabolites as a powerful defense mechanism, of which prominent mesograzers, such as amphipods, take advantage by seeking refuge in the algae. The chemistry produced by *P. cartilagineum* has been well investigated over the past four decades showing that the most prominent natural products produced are polyhalogenated monoterpenes. A particular amphipod species, *Paradexamine fissicauda,* not only inhabits the algae, but also ingests the thallus as a food source. This rare behaviour was investigated to show that *P. fissicauda* has utilized the defensive chemistry of *P. cartilagineum* by sequestering the compounds themselves upon ingestion, rather than just inhabiting the algae for protection. Feeding assays were done with a prominent omnivorous Antarctic fish species to show that the *P. fissicauda* fed on *P. cartilagineum* was rejected versus *P. fissicauda* fed on non-chemically defended algae, *Palmaria decipiens*, which was readily eaten. Due to the volatility of the compounds, the analysis of these sequestered compounds was done via GC/MS QToF. The resulting data shows that compounds prevalent in *P. cartilagineum* are seen in the hydrophobic extracts of the amphipods and nonexistent in the extracts of *P. decipiens* fed amphipods, making *P. fissicauda* the only known crustacean to sequester defensive chemistry from its diet.

SYMPOSIUM TOPIC 3: RHIZOSPHERE ECOLOGY

Topic Coordinators:
Leslie Weston (Charles Sturt University, Australia) and **Ulrike Mathesius** (Australian National University, Australia)

DEVELOPMENT AND APPLICATION OF A METHOD STUDYING THE CHEMICAL COMPOSITION OF VOLATILE AND NON-VOLATILE ROOT EXUDATES

Elisabeth EILERS[1,2], Gerhard PAULS[3], Bill HANSSON[2], Monika HILKER[1], Andreas REINECKE[2,4]

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[2]*Max-Planck-Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Jena, Germany*
[3]*Max-Planck-Institute for Chemical Ecology, Department of Bioorganic Chemistry, Jena, Germany*
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Plant root-derived chemicals are involved in numerous interactions among soil biota. Yet, analysing these compounds raises difficulties: the major constraints are low concentrations of root-derived signalling compounds, high decomposition rates, and high levels of non-target compounds extracted from the surrounding matrix. Previous methods did not allow the simultaneous study of volatile and non-volatile rhizosphere chemicals. Due to these constraints, little is known about how biotic and abiotic factors interact in affecting the blend of root-released chemicals. We designed a rhizosphere chamber from which rhizosphere chemicals were extractable without root damage. Volatile compounds and sugars were extracted from rhizospheres and analysed by coupled gas chromatography-mass spectrometry. Our extraction method was tested by comparing the relative extractability of typical rhizosphere chemicals from differentially adsorptive substrates. The ubiquitous ruderal plant dandelion (*Taraxacum* sect. *ruderalia*) was chosen as model plant to study the impact of biotic (mycorrhizal colonisation, plant age) and abiotic factors (substrate type, light intensity) on rhizosphere chemical profiles. Our results show that our newly developed sampling technique is suitable for extraction of volatile and non-volatile compounds from undisturbed rhizospheres. The interactions of the environmental test factors caused specific patterns of volatile compounds (mainly sesquiterpenes) and sugars (glucose, fructose, and sucrose) in the rhizospheres of dandelion plants. A random forest analysis revealed that the factor plant age *per se* and in interaction with other factors was a powerful predictor of the chemical profile of dandelion rhizospheres. Furthermore, sugars (glucose, sucrose) and their quantities contributed most to the specificity of rhizosphere chemical profiles.

SYMPOSIUM TOPIC 4: PLASTICITY OF CONSTITUTIVE PLANT DEFENCES: MICROBES TO CLIMATE

Topic Coordinators:

Daniel Ballhorn (Portland State University, USA) and **Ros Gleadow** (Monash University, Australia)

TRANSITIONS OF TERPENE COMPONENTS AND ANTIFUNGAL PROPERTIES BY THE MILD HEAT TREATMENTS OF CONIFER LEAF OILS

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[2]*Faculty of Agriculture, Yamagata University, Tsuruoka, Japan*

Conifer leaf oils are complex mixtures of volatile terpenoids, and several defensive roles have been reported. Each constituent is well-studied; however, the properties of leaf oils themselves are still unknown. In this study, the qualitative and quantitative transitions of leaf oils by mild heat treatments (MHT) and their antifungal properties against wood-rot fungi were demonstrated. Leaf oils were collected from three Japanese domestic conifers, *Cryptomeria japonica* D. Don, *Pinus thunbergii* and *Thujopsis dolabrata* var. hondai by using the steam distillation apparatus. As for the MHT, 10 mL of each leaf oil was prepared in the open-air screw vials and separately treated at 50, 70 and 90 ^{o}C on hot stirrer. Non-heat (17 ^{o}C) treatments (NHT) were also performed. Samples were collected every 24 hours, and GC-MS and GC-FID analyses were performed on every sample. Mycelial growth inhibition of different composited samples were tested against one white-rot fungus and two brown-rot fungi at a 50 μg/cm^2 concentration according to the previously reported methods. In a process of MHT, antifungal activities significantly increased on all leaf oils according to the decrease of monoterpene hydrocarbon proportions. MHT promoted the results of NHT in each oil; however, oils turned into tar-like conditions when continued a certain period, and activities significantly declined due to such degradation. Therefore, the proportions of higher volatility constituents, mainly monoterpene hydrocarbons in each oil, are suggested as being the important factor for the expression of antifungal properties of conifer leaf oils. This research is supported by LIXIL JS Foundation (12-71).
Kusumoto et al. 2010. *J. of Chem. Ecol.* 36,1381-1386.

(Z)-3-HEXEN-OL MAY ACT AS AN EICITOR IN DEFENSE RESPONSE OF DISEASE- SENSITIVE MAIZE GENOTYPES BY INTER-CROPPING WITH RESISTANCE MAIZE GENOTYPES

Tianxue LIU[1], Xiuping WANG[1], Chaohai LI[1]

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Intercropping is regarded as an effective and environment friendly cultivation practice in disease control. In this study, field investigation, environment control study and laboratory analyses were conducted in order to explore: 1) the effects of inter-cropping with different maize genotypes on air-borne diseases control; 2) the role of VOCs (volatile organic compounds) in air-borne diseases control in intercropping system. The results showed that, compared to mono-cropping, disease grade of SCR (southern corn rust) in sensitive genotypes decreased from 5 and 7 to 3 and 3 in 2006 and 2007, respectively, when inter-cropped with SCR-resistant genotypes. Activities of defense-related enzymes under SCR inoculation in SCR sensitive genotype were higher when inter-cropping with SCR resistance genotypes than in mono-cropping. Treatment with green leaf volatile component (Z)-3-hexen-ol also improved the activity of PAL and the content of total phenols. Gene expression was also induced by (Z)-3-hexen-ol treatment. The result indicated that the plant disease resistance is not only related to its hereditary but also could be affected by the genetic diversity of the cropping community, and (Z)-3-hexen-ol may act as an elicitor in air-borne diseases control in intercropping system.

SYMPOSIUM TOPIC 5: CHEMICAL ECOLOGY OF POLLINATION

Topic Coordinators:

Rod Peakall (Australian National University, Australia), **Bjorn Bohman** (Australian National University, Australia) and **Anna Karin Borg-Karlson** (Royal Institute of Technology, Sweden)

CONSTITUENTS FROM LABIAL GLAND EXTRACT OF THAI STINGLESS BEE

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[2]*Natural Product Research Unit, Faculty of Science, Chulalongkorn University, Bangkok, Thailand*

Insect attractants play an important role in agriculture for increasing crop yields and decreasing loss of stored products. The use of trail pheromone of stingless bee may affect on their pollination in crop fields. This study aims to analyze trail-scent compounds from labial glands of *Tetragonula pagdeni*, a common stingless bee in Thailand by gas chromatography-mass spectrometry (GC-MS). Trail-following bioassay was used to confirm the response behaviour of *T. pagdeni*. Six hydrocarbons were detected. Among these, three unidentified compounds appeared in scent-marked filter paper extract that the stingless bees rubbed; one of them displayed the trail-following attractant to this species. This possible trail scent was the compound in peak no.2 that might be unsaturated hydrocarbon with short chain. Further characterization of this compound needs to be conducted to confirm its behavioral activity. This active compound differed from the previous findings that trail pheromone of stingless bees were terpene- and wax-esters. The first report of this study might be the promising alternative tools for supporting crop field pollination in Thailand.

SYMPOSIUM TOPIC 6: EVOLUTION OF CHEMICAL COMMUNICATION IN THE ERA OF GENOMICS AND TRANSCRIPTOMICS

Topic Coordinators:
Christer Löfstead (Lund University, Sweden) and **Astrid Groot** (Institute for Chemical Ecology, Germany)

TRANSCRIPTOME ANALYSIS OF THE CHEMOSENSORY GENE FAMILIES OF THE LEGUME POD BORER, *MARUCA VITRATA* (LEPIDOPTERA: CRAMBIDAE)

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[1]*AVRDC – The World Vegetable Center, Tainan, Taiwan*

Insect chemoreception plays an essential role in host-plant selection, and mate-seeking behaviors that make significant contributions to proliferation and survival. Moreover, chemosensory proteins modulate pheromone perception, which is closely associated with reproduction and speciation, and may shape insect evolution, so they are thus of great interest to entomologists. The chemosensory gene families are comprised of odorant binding proteins (OBP) and chemosensory proteins (CSP) of the peripheral olfactory processing pathway; odorant receptors (OR) and gustatory receptors (GR) from the chemoreceptor superfamily; and other members like ionotropic receptors (IR), sensory neuron membrane proteins (SNMP), and pheromone binding proteins (PBP). RNA sequencing has been well developed for high-throughput transcriptome analysis. The present study examined transcripts obtained from *de novo* transcriptome sequence assembly that were annotated under the gene ontology (GO) system with terms related to the chemosensory function in *Maruca vitrata*, a major lepidopteran pest of leguminous crops. We performed a comparative genomic analysis of the chemosensory gene families with other Lepidoptera insects with fully sequenced transcriptomes in order to validate the birth-and-death evolutionary model and to unveil their origins and diversifications. The findings will not only represent a significant step forward in our understanding of the architecture of chemosensory gene families, but also lead to the possibility of targeting chemosensory systems for managing *M. vitrata*.

MOLECULAR EVOLUTION OF SEX PHEROMONE RECEPTORS IN NOCTUID MOTHS OF THE GENUS *SPODOPTERA*

Emmanuelle JACQUIN-JOLY[1], Olivier MIRABEAU[1], Arthur DE FOUCHIER[1,2], Annick MARIA[2], Marie-Christine FRANÇOIS[1], Nicolas MONTAGNÉ[2]

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Female moths of closely related species emit slightly different blends of sex pheromone components, which is necessary (and in many cases sufficient) to maintain premating isolation. Thanks to rapid progresses in sequencing technologies, genomic and transcriptomic data sets are now available in numerous Lepidoptera species and a variety of pheromone receptors (PRs) have been annotated. This opened new routes to understand the molecular bases of the pheromonal communication system, and the study of PR evolution is of particular interest to highlight mechanisms at the base of reproductive isolation and speciation. Only a dozen of PRs have been functionally characterized in moths, but they all share a common origin, as exemplified in phylogenies of moth odorant receptors. In that context, we aim at characterizing candidate PRs in the genus *Spodoptera* (containing important crop pest moths) and studying their evolutionary history, as a first step toward the exploration of structure-function relationships among moth PRs. Transcriptomic, homology cloning and genomic data analyses are being used to identify PRs in different species from the genius *Spodoptera* (*littoralis, exigua, frugiperda*) and their functional characterization is in progress. Phylogenies of candidate PRs are being constructed using probabilistic methods (Maximum Likelihood and Bayesian Inference) to identify potential deletion/duplication events that could be linked to the differences in pheromone blend composition of these three species. Finally, we aim at identifying PR regions under positive selection, which may be responsible for the emergence of new ligand receptivity, and may contribute to premating isolation and speciation in these model pests.

GENOMIC AND INFORMATIC CAPABILITY FOR THE CHEMICAL MOLECULAR ECOLOGIST

Alexie PAPANICOLAOU[1], the i5k Curation Working Group[2]

[1]*CSIRO Ecosystem Sciences, Canberra, Australia*
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This paper presents the genomic capability recommended for chemical ecologists working on "non-model" species. Genomic data underpins our ability to dissect the molecular mechanisms of how an organism perceives, interacts and reacts with its environment. Further, by using multiple species with a well characterized ecology we can understand how these mechanisms came to exist. Traditionally, such species have been referred to as 'non-models' due to the lack of genomic data. Next Generation Sequencing (NGS) has removed the data bottleneck of genomics, in order to produce a bioinformatic one. To avoid a "death by sequencing", research labs will benefit from a standardized capability that converts NGS data to hypotheses which can be tested in the laboratory. First, understanding the characteristics of each species minimizes sequencing costs and places them well within the reach of a single project budget. Second, deep RNA-Seq and educated use of bioinformatic software underpins a robust and rapid automated annotation. Third, transcriptional profiling drives hypothesis generation which can be further enhanced by the use of a phylogenetic framework. Fourth, friendly graphical user interfaces - coupled with education and outreach - allow scientists to reclaim their own data, seamlessly access community data, and curate gene models so that downstream analyses are improved. Finally using examples from insect genomes, we show how the genomics of chemical ecology can immediately benefit by access to sequence information from multiple species and the careful use of informatics.

SYMPOSIUM TOPIC 7: THE CHEMICAL STIMULUS – ITS ANALYSIS AND SYNTHESIS

Topic Coordinators:
Stefan Schulz (Technische Universitat Braunschweig, Germany) and **Ales Svatos** (Institute for Chemical Ecology, Germany)

COST-EFFICIENT SYNTHESIS OF CERALURE B1; AN EFFICIENT ATTRACTANT FOR THE MEDITERANEAN FRUITFLY (*CERATITIS CAPITATA*), A DEVASTATING AGRICULTURAL PEST

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[1]*Linnaeus University, Kalmar, Sweden*
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The Mediterranean fruitfly (Medfly; *Ceratitis capitata*) is a serious pest in fruit agriculture. It is a very efficient invader and it is under surveillance in several areas of the world, in order to stop the spreading. More than 100 000 traps with the para-pheromone attractant trimedlure are for example used every year by the U.S. Department of Agriculture in control of the Medfly. A more efficient attractant, ceralure B1, is available but it is currently too expensive for commercial use. The most efficient enantiomer, (-)-ceralure B1, is depicted in Figure 1. Racemic ceralure B1 has however, been shown to be almost as efficient in field trials. The present methods for the synthesis of ceralure B1 are based on assymetric Diels-Adler reactions and include about 9 steps, with over-all yield of 15-26%. A completely new synthesis route of ceralure will be presented. The aim is to achieve a cost-efficient synthesis of ceralure B1, as well as different modifications of the ceralure para-pheromone that can be tried in the field.

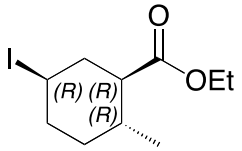

Figure 1. (-)-Ceralure B1, the most efficient Medfly attractant known today

IDENTIFICATION OF THE SEX PHEROMONE OF THE SCALE INSECT *AULACASPIS MURRAYAE* TAKAHASHI

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[1]*Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan, Republic of China*
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[3]*Department of Entomology, University of California, Riverside, CA, USA*

The *Aulacaspis murrayae* Takahashi (Hemiptera: Diaspididae) is a serious pest damaging orange jasmine *(Murraya paniculata* L.) Jack**)**, that is used widely as hedge plant in Taiwan. A female specific compound was identified from aeration extracts of virgin female scale insect, *Aulacaspis murrayae* Takahashi and the stereochemistry of the compound was confirmed by comparison with authentic synthesized compound. Identification and synthesis of the compound will be presented. Bioactivity of the identified compound is under investigation.

STRUCTURE DETERMINATION OF THE SEX PHEROMONE SECRETED BY *LOEPA SAKAEI*, A GIANT SILKWORM MOTH INHABITING IN OKINAWA ISLAND

Akiko KANEGAE[1], Hideshi NAKA[2], Haruki TATSUTA[3], Yan QI[1], Tetsu ANDO[1]

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[3] *Faculty of Agriculture, University of the Ryukyus, Japan*

The lepidopteran family of Saturniidae includes more than 1,000 species found all over the world, and sex pheromones have been reported for 16 species. While Japan is inhabited by 11 species, no pheromones of the endemic species have been chemically identified. Therefore, we put the study of saturniid pheromones into practice, starting with *Loepa sakaei*, which is found only in Okinawa and Amami-Ohshima Islands and is designated as a quasi-endangered species. A pheromone gland extract was prepared with two virgin female moths. GC-EAD analysis with an aliquot of the extract (0.25 female equivalent, FE) revealed one EAG active component, and the mass spectrum was obtained by GC-MS analysis with another aliquot (0.25 FE). The highest ion was detected at *m/z* 192, which was estimated to be $[M-60]^+$ of tetradecadienyl acetate. The spectral pattern was similar to that of 6,11-hexadecadienyl acetate (6,11-16:OAc) published for the saturniid species *Antheraea polyphemus*. Based on the similarity, we estimated the EAG-active component to be 4,9-tetradecadienyl acetate (4,9-14:OAc), which included two double bonds at the same ω5- and ω10-positions as 6,11-16:OAc. A field test with the synthetic lures was carried out from May to June 2012 in Okinawa Island. Male moths of *L. sakaei* were attracted only by the (4*E*,9*Z*)-isomer, indicating that mating communication of this species was mediated by E4,Z9-14:OAc. It would be interesting to know whether the C_{14}-chain compound is a species-specific pheromone component of Japanese saturniid species. Therefore, we are analysing pheromone components of other species, such as *Aglia japonica* and *Rhodinia fugax.*

AEDES AEGYPTI LARVAE RESPOND TO NATURAL AND SYNTHETIC ODORANTS

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The ability to detect a wide range of sensory cues is essential for the survival and vectorial capacity of mosquitoes. This investigation focuses on the analysis of olfactory-driven behavior in dengue vector mosquito *Aedes aegypti* (L.) larvae to respond to synthetic or natural odorant stimuli. We established a sensitive olfaction-based behavior assay, following a previously established technique. In this assay, the distribution of 100 of 2nd or 3nd instar larvae of *Ae. aegypti* was monitored to a range of odorant stimuli as well as appropriate negative controls throughout a 60 minute time course. The number of larvae in both test and control zone was determined throughout. All time-point and performance index (PI) at 40min were calculated to represent the characteristic response of each odorant with +1 indicative of full attraction and –1 of complete repulsion. The widely used insect repellent N, N-diethyl-m-toluamide (DEET), consistently evoked highly significantly negative PI as also did Indole and Acetophenone. When using yeast extract, a known food source, the larvae responded with a significantly positive PI as also with 2-methylphenol, 1-octen- 3-ol, 3-meyhylphenol and fish food. We also found that consistent with the olfactory function, ablation of the larval antennae eliminated these behavioral responses. It is important to study the behaviour of larvae attractants and repellents, as a contribution to the understanding of the mechanism of olfaction in *Ae aegypti* larvae.

ELECTROPHYSIOLOGICAL AND BEHAVIORAL RESPONSE OF *N*-HENEICOSANE, OVIPOSITION PHEROMONE OF *AEDES AEGYPTI,* ON *AEDES ALBOPICTUS*

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Aedes aegypti and *Aedes albopictus* are highly anthropophilic mosquito species and potential vectors of dengue and yellow fever. The location of suitable sites for oviposition requires a set of visual, tactile and olfactory cues that interact with the female before laying their eggs. In this work we study the effect of *n*-heneicosane, recognized oviposition pheromone of *Ae. aegypti*, on the olfactory receptors on the antennae of *Ae. aegypti* and *Ae. albopictus* using electroantennographic detection coupled to gas chromatography (GC-EAD). We also analyzed the effect of this compound on the oviposition response of *Ae. albopictus* in order to determine whether *n*-heneicosane also influences its behavior. We observed a significant electroantennographic response to *n*-heneicosane in adult females of both species of mosquitoes and repellency at certain doses in the oviposition behaviour on *Ae. albopictus.* Furthermore, *n*- heneicosane was identified in cuticles of *Ae. albopictus* larvae by GC-MS technique. We found that the oviposition behaviour for both species is influenced by the existence of larvae of one species in presence of the adult of the other. These results could determine whether this pheromone could be used in baited traps to improve monitoring on control strategies against both species of mosquitoes.

ANTITERMITIC ACTIVITIES OF BRANCH HEARTWOOD EXTRACTS OF *CHAMAECYPARIS OBTUSA*

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Hinoki (*Chamaecyparis obtusa* Endl.) is an important Japanese conifer tree which has been used as wooden structures because of its durability. It is well known Hinoki timber has antitermitic properties. At present, branch wood of Hinoki is wasted because it is considered to be a valueless part. Therefore, it would be useful to develop a method to use this material. In this study, we investigated the bioactivities of components of Hinoki branch against Japanese termites *Reticulitermes speratus*. We separated germacra-1-(10),5-dien-4β-ol, *t*-cadinol, α-cadinol, hinokiresinol, and hinokinin from branch extracts and investigated the antitermitic activity of these compounds by no-choice feeding, dual choice feeding, non-contact, no-choice contact, and dual choice contact methods. The sesquiterpenoids, germacra-1-(10),5-dien-4β-ol, *t*-cadinol and α-cadinol were strongly termiticidal by no-choice and dual choice feeding methods. On the other hand, there was no, or very little activity by non-contact test. The sesquiterpenoids showed strongly termiticidal activities on no-choice contact method. In dual choice contact test, these sesquiterpenoids were strongly repellent. Thus, these activities were provided that only on the direct contacting conditions and active component were didn't provide by feeding. Therefore, the sesquiterpenoids had strong termiticidal, antifeedant, and repellent activities. The norlignan, hinokiresinol had weak termiticidal, while it showed a strong antifeedant, and repellent. Germacra-1-(10),5-dien-4β-ol and hinokiresinol were present in branch characteristically. These compounds would protect Hinoki branches from termites. Hinoki branch heartwood, which is usually unused, is a potential source of active antitermitic compounds.

CHARACTERISATION OF POSTICLURE AND THE STRUCTURE-RELATED SEX PHEROMONE CANDIDATES PREPARED BY EPOXIDATION OF (6*Z*,9*Z*,11*E*)-6,9,11-TRIENES AND (3*Z*,6*Z*,9*Z*,11*E*)-3,6,9,11-TETRAENES

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Trans-11,12-epoxy-(6*Z*,9*Z*)-6,9-henicosadiene (posticlure) has been identified from a pheromone gland of a lymantriid species, *Orgyia postica*. Since the diversity of Lepidoptera suggests that some species utilize the structure-related epoxy compound as a sex pheromone component, we systematically synthesized epoxydienes and epoxytrienes from (6*Z*,9*Z*,11*E*)-6,9,11-trienes and (3*Z*,6*Z*,9*Z*,11*E*)-3,6,9,11-tetraenes with a C_{19}–C_{21} chain and accumulated the chemical data in order to utilize for new pheromone research. Peracid oxidation of each triene and each tetraene produced a mixture of three epoxydienes (*cis*-6,7-epoxy-9,11-diene, *cis*-9,10-epoxy-6,11-diene, and *trans*-11,12-epoxy-6,9-diene) and four epoxytrienes (*cis*-3,4-epoxy-6,9,11-triene, *cis*-6,7-epoxy-3,9,11-triene, *cis*-9,10-epoxy-3,6,11-triene, and *trans*-11,12-epoxy-3,6,9-triene), respectively. While the 9,10-epoxy compounds were unstable and interestingly converted into 9-ketones after chromatography over SiO_2, each positional isomer could be isolated by HPLC equipped with an ODS column and the chemical structure was determined by NMR analyses. On the GC-MS analysis with a polar column, the positional isomers were also eluted separately and proposed characteristic mass spectra. By comparing the spectral data of the epoxy compounds with a different carbon chain, diagnostic fragment ions reflecting the chemical structure were determined as follows: *m/z* 79, 109, 113, and M-114 for the 6,7-epoxydienes; *m/z* 69, 97, 111, 139, and M-111 for the 9,10-epoxydienes; *m/z* 57, 79, 109, 136, M-151, and M-111 for the 11,12-epoxydienes; *m/z* 79, 91, 105, and 119 for the 3,4-epoxytrienes; *m/z* 79, 124, M-124, M-96, and M-69 for the 6,7-epoxytrienes; *m/z* 79, 95, 109, 137, and M-108 for the 9,10-epoxytrienes; *m/z* 79, 134, M-149, M-109, and M-95 for the 11,12-epoxytrienes.

EVALUATION OF SIZE ASYMMETRIC COMPETITION OF BARLEY SEEDLINGS AGAINST ANNUAL RYEGRASS

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Size asymmetric competition (SAC) indicates the disproportionate advantage of larger plants interacting with their smaller neighbours. Strong chemical interferences were reported between barley (Hordeum vulgare L.), and weeds. However, researches are limited on SAC of barley.
Five different densities (0, 5, 10, 15, and 20 barley seedlings/beaker) of barley seedlings (cv Buloke) were grown separately in 50 mL agar solution of half of glass beaker, after 9 days five pre-germinated ryegrass seeds were transplanted other half agar surface of each against each barley density. The beakers were kept in a controlled growth chamber for further 7 days co-growth to know SAC effect of barley seedling on receiver weed species. The root and shoot length of the ryegrass was measured and the root surface area and volume was assessed by scanning and image analysis using WinRhizo software. Results demonstrated seedling inhibition was greatest under SAC. This inhibition enlarged considerably with increased density of barley. Strong SAC effect was detected at the density of 30 barleys /beaker, where the highest root (83.5%) and shoot (62%) inhibition of ryegrass was observed. At such point the root surface area (cm3/plant) and root volume (cm3/plant) of ryegrass recorded 2.80 and 0.018 accordingly, which was significantly lower than parameters counted at other densities. This result suggests barley is a candidate competitive crop against small neighboring weeds where density enhanced such interference (competition and allelopathy).

TOWARDS ELUCIDATING THE ROLE OF FAS GENES IN PLANT PATHOGENESIS

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Phytohormones are pivotal in regulating plant growth and development, among which cytokinins (CKs) are essential for cell division, leaf senescence and other plant developmental processes. Many plant pathogens interfere with plant hormonal balance and alter the host to their advantage by either producing the phytohormones themselves or by modifying the host plant's metabolism. The phytopathogenic actinomycete, *Rhodococcus fascians* induces malformations ranging from leaf deformation, witches' broom and leafy galls that provoke shoot meristem amplification in many plants. The disease symptoms induced by *R. fascians* resemble typical CK effects and can be partially mimicked by exogenous application of CKs. However, no clear correlation between CKs and symptom development is known. *R. fascians* pathogenicity has been linked to a linear plasmid, pFiD188. Three loci (*fas*, *att*, *hyp*) are involved in virulence. The *fas* operon has six genes FAS1-6. FAS4 is homologous to isopentenyltransferase, an important enzyme in CK biosynthesis. FAS1 is similar to cytochrome P450 monoxygenase and N-terminal region of FAS2 is similar to 4Fe-3S type ferredoxins. FAS1 is hypothesized to hydroxylate the isopentenyl side chain of the CK produced by FAS4, while FAS2 and FAS3 deliver the energy for this reaction *via* the ferredoxin-like domain. The unusual strong infection by these bacteria compared to low levels of CKs in culture and infected plant tissues, indicates a synergy of different CKs and possibly new CK-type molecules employing basic CK biochemistry. Analysis of new CK type metabolites from *R. fascians* will be presented. Further progress on the function of FAS genes will be discussed.

IDENTIFICATION OF THE SEX PHEROMONE SECRETED BY *PALPITA NIGROPUNCTALIS* (LEPIDOPTERA: CRAMBIDAE)

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The lilac pyralid, *Palpita nigropunctalis* Bremer (Crambidae: Pyraustinae), is a common pest of Oleaceae. A crude pheromone extract of the females was examined by GC-EAD and GC-MS. The GC-EAD analysis indicated three EAG-active components (I–III), and a GC trace by a flame ionization detector indicated a mixing ratio of about 5:1 of I and II and a very low content of III. Mass spectra of I and II were successfully recorded. Ions at *m*/*z* 238 (M^+) and 220 ($[M-18]^+$) detected for I indicated the structure of a monoenyl aldehyde with a 16-carbon chain. While M^+ was not detected for II, ions at *m*/*z* 222 ($[M-60]^+$) and 61 ($[AcOH+1]^+$) suggested that II was a monoenyl acetate with a 16-carbon chain. GC-MS analysis of the extract treated with DMDS showed mass spectra of the adducts derived from I and II. Ions at *m/z* 332, 215, and 117 for the adduct of I and ions at *m/z* 376, 259, and 117 for the adduct of II revealed double bonds at the same 11-position. Furthermore, the pheromone extract was examined by GC-FT-IR. An IR spectrum of I showed characteristic absorption at 1716 and 966 cm^{-1}, which indicated a formyl group and *E* configuration of the double bond, respectively. In the case of II, absorption at 1745 and 968 cm^{-1} indicated an ester carbonyl and *E* configuration. As facilitated by comparison with authentic standards, we concluded that I and II were (*E*)-11-hexadecenal and (*E*)-11-hexadecenyl acetate, respectively, and speculated that III was (*E*)-11-hexadecen-1-ol.

SYMPOSIUM TOPIC 8: MICROBIAL-CHEMICAL ECOLOGICAL INTERACTIONS AMONG MICRO-ORGANISMS AND THEIR ENVIRONMENTS

Topic Coordinators:
Junwei (Jerry) Zhu (USDA, USA) and **Anna Karin Borg-Karlson** (Royal Institute of Technology, Sweden)

ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL AND ITS COMPOSITIONS FROM *POLYGONUM ODORATUM* LOUR. AGAINST RICE PATHOGENIC FUNGI

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Rice diseases establish the major obstacle of high and suffering rice potency. The most effective and frugal way to control diseases were poor deploying resistant varieties. Plant natural products are currently explored as potential sources of fungicides and as a possible way to control rice diseases. This study was pledged to evaluate the *in vitro* antifungal activity of essential oil and its compositions of *Polygonum odoratum* Lour. against rice pathogenic fungi. The oil displayed potential antifungal activities *in vitro* as percentage of mycelia inhibition (and spore germination) against *Rhizoctonia solani* and *Bipolaris oryzae* of 77% (75%) and 60% (92%) inhibition with IC_{50} values of 0.066 and >2.5 mg/mL, respectively. GC/MS analysis of oil allotted identification of dodecanal (54%), decanal (15%), *trans*-caryophyllene (8%), cyclododecane (7%), and α-humerene (5%) as main compounds. Dodecanal strongly inhibited the growth of *R. solani* and *B. oryzae*, with IC_{50} values 0.851 and >3.0 mg/mL, respectively.

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THE ROLE OF *EWINGELLA* SP. IN THE GUT OF THE LARGE PINE WEEVIL, *HYLOBIUS ABIETIS*

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The large pine weevil, *Hylobius abietis*, is one of the economically most important pests in reforestation areas in large part of Europe and Asia. The female weevil excavates a cavity in the root bark with her snout and places an egg, adds her feces and closes the cavity with chewed bark to protect it from conspecific predation. We have focused on the intestinal bacteria of *H. abietis* for a screening of repellents. Among 54 isolated strains of microorganisms from the gut and feces of *H. abietis.* A bacterium, when growing in natural frass medium, produced isoamyl alcohol, phenol, 2-methoxyphenol, and 4-vinyl guaiacol was identified as *Ewingella* sp. NBRC109474 based on a rDNA sequence. This strain could convert ferulic acid and vanillin to 4-vinyl guaiacol and vanillyl alcohol respectively. On a medium included 0.5% glucose and yeast extract, pH7.0, this strain produced ammonia. On the nitrogen deficiency medium, the *Ewingella* strain could grow but acetylene reduction activity was low. Bacteria which have a high homologous sequence of the rDNA with this strain are found in one other type of bark beetle and longicorn beetles. We consider *Ewingella* sp. to be an important strain for the pine weevil and as such a tool for developing control methods of this pest. The repellent and antifeedant effects of 4-vinyl guaiacol and vanillyl alcohol against *H. abietis* are now in progress and promising.

THE EFFECT OF *FUSARIUM* SPP. ON THE GROWTH AND DEVELOPMENT OF *BUSSEOLA FUSCA* LARVAE

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Fusarium spp. commonly infect maize plants, causing ear rots and mycotoxin production in maize. The effect of these *Fusarium* spp. on the growth and survival of *Busseola fusca* larvae, a common pest of maize plants, is not known. To investigate this, pure cultures of *F. verticillioides* (MRC 826 and GCI 282 strains), only one isolate of *F. subglutinans, F. proliferatum* and *F. graminearum* were cultured on maize grain for 3 weeks. The maize grain was then milled and mixed into an artificial *B. fusca* diet at a 10% inclusion rate. Five *B. fusca* neonate larvae were placed onto 50 g of the amended *B. fusca* diet in a polyethylene bottle with a wire mesh lid at room temperature, replicated 20 times per treatement. The larvae were allowed to feed for 3 weeks, after which survival and mean larval mass were determined . Results showed that the inclusion of *F. subglutinans* in the diet significantly reduced the growth and survival of *B. fusca* larvae compared to the other species and control. *Fusarium subglutinans* possibly produces compounds that have toxic or feed refusal properties on *B. fusca* larvae. *Fusarium verticillioides* MRC 826 and GCI 282*, F. proliferatum* and *F. graminearum* had no significant effect on the growth and survival of *B. fusca* when compared to the control. These results indicate the existence of a *Fusarium* spp. x *B. fusca* interaction that needs to be further investigated.

ISOLATION AND SCREENING OF ACTINOMYCETES FROM MANGROVE SOILS IN THAILAND FOR ANTIBACTERIAL ACTIVITY

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The mangrove ecosystem is a potent source for actinomycetes with the capability to produce wide range of bioactive secondary metabolites. Actinomycetes are a prokaryotic group of Gram-positive and filamentous bacteria. They are major source for the production of numerous novel compounds with diverse biological activities, including antimicrobial properties. Objective was to evaluate the antibacterial activity of actinomycetes isolated from mangrove soil from different sites around Chantaburi province, Thailand. Actinomycetes were isolated from mangrove soil samples using modified starch casein agar medium. Preliminary screening of isolates for antibacterial activity against the test microorganisms was performed by conventional spot inoculation method on agar medium. In this study, twenty-three actinomycetes were isolated from five mangrove sites. The isolates were grouped in five color series based on their spore color. Twenty-two actinomycete isolates (95.7% of the isolates) exhibited antibacterial activity against at least one of the test microorganisms. Most the isolates inhibited growth of the Gram-positive bacteria tested. Five isolates were highly active against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 with an inhibition zone more than 25 mm in diameter. In addition, five isolates showed moderate activity against Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). The results of the research show a diversity of actinomycetes in mangrove ecosystems and the isolated strains have a broad spectrum antibacterial activity which show potential as a source of novel antibiotics.

SYMPOSIUM TOPIC 9: MULTIMODAL COMMUNICATION: (INTEGRATION OF OLFACTION, TASTE, VISION, ACOUSTICS, MECHANORECEPTION)

Topic Coordinators:
Gerhard Gries, (Simon Fraser University, Canada) and **John Hildebrand** (University of Arizona, USA)

COMMUNICATION BY CHEMICAL CUES OF AGROBIONT SPIDER *PARDOSA AGRESTIS* (WESTRING)

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Wolf spider *Pardosa agrestis* (Westring) (Lycosidae) is an abundant predator of pests in the agroecosystems in central Europe. Juvenile spiders were collected in the cereal field and were reared until maturity for laboratory investigation. The ability to detect female airborne pheromones and the pheromones deposited in dragline silk were tested on the adult virgin males. The two-choice olfactometer and the two-choice dragline silk tracking tester were used in laboratory experiments. Virgin males (N = 30) did not prefere tunnel with female airborne cues in the two-choice oflactometer. The number of chosen tunnels with female cues did not differ from the number of control tunnels (0.53 vs. 0.47). However, virgin males (N = 30) significantly prefered tunnels with female pheromones deposited in dragline silk (0.83 vs. 0.17). Our investigation revealed the intersexual communication of *P. agrestis* via cues located in draglines and we found that *P. agrestis* does not communicate via airborne cues.

THE ROLE OF VIBRATIONAL AND OLFACTORY SIGNALS IN COURTSHIP AND MATE SELECTION OF *AACANTHOCNEMA DOBSONI*

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Psyllids (Hemiptera: Psylloidea) have been shown to rely on either substrate-borne vibrational signals (e.g. *Schedotrioza apicobystra* (Triozidae)), chemical signals (e.g. *Cacopsylla bidens* (Psyllidae)), or both (e.g. *Diaphorina citri* (Psyllidae)) for courtship and mate recognition. Most of the past studies have focussed on how a single signal is used to achieve mating. However, it is possible that the use of more than one signal (multimodal signalling), as in *D. citri,* for mate attraction maybe a widespread phenomenon in Psylloidea. This study focusses on courtship and mate recognition signals/cues utilised by *Aacanthocnema dobsoni* (Hemiptera: Triozidae) and aims to clarify their role. Vibrational signalling was quantified using a laser vibrometer. Gas chromatography-mass spectrometry (GC-MS) was used to quantify gender-based differences in cuticular hydrocarbons. Y-tube olfactometer bioassays were used to assess psyllid responses to volatile olfactory cues produced by conspecifics. Psyllid responses of males to dead females with their cuticular hydrocarbons intact or removed by solvent were also conducted. Laser vibrometer recordings reveal male and female acoustic duets during courtship. GC-MS results revealed qualitative and quantitative gender-based differences in cuticular hydrocarbons. Our olfactometer results provide no evidence for existence of a volatile sex pheromone. The biological activity of psyllid cuticular hydrocarbons is being assessed. Our results suggest that *A. dobsoni* may not rely on a sex pheromone for long-range mate attraction but vibratory signalling instead. Vibratory signals may be complemented at close range by cuticular hydrocarbons that provide cues for mate recognition and possible assessment of reproductive status.

ANTENNAL MORPHOLOGY AND SENSILLAR ULTRASTRUCTURE OF *DASTARCUS HELOPHOROIDES* (FAIRMAIRE) (COLEOPTERA: BOTHRIDERIDAE)

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The parasitoid beetle *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) is an important parasite of longicorn beetles (Cerambycidae), and has been used in China for the biological control of the Asian longicorn beetle (*Anoplophora glabripennis*) and the Japanese pine sawyer (*Monochamus alternatus*). In this study the antennal morphology and sensillar ultrastructure of *D. helophoroides* were observed using scanning electron microscopy and transmission electron microscopy. Two types of sensilla trichodea (Tr. 1 and Tr. 2), two types of sensilla basiconica (Ba. 1 and Ba. 2), three types of sensilla chaetica (Ch. 1, Ch. 2 and Ch. 3), and Böhm's bristles were identified according to the morphology and fine structure of each type of sensilla in both sexes. Ultrastructural studies revealed porous structures on the cuticle wall and dendritic branches in the inner lumen of Tr. 1, Tr. 2, Ba. 1, and Ba. 2, thereby suggesting chemoreceptor functions. No difference in shape, structure, sensilla distribution and typology was observed between the sexes. These structures likely have roles in the host locating and habitat searching behaviour of adult *D. helophoroides*, and suggest future studies on the olfaction mechanism and host location behaviour of *D. helophoroides* and other coleopteran parasitoids.

CHIRAL DISCRIMINATION OF AGGREGATION PHEROMONE AND POPULATION DIVERGENCE OF *IPS SUBELONGATUS* IN NORTHEASTERN CHINA

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We investigated thirteen geographic populations of *Ips subelongatus* in northeastern China based on electrophysiological and behavioral characteristics and mtDNA COI sequences in order to explore the possibility of existing pheromone races and the extent of population divergence. Antennae responded most strongly to (*S*)-(–)-ipsenol (100% detection; 0.35–0.73 mV) at the 50-ng stimulus level in gas chromatography (GC)–electroantennographic detection (EAD) analyses, while its antipode, (*R*)-(+)-ipsenol was antennally inactive. Populations varied in their responses to (*R*)-(–)- and (*S*)-(+)-ipsdienol in GC-EAD analyses. Electroantennogram sensitivity to 97%-(*S*)-(–)-ipsenol and 97%-(*S*)-(+)-ipsdienol was 0.1 µg in dose-response analyses, with no quantitative differences among the six populations tested. A binary blend of 97%-(*S*)-(–)-ipsenol and 97%-(*S*)-(+)-ipsdienol was critical for mass trapping across northeastern China; attraction was interrupted by (*R*)-(+)-ipsenol and/or (*R*)-(–)-ipsdienol. Furthermore, 27 mitochondrial haplotypes were found among the thirteen populations (intraspecific nucleotide divergence, 0.1–1.1%). Analyses of molecular variance and haplotype networks indicated that different geographic populations had generated some genetic variation but did not form completely independent groups. Although some chiral discrimination of pheromone enantiomers was observed, true pheromone races were absent. 97%-(*S*)-(–)-ipsenol and 97%-(*S*)-(+)-ipsdienol can be used directly to control *I. subelongatus* in northeastern China.

SUGAR RECEPTORS IDENTIFIED FROM *HELICOVERPA ARMIGERA* AND *BOMBYX MORI*

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The gustatory system mediating food selection and preference in caterpillars is of great interest, since many economically important agricultural pests are members of the order Lepidoptera. Many lepidopteran larvae have specialised feeding habits. Sugars are known to be strong phagostimulants in phytophagous insects. Previously, we compared electrophysiological responses to sugars between fifth instars of *Helicoverpa armigera*, a polyphagous generalist pest, and *Bombyx mori*, an oligophagous specialist beneficial. GRNs located in styloconic sensillum were highly activated by m*yo*-inositol and sucrose in both *B. mori* and *H. armigera*. Further studies showed that the number of sugar receptors in both species is similar. Calcium imaging studies confirmed that in *B. mori* BmGR8 identified from maxillary galea is a specific *myo*-insoitol receptor, while a fructose receptor (BmGR9) was reported at the same time. In *H. armigera*, HaGR9 sharing high levels of amino acid identity with BmGR9 and DmGR43a showed broader responses to sugars including D-galactose, D-maltose, and D-fructose.

SYMPOSIUM TOPIC 10: NEW STUDIES IN CHEMICAL ECOLOGY INVOLVING VERTEBRATES AND OPPORTUNITIES FOR SOLVING PRACTICAL PROBLEMS OF LIVESTOCK PRODUCTION AND HUMAN HEALTH

Topic Coordinators:

John Pickett (Rothamsted Research, UK) and **Mike Birkett** (Rothamsted Research, UK)

IDENTIFICATION OF VOLATILES IN THE URINE OF LITTLE INDIAN FIELD MOUSE, *MUS BOODUGA*

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Pheromones (chemo signals) play a significant role in controlling both the behaviour and physiology of mammals. Rodents, being largely nocturnal and often moving through dense cover, rely heavily on these species-specific chemical signals to attract mates, warm rivals, aid navigation and control the sexual development of other members of the same species. In our study, Gas Chromatography linked Mass Spectrometry was used to identify the volatiles in the urine and faeces of pest rodent Little Indian field mouse, *Mus booduga*. A total of 21 compounds were identified in the urine of male mouse. Among the compounds, dimethyl sulfone, cholest-5-en-3-ol (3.beta.), 1-iodotetradecane, 5-sec-butyl-2-hydroxy-m-xylene-.alpha.,.alpha.'diol, betulin and cholestan-3-ol, (3.beta.,5.beta.) are the major compounds and are reported as pheromones in various animals. In faeces, 26 compounds were identified. Of these four compounds namely 9-Octadecenoic acid, Z,Z-8,10-Hexadecadien-1-ol, 9,12-Octadecadienoic acid and Ethyl 13-docosenoate appeared as major compounds. In urine, the pheromone carrying protein (19 kDa- Major Urinary Protein) was also identified. Further behavioural assessment of identified compounds would be helpful to develop a powerful and species-specific pheromone trap for managing rodent populations.

ASSESSMENT OF ESTRUS-SPECIFIC FAECAL VOLATILES IN ESTRUS INDUCED GOAT, *CAPRA HIRCUS*: AN INVENTIVE STEP IN LIVESTOCK IMPROVEMENT

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Species-specific chemical signals evolved in mammalian systems favors communication. The diverse origin of these signals includes urine, faeces, saliva, vaginal mucus etc. Among those, faeces have been believed to act as a potent source of chemo-signals. With this background, the present study was executed to identify the estrus-specific volatiles in faeces of estrus induced female Indian goat, *Capra hircus*. The goats (n=6) were inserted with intra-vaginal sponges for the induction of estrus. After 15 days, the sponges were removed, the phase was considered as pro-estrus. One day after pro-estrus considered as estrus (confirmed with the exhibition of estrus behaviours such as restlessness, vaginal mucus discharge, frequent urination, homosexual behaviours in female goats and co-incident behaviours such as flehmen and mounting behaviours from male goat) and three days after pro-estrus considered as post-estrus. Faecal samples were collected accordingly in all the phases and analysed with Gas Chromatography-Mass Spectrometry. GC-MS analysis revealed 27 compounds, among them three were common to all phases and seven as estrus-specific. Of note among estrus-specific volatile compounds, 1-octadecanol, tetradecanoic acid and 1-tetradecanol were suspected to have pheromonal effects. These compounds combined with other all other compounds may have a role in advertising estrus of female to male goats. Thus, these three compounds may be considered as estrus indicators in goat, and used to develop non-invasive estrus detection kit. Overall, this basic research will be useful in the easy identification of estrus in goat, and thereby would pave a milestone in livestock improvement.

SYMPOSIUM TOPIC 12: MOLECULAR MECHANISMS IN PERCEPTION OF SEMIOCHEMICALS

Topic Coordinators:
Judith Reinhard (University of Queensland, Australia) and **Charles Claudianos** (University of Queensland, Australia)

ANTENNAL TRANSCRIPTOME ANALYSIS AND FUNCTIONAL CHARACTERATION OF PHEROMONE RECEPTORS IN THE COTTON BOLLWORM *HELICOVERPA ARMIGERA*

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Male moths can accurately perceive the sex pheromone emitted from conspecific females by their highly accurate and specific olfactory sensory system. Pheromone receptors (PRs) are of special importance in moth pheromone reception because of their central role in chemosensory signal transduction processes that occur in the male antennae. A systematic analysis of olfactory receptors (ORs) including PRs is still largely absent in most insect species because of the difficulties in gene cloning. Recently, RNA-seq approaches have been used to identify olfactory genes in species where a genome sequence is not yet available. In this study, we identified the olfactory gene repertoire of the economically important agricultural pest moth, *Helicoverpa armigera*, by assembling the adult male and female antennal transcriptomes. Within the transcriptomes we identified a total of 47 OR candidate genes containing 6 PR candidates. After that, we cloned six full-length PR genes. Realtime PCR showing all genes exhibited male-biased expression in adult antennae. Functional analyses of the six PR genes were then conducted in the heterologous expression system of *Xenopus* oocytes. HarmOR13 was found to be a specific receptor for the major sex pheromone component Z11-16:Ald. HarmOR6 was equally tuned to both of Z9-16:Ald and Z9-14: Ald. HarmOR16 was sensitively tuned to Z11-16: OH. HarmOR11, HarmOR14 and HarmOR15 failed to respond to the tested candidate pheromone compounds. Our experiments elucidated the functions of some PR genes of *H. armigera*. These advances may provide remarkable evidence for intraspecific mating choice and speciation extension in moths at molecular level.

INVOLVEMENT OF A CYSTEINE-RICH INTRACELLULAR LOOP IN THE ACTIVATION OF *DROSOPHILA MELANOGASTER* ODORANT CO-RECEPTOR (ORCO)

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Insect odorant receptors function as heteromeric odorant-gated cation channels comprising a conventional odorant-sensitive tuning receptor, and a conserved co-receptor (Orco). A synthetic Orco agonist, VUAA1, is able to activate both heteromeric and homomeric Orco-containing channels. Very little is known about specific residues in Orco that are required for channel activation. DmelOrco contains 8 cysteine amino acid residues, 6 of which are highly conserved in other insect taxa. To investigate the importance of these amino acid residues, individual cysteines were substituted, one at a time, with either alanine or serine. Each Orco variant was expressed in FlpIn 293 T-Rex cells and activity determined by changes in intracellular Ca^{2+} levels in response to VUAA1. Substitution of two cysteines in intracellular loop 3, individually or both together, gave rise to variants where the kinetics of Ca^{2+} influx were much faster than wild-type DmelOrco. We suggest that these two cysteine residues are normally linked through a disulphide bond. The inability of cysteine substitution mutants to form a disulphide bond may facilitate early conformational changes involved in the mechanism required for VUAA1 to activate the channel.

IDENTIFICATION OF CANDIDATE ODORANT RECEPTORS IN THE ASIAN CORN BORER *OSTRINIA FURNACALIS*

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Odorant receptors (including pheromone receptors) play important roles in many insect behaviors such as mate finding and host-plant selection. In the Asian corn borer, 10 pheromone receptors have been identified so far. However, because these receptors were cloned by degenerate PCR based on the expected homology to *Bombyx mori* pheromone receptor, it was not clear whether there are additional pheromone receptors that play important roles in pheromone perception. Furthermore, general odorant receptors were totally unknown. In this study, to identify all the odorant receptors expressed in the antennae, RNA-seq analysis was carried out. Reads obtained from male and female antennae were assembled and screened by the homology to the odorant receptors of *B. mori*, *Drosophila melanogaster* and other insects. As the result, 1) additional new candidate odorant receptors were identified, and 2) expression levels of these candidates were examined by quantitative RT-PCR in various tissues. It is suggested that these candidates can be classified into potential pheromone receptors and general odorant receptors.

SYMPOSIUM TOPIC 14: CHEMICAL ECOLOGY OF INVADING SPECIES

Topic Coordinators:
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TRACKING INDIVIDUAL FLY RESPONSES TO SEMIOCHEMICAL LURES: RADIO-FREQUENCY IDENTIFICATION OF *BACTROCERA DORSALIS* AND *BACTROCERA CUCURBITAE*

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Tephritid fruit flies respond to a number of semiochemical cues including volatiles from host plants, food (protein) odors, and compounds related to mating (pheromones and parapheromones). Olfactory responses are fundamental to detection and control of pest tephritids, and vary with age, physiological state, and environmental conditions. In order to study behavioral responses to olfactory cues it is often important to be able to identify individual insects and record information about their activity over time. To this end, insects are usually marked and then followed by direct observation or video. Shortcomings of these techniques include the limited number of individuals that can be monitored, limited experiment duration, and requirements for observation under artificial conditions. It is now possible to collect insect behavioral data by use of radio-frequency identification (RFID) technology. RFID is used to study eusocial insects, bees and ants, by attachment of an RFID tag to individual insects, which is then read when the marked insect passes close to a reader. In order to study tephritid olfactory response behaviour, we have developed methods for tagging flies with RFID and automated reader stations (reading stations record proximal flies and release attractant compounds at pre-programmed time intervals). These methods have the potential to provide large behavioral data sets while addressing experimental variables such as age, diurnal patterns, environmental conditions, physiological state, multiple attractants, etc. Our application of RFID to tephritids allows analysis of behaviour with a degree of detail not obtainable via other observation techniques.

ALLELOPATHY AND ALLELOPATHIC ACTIVE SUBSTANCE IN INVASIVE AQUATIC PLANT WATER HYACINTH

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Water hyacinth (*Eichhornia crassipes*) is one of the most noxious invasive aquatic plants in many countries due to its rapid growth and reproduction rate. Most practical methods to control water hyacinth are mechanical removal of the plants, and chemical and biological controls with large expense. We investigated possible allelopathic property and substances in water hyacinth. An aqueous methanol extract of water hyacinth inhibited the growth of roots and shoots of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), timothy (*Phleum pretense*) and ryegrass (*Lolium multiflorum*). Increasing extract concentration increased the inhibition. These results suggest that water hyacinth may have allelopathic property and contain allelopathic active substances. The extract was then purified by several choromatographies with monitoring the inhibitory activity and the main allelopathic active substance was isolated. The chemical structure of the substance was determined by spectral data as loliolide. Loliolide inhibited the growth of cress roots and shoots at concentrations greater than 0.3 mM. The concentrations required for 50% inhibition of root and shoot growth of these test plants ranged from 0.44-0.52 mM, respectively. These results suggest that loliolide may be allelopantic substance and the main contributor to the growth inhibitory effect of water hyacinth. The water extract of water hyacinth also inhibited all test plant species concentration dependently. Therefore, water hyacinth is allelopathic and this property may work to grow in natural ecosystems.

COMPETITION BETWEEN INASIVE ANTS AND NATIVE ANTS—CHEMICALS JETTED BY THE GHOST ANT *TAPINOMA MELANOCEPHALUM* FABRICIUS SUPPRESSED THE ACTIVITY OF RED IMPORTED FIRE ANT *SOLENOPSIS INVICTA* BUREN

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The red imported fire ant *Solenopsis invicta* Buren was found to be an alien invasive species in mainland China in Sept., 2004, and has infected more than 8 provinces and special administrative regions now. Damage, especially to human health, caused by the fire ant has been obvious in the above regions. The influence of the fire ant on the native biodiversity of South China has also been paid attention to. The obvious diversity reduction of native ant community in typical habitats of south China was revealed in some reports, and several native ants could compete and coexist with the fire ant. Competitive advantage of the ghost ant *Tapinoma melanocephalum* Fabricius to the fire ant at the individual and population levels was observed. Of those, the chemicals jetted by *T. melanocephalum* played a role in its competition with *S. invicta*. The repellent effect of the chemicals on the fire ant was obvious, and the ghost ant suppressed the activity and attack of the fire ant workers by utilizing this chemical weapon. The fire ant workers disliked the secretions from the ghost ant very much, and dodged and ran away rapidly when the ghost ant jetted the chemicals. That was the probable reason why the ghost ant could coexist with fire ant and be one of the dominant ant species in south China.

SEED VOLATILE ALLELOCHEMICALS OF INVASIVE *HERACLEUM SOSNOWSKYI* MANDEN

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Heracleum (Apiaceae) is of particular interest as one of the most invasive species in European countries. It is recognized as rapidly spreading plants due to high seed productivity, high germination percentage, toxic and allelopathic characteristics. *Heracleum* usually produce dense coverage of land by their seed, and specific unpleasant flavor, however, information on seed volatile compounds and their role in invasiveness is limited. This study focuses on the determination and identification of volatile chemicals from the seed of *Heracleum sosnowskyi* Manden and their allelopathic activity. Seeds of *Heracleum sosnowskyi* Manden were collected between August-September, 2011-2012 from different locations of Western Russia, Far East of Russia and central part of Belarus, where this plant is reported to be invasive. The dish pack method was implemented to assay the growth inhibitory effect of volatile compounds of Heracleum seeds. Cotton swab method was used to determine the individual contribution of major components in a total inhibitory activity. The volatile composition of the seeds were analysed by GC-MS (model QP 2010 Plus, Shimadzu, Japan). Bioassay with the dish pack method showed that volatile chemicals from the seeds of *Heracleum sosnowskyi* Manden demonstrated inhibitory effect with location-specific variations. GC-MS determined that the major volatile components from *Heracleum sosnowskyi* Manden seeds are octanal, 1-octanol and 1-octyl acetate. The other volatiles detected in trace amounts include hexanol, propan-1-yl 2-methylbutanoate, isopropyl isovaleriate, n-hexyl acetate, *o*-cymene, n-hexyl isobutanoate, hexyl 2-methyl butyrate. Using cotton swab method, we have concluded that octanal is the main volatile chemical responsible for the inhibitory activity by *Heracleum sosnowskyi* Manden seed.

KAIROMONE ATTRACTANTS FOR THE COCONUT RHINOCEROS BEETLE, *ORYCTES RHINOCEROS*

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The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros*, is an invasive pest of coconuts and other palms. Native to Southern Asia and distributed throughout Asia and the Western Pacific, adults burrow into the crowns of coconut trees, causing frond damage which appears as characteristic triangular cuts and can eventually kill trees. An aggregation pheromone, ethyl 4-methyloctanoate, has been previously identified and is used widely for CRB trapping. However, some populations of CRB appear to be less responsive to ethyl 4-methyloctanoate. Additionally, trap captures of CRB are enhanced when the aggregation pheromone is combined with coconut material as an attractant. For these reasons, CRB associated materials were investigated for potential attraction. Y-tube bioassays were conducted, and several plant materials were found to be attractive. Headspace collections from these materials were analysed by gas-chromatography electroantennographic detection (GC-EAD) with a number of compounds showing responses. Behavioral bioassays of EAD-active compounds are ongoing. It is hoped that the identification of kairomones attractants for CRB will increase trapping efficiency and lead to greater control of this invasive pest.

ERADICATION WITH FINESSE: USE OF MULTIPLE TACTICS FOR IMPROVED ERADICATION SUCCESS

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Biosecurity managers are grappling with accelerating rates of pest invasion and establishment and there are limited tools that they can use to attempt eradication once a response is initiated. Tools such as broad-spectrum insecticides are often deemed too hazardous or may not be appropriate in sensitive areas, so 'softer' tools may be required. Tools may be density dependent or independent and may target different life stages of the pest under eradication. Eradication attempts using multiple tactics used concurrently or in a stepwise fashion, particularly when only density-dependent tools are available for use, may increase the chance of eradication success by increasing public palatability and through additive and synergistic results of the tools being used. Currently, the Global Eradication and Response Database (GERDA) contains records from 103 countries on 972 arthropod and plant pathogen incursion responses, of which 889 became eradication programmes. We used the database to determine the likelihood of eradication success based on the number of tools used for eradication and how the tools were used (i.e. stepwise or concurrently), highlighting the compatibility of particular tools for use in eradication attempts. This project includes strong international collaboration between Better Border Biosecurity B3 (New Zealand), National Center for Ecological Analysis and Synthesis (NCEAS) (USA), US Forest Service (USA), PRATIQUE: enhancements of pest risk analysis techniques (EU), and the Plant Biosecurity Cooperative Research Centre (PBCRC). Such global collaboration in eradication science should improve our ability to respond effectively and efficiently to future invasion pressures.

SYMPOSIUM TOPIC 15: APPLIED CHEMICAL ECOLOGY: NEWEST RESEARCH AND DEVELOPMENT

Topic Coordinators:
Alex IL'ichev (Department of Environment and Primary Industries, Australia)
and **Jerry Zhu** (USDA, USA)

ATTRACTION OF BRACONID *APANTELES* WASPS, PARASITOIDS OF THE TEA GEOMETRID LARVAE, TO COMBINATIONS OF VOLATILE AND COLORS RELATED TO TEA PLANTS

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The tea geometrid, *Ectropis oblique* (Prout), is one of the most important pest insects in tea plantations. Two species of braconid wasps, *Apanteles* spp. are major larval parasitoids of the tea geometrid. Compared with those from the intact tea shoots, the contents of (*E*)-2-hexenal, (*E*)-ocimene, linalool, *α*-farnesene, benzaldehyde or (*E*)-2-pentenal were higher in the damaged tea shoots. EAG test indicated that following tea shoot volatiles: (*E*)-2-hexenal, (*E*)-2-pentenal, benzaldehyde, *α*-farnesene, phenethyl alcohol, linalool, methyl salicylate and methyl jasmonate, as well as a mixture of (*E*)-2-hexenal, linalool and *α*-farnesene (mixture 1) were strongly active by the *Apanteles* wasp antennae, with the mixture 1 being the strongest. Among the 12 tested colors, bud green and yellow jasmine displayed significantly stronger attraction to *Apanteles* spp. than did the other colors. In tea plantations, the attraction of synthetic mixture 1, *E*-2-hexenal, (*E*)-2-pentenal, benzaldehyde, *α*-farnesene, and other nine individual volatile candidates to *Apanteles* wasps were compared through trapping experiments, with the mixture 1 being the strongest. When the bud green board (non-sticky) baited with the synthetic mixture 1 was applied in the field during the early geometrid larval stages in the third generation (that normally causes the most damage on the tea plants), the control effect via increased parasitism by *Apanteles* wasps exceeded 50 %. Our results showed that the synomone-baited colour boards, attractive to *Apanteles* wasps, might have a potential for an enhanced biological control against the economically important tea geometrid moth in the tea plantations.

ATTRACTION OF TURNIP SAWFLY, *ATHALIA ROSAE* (HYMENOPTERA: TENTHREDINIDAE) TO METHYL SALICYLATE

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In the process of searching attractant(s) for *Lygocoris* (*Apolygus*) *spinolae* (Hemiptera: Miridae), we found that methyl salicylate (MeSA) attracted turnip sawfly, *Athalia rosae*. Turnip sawfly is distributed in Asian and European countries, and feeds on leaves of cruciferous vegetables. We did two years' field study. In the 1st year, we tested the attractiveness of four chemicals, *n*-tridecane, acetaldehyde, MeSA, and phenylethyl alcohol, using yellow sticky traps. Among them, MeSA attracted significantly more number of turnip sawflies than others did. There was no sexual difference in the number of sawflies attracted. We tested four different amount of MeSA in the 2nd year. However no difference was found in sawfly catches among lure amounts from 10 ul to 180 ul per rubber septum. We could not find any attractiveness of MeSA to *Coccinella septempunctata* (Coleoptera: Coccinellidae) and *Chrysopa nigricornis* (Neuroptera: Chrysopidae), even though they have been known to be attracted to MeSA. Thus, MeSA may be used as an attractant for monitoring the turnip sawfly.

THE INTERACTION BETWEEN *DIATRAEA SACCHARALIS* (LEPIDOPTERA, CRAMBIDAE) AND SUGARCANE: CHANGES IN PLANT'S PROTEOME

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The crescent global demand for renewable energy sources to replace fossil fuels has given a great interest to sugarcane (*Saccharum* sp.). Brazil is the main world producer, where sugarcane has been cultivated in 8.5 million hectares producing up to 600 million metric tons in 2012/2013. Biotic stress is responsible for significant sugarcane losses and it has been estimated that around 10% of this crop losses are caused by insect pests, from which the sugarcane stem borer (*Diatraea saccharalis*) is the most important. In order to reduce insect damage, plants have evolved complex and varied defense mechanisms, including, physical barriers, toxic and volatile metabolites, and defense proteins. Here, by using a two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/TOF), we identified the proteins in phenolic extracts of leaves that both were wounded and treated with oral secretion (OS) of *Diatraea saccharalis*. The phenolic extracts yielded approximately 650 protein spots, and 169 of them were altered by elicitation. In general, proteins that had an increased expression are involved in primary metabolism, defense, and transcriptional and translational regulation; while those that had a decreased expression are involved in photosynthesis. Systemic suppression of photosynthesis in herbivory by caterpillars has often been described for other plants. We concluded that the the response of the plant´s proteome to herbivory is complex, however the integration of proteomics and the chemical ecology may facilitate the understanding of this ubiquitous ecological interaction and so enable the pest management.

PRACTICAL APPLICATION OF A SEX PHEROMONE FOR MONITORING THE JAPANESE MEALYBUG, *PLANOCOCCUS KRAUNHIAE* (HOMOPTERA: PSEUDOCOCCIDAE)

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The Japanese mealybug, *Planococcus kraunhiae* is distributed in Japan (west from the Southern Kanto district), China, Eritrea, and North America. It is a serious pest of persimmons and grapes in Japan. Fruit damage by this species has increased gradually since the 1990s, so establishment of monitoring by attractant-baited traps has been desired. 2-isopropyliden-5-methyl-4-hexen-1-yl butyrate has been identified as a sex pheromone of the Japanese mealybug. The sex pheromone is emitted by females to attract males. Narai et al. gave a poster presentation on sex pheromone quantity per lure at APACE 2009 in Hawaii, so we studied a pheromone trap with the sex pheromone for monitoring. Experiments to clarify pheromone quantity per lure, active life, and shelf life were conducted in grape orchards and a persimmon orchard in Shimane prefecture (Western Japan). A red rubber septum (8 mm o. d., Sigma-Aldrich Co.) containing the sex pheromone was used as a lure. A triangular trap with the lure was hung from a branch of a tree at a height of 1.5 m. The interval between traps was about 10 m. A pheromone trap had a capturing efficacy of 90% four weeks after placement (80% eight weeks later) when a lure with 100 µg of the sex pheromone was used. Lures in an air-tight bag could be stored for one year after manufacture in a refrigerator. However, capturing efficacy decreased to 60%, when a lure was kept at 25 degrees Celsius in a dark place.
Sugie et al. 2008. *Appl. Entomol. Zool.* 43, 369-375.

FROM CHEMICAL ECOLOGY TO APPLICATION: A FLASHBACK OF OLFACTORY RESPONSE LEADING TO AREA-WIDE MANAGEMENT OF THE FRUIT FLY, *BACTROCERA DORSALIS* IN INDIA

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The Oriental Fruit Fly (OFF) *Bactrocera dorsalis* Hendel is a pest of mango, (*Mangifera indica*) in India, causing 20-80% loss. Since the discovery of methyl eugenol (ME) as a male attractant of OFF by Howlett in India in 1912, global interest in this compound has resulted in utilizing it in male annihilation. Almost 90 years after the discovery of ME, a joint India (ICAR)–UK (DFID) project began to investigate OFF control options using the ability of ME to lure Tephritids. An ecofriendly dispenser of the lure was found to be plywood blocks (1.5 x1.5 x 3 cm) with maximum half–life of catch decays of 109 days. This dispenser when placed in a holed plastic receptacle (750 mL capacity) served as a trap that could significantly attract more males than many other trap designs. A series of field investigations showed that at least 3 mL/acre of ME spaced through 0.5ml/lure trap was optimally needed to attract almost all the males in a unit area. Thus six traps/acre of mango orchard was recommended. This when extended on an area wide base annihilated males resulting in >95% control of OFF. When jaggery (a secondary product of sugar industry) baits were supplemented, the control was >99%. In 2011 and 2012, the loss saved was to the tune of ₹4800 and ₹5200 crores (861.06 and 932.82 million Aus$) respectively in South India. This is one of the best examples of application of a facet of chemical ecology in insect management.

MALE RESPONSES TO CHEMICAL MATING CUES IN DIFFERENT POPULATIONS OF WHITE-SPOTTED LONGICORN BEETLES, *ANOPLOPHORA MALASIACA: HYPOTHESIS OF MATE LOCATION AND RECOGNITION SYSTEM*

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Host plant range of *Anoplophora malasiaca* is very wide and this species feeds on a variety of horticultural crops such as citrus, apple, and blueberry, and, urban landscape trees such as maple, poplar, willow etc. There is no evidence of having a volatile attractant pheromone in this species. How do adults find their mates? We'll review the main findings of our study and propose a hypothesis for the mate location and recognition system of *A. malasiaca*. Adult *A. malasiaca* were collected from field populations inhabiting three different host plants: *Citrus, Salix* and *Vaccinium*. Males of all three populations were attracted most frequently to odors of their wounded original hosts, indicating that male orientation behaviour is induced by the odors of the fed male's host plants. We also observed the mating responses of the males to elytra extracts from young and mature females of the three populations and chemically analysed those extracts. Males of three populations differentially responded to various female extracts but responded more to extracts from mature female extracts, indicating that males might distinguish female origin and maturity. All eight hydrocarbons, four ketones, and three lactones that were previously identified as female contact sex pheromones were found in all the extracts. Higher amounts of lactones on mature females likely caused greater male responses. These results suggested that males would be more likely to approach a female feeding on the same host plant and subsequently recognize her sexual maturity of females based on the profile of contact sex pheromones on her elytra.

SYMPOSIUM TOPIC 16: STUDENT TRAVEL AWARDS SYMPOSIUM (YOUNG CHEMICAL ECOLOGISTS)

Topic Coordinators:
Ring Cardé (University of California, USA), **Jocelyn Millar** (University of California, USA) and **Yongping Huang** (Chinese Academy of Science, China)

RESPONSE OF THE FEMALE OF *BACTROCERA CUCURBITAE* TO HOST FRUIT ODOURS AND TO SELECTED SEMIOCHEMICALS FROM CUCURBIT FRUITS

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The Melon fly, *Bactrocera cucurbitae* (Diptera, Tephritidae) is one of the most important pests attacking Cucurbitaceae crops in tropical regions of the world such as Reunion Island, causing high level of damage. Host plant semiochemicals, which are critical in host finding behaviour of females, can be considered as promising sources of new attractants. A first part of this study aimed at evaluating in small test cages the relative attractiveness for the females of the odours of various host fruit species in the Cucurbitaceae family. Large differences in attractiveness were observed between the tested species, and, for a given species, between varieties and between phenological stages. Best responses of the females were for instance observed with mature fruits of *Luffa cylindrica* (sponge gourd) and *Cucumis sativus* (cucumber). Depending on the cucurbit species, the most attractive was the odour of young fruit or the odour of mature fruit. For a given cucurbit species, the nature of the variety also had a strong influence on the female response, as was observed in cucumber. In a second step, the volatile emissions of the fruits were characterized according to their stage of ripening using headspace solid-phase microextraction (HS-SPME) collection and gas chromatography/mass spectrometry (GC/MS) detection. Finally, ten volatile compounds (alcohols, aldehydes and a terpene) selected according to the chemical analysis were tested individually or in blends in olfactometer tests. The most promising compounds or blends should be further tested for their attractiveness in field experiments in the future.

DEFENSIVE BEHAVIOUR OF LARVAE OF GREEN LACEWING AGAINST APHID-TENDING ANTS: APHID CARCASSES ON THEIR BACKS FUNCTION AS CHEMICAL CAMOUFLAGE

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In ant-aphid interactions, ants attack and exclude natural enemies of aphids. However, larvae of the green lacewing *Mallada desjardinsi* (Neuroptera: Chrysopidae) prey on the aphid, *Aphis craccivora* (Homoptera: Aphididae), without exclusion by ants, and the lacewing larvae carry carcasses of aphids preyed by themselves on their backs on aphid-colonized plants. We, therefore, tested the defensive role of the aphid carcasses on the back of lacewing larvae against aphid-tending ants. When we removed aphid carcasses from the back of lacewing larvae, time spent by the larvae on aphid-colonized plants was significantly reduced due to an increase of ant aggressions, resulting in a decreased consumption of aphids. Furthermore, we focused on the role of chemicals on the aphid carcasses as a factor to reduce ant aggressiveness. Lacewing larvae, “living” aphids, and aphid carcasses on the back of lacewing larvae were extracted with *n*-hexane, and the extracts were purified with silica gel column. We measured aggressiveness of aphid-tending ants to the lacewing larvae which were carried a piece of cotton wool applied with above extracts. The ants attacked lacewing larvae with the extract of the conspecifics more than ones with the extract of aphids regardless of “living” or preyed. Chemical analysis with GC-MS showed the difference of chemicals between the extracts of lacewing and aphids irrespective of the types of aphids. These results suggest that the aphid carcasses on the back of lacewing larvae function as chemical camouflage against aphid-tending ants and enable the larvae to prey on aphids without exclusion by ants.

MOLECULAR EVOLUTION OF SERINE PROTEASE SUPERFAMILY AND ADAPTIVE RESPONSE TO SOYBEAN KUNITZ TRYPSIN INHIBITOR IN *HELICOVERPA ARMIGERA*

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Herbivorous lepidopteran insects rely on proteases for digestion of plant material that is generally low in protein content. To deter herbivory, plants produce protease inhibitors (PIs) to inhibit insect digestive proteases. Insects adapt to overcome PI, by increasing production of their proteases and/or evolving PI-insensitive proteases. We are interested in understanding the regulation of PI-sensitive and PI-insensitive proteases of *Helicoverpa armigera,* in response to SKTI, a Kunitz-type trypsin inhibitor from soybean. First we annotated a total of 113 serine protease genes. Five major groups are inferred by phylogenetic analysis; one azurocidine-like while two chymotrypsin groups have different pro-peptide lengths. Protein sequences are highly conserved at their N-terminal signature sequence and catalytic residues H_{57} D_{102}, S_{195}, although catalytic residues are mutated in some sequences. Midgut transcriptional response of 4th instar *H. armigera* to SKTI in artificial diet measured by microarray shows up-regulation of several trypsins at first, but their expression decreases gradually, while expression of up-regulated chymotrypsins and their number increases over time. Enzyme specific activity assays over time confirms this pattern. Affinity chromatography, 2D gel electrophoresis and mass spectrometry was used to identify sensitive/insensitive protease from the lumen of 5th instar *H. armigera* fed on SKTI containing artificial diet. Some of the alkaline and diverged serine proteases appear to be insensitive to SKTI, although it is difficult to distinguish among highly similar genes. Experiments are ongoing to quantify the expression of identified PI sensitive/insensitive proteases from affinity purification.

FUNCTIONAL DIFFERENTIATION OF PHEROMONE-BINDING PROTEINS IN THE COMMON CUTWORM *SPODOPTERA LITURA*

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Pheromone-binding proteins (PBPs) are thought primarily to bind and transport sex pheromones in moth antennae. Considering multiple components of sex pheromone and multiple PBP genes exist in a single species, it is hypothesized that PBPs may contribute to the discrimination of different sex pheromone components. To test this hypothesis, we conducted a comparative study on three PBPs from *Spodoptera litura*. Quantitative real time PCR showed that three SlitPBP genes were transcribed at different levels in male (PBP1 = 3.0×PBP2 = 9.7×PBP3) and female (PBP1 = 1.6×PBP2 = 2.6×PBP3) antennae, thus exhibited a very different sex-biased transcription in adult antenna with male/female ratio of 2.7, 1.4 and 0.7 for PBP1, PBP2 and PBP3, respectively. Furthermore, ligand-binding assays showed that three SlitPBPs exhibited an obvious difference in binding affinities to female sex pheromone components (PBP1 > PBP2 >>PBP3), but these SlitPBPs were not capable of discriminating a specific sex pheromone component. Similar results were also obtained from studied pheromone analogs. In addition, binding assays indicated that although three SlitPBPs showed a similar pH-dependent conformational change, behaved differently across a pH 4.0 to 9.0. Taken together, our data suggest that in *S. litura* PBP1 and PBP2 may play critical roles in the perception of female sex pheromones, but do not show an obvious discriminative ability among different sex pheromone components; whereas PBP3 may play minor roles or have other functions.

FUNCTIONAL ANALYSIS OF HOST PLANT VOLATILES IN THE REGULATION OF OVIPOSITIONAL BEHAVIOR IN THE YELLOW PEACH MOTH, *CONOGETHES PUNCTIFERALIS*

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Host plant volatiles induce oviposition in the yellow peach moth *Conogethes punctiferalis* (Honda and Matsumoto, 1984), but the detailed functions of the volatiles in a series of oviposition behavioral responses are still unknown. The complete process leading to oviposition was explored in wind tunnel tests and divided into five behavioral components; take off flight from release point, half way flight, hovering close to source, landing and egg-laying. Subsequently, the effects of host plant odors on these behavioral components were analysed by providing continuous or transient odor plumes during each behavioral component. Plant odors accelerated the take off flight and increased orientation to stimulus source in hovering and landing. These odors also stimulated females to lay more eggs resulting in a significantly longer time for egg-laying with no decrease in the time necessary to lay each egg. The absence of plant odors delayed take off flight and interrupted the subsequent behavioral responses. These results indicate the host plant stimuli induce orientation by flight and stimulate egg-laying, and may also independently regulate each component of the behavioral responses by the central nerve system for oviposition in the yellow peach moth.

SYMPOSIUM TOPIC 17: CHEMICAL ECOLOGY (GENERAL TOPICS)

Topic Coordinators:
Myron Zalucki (University of Queensland, Australia) and **Alex IL'ichev** (Department of Environment and Primary Industries, Australia)

TOXICTY EFFECT OF WEED *CHROMOLAENA ODORATA* AGAINST TERMITES *MACROTERMES CARBONARIUS* AND *GLOBERTERMES SULPHUREUS*

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The essential oils of leaves and stem of the weed *Chromolaena odorata* in Malaysia were examined for insecticidal properties against termites *Macrotermes carbonarius* and *Globertermes sulphureus.* In fumigant assays, LC_{50} value for *M. carbonarius* worker was 1.54 ppm/termite after 12 hours exposure while for worker *G. sulphureus* worker the LC_{50} was 6.33 ppm/termite iindicating that *G. sulphureus* was less susceptible The LC_{50} for soldier *M. carbonarius* was 1.05 pm/termite while the LC_{50} soldier *G. sulphureus* was 4.76 ppm/termite. Analysis using GCFID and GCMS revealed a number of potential compounds responsible for the mortality of both species of termites. Both stems and leaves of *C. odonata* essential oil could be used as an alternative control for termites.

OVIPOSITION PREFERENCE AND LARVAL PERFORMANCE OF *HELICOVERPA ASSULTA* IN HOST AND NON-HOST PLANTS

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The oriental tobacco budworm, *Helicoverpa assulta*, is an oligophagous insect feeding on a few species of Solanaceae plants, including *Nicotina tabacum*. This study investigated the relationship between *H. assulta* and plants, focusing on oviposition preference and larval performance of the specialist in host and non-host plants. (1) In choice experiments, *N. tabacum* and *Phaseolus vulgaris* were put in a cage with 5 females and 10 males. Most of eggs (ca. 98%) were layed on *N. tabacum*, whereas few eggs on *P. vulgaris.* (2) In non-choice experiments, *N. tabacum*, *Datura stramonium*, or *P. vulgaris* was individually put in a cage with 3 females and 6 males, resulting that *N. tabacum* was the most preferred host, followed by *D. stramonium* and *P. vulgaris*. Oviposition was delayed about 2 days on *P. vulgaris* (a non-host plant) compared to the two host plants. (3) Larval performances were also compared with the three plants. Larval growth was better on *N. tabacum* followed by *D. stramonium* and *P. vulgaris*. High mortality (ca. 100%) of larvae was observed on *P. vulgaris*, suggesting toxic component(s) derived from the non-host plant might cause the death. The ovipositional avoidance on *P. vulgaris* is currently being investigated to search for the oviposition deterrent, which could be useful for the environment-friendly pest management of *H. assulta*.

EFFECT OF INTERTRAP DISTANCE ON THE CAPTURE OF LARGE WOODBORERS (COLEOPTERA: CERAMBYCIDAE)

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In North America, woodboring beetles from the family Cerambycidae can have significant negative economic and ecological effects on forest ecosystems, and the rate of introduction and/or detection of exotic species, is increasing. Monitoring programs for exotic and native forest insects frequently rely on traps baited with odorants, that mediate the orientation of target taxa, towards a resource (e.g., mates, host material). Effective survey and detection tools are essential because: 1) as populations of the invasive species increase, the probability of containment and eradication decreases; 2) successful containment and eradication requires that the distribution of the target species be accurately defined, on an ongoing basis; and 3) evaluation of the success of management efforts is not possible without good monitoring tools. Little is known about how spatial factors (e.g., distance between traps) influence the efficacy of survey and management programs that employ intercept traps. This study used hexagonal arrays of odorant baited intercept traps to examine the impact of intertrap distance on the efficacy of intercept traps for large woodboring beetles (Coleoptera: Cerambycidae).

ACARICIDAL ACTIVITY OF *CRYPTOMERIA JAPONICA* LEAF COMPONENTS AGAINST SPIDER MITES

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Spider mites are known as pest for soybean, tea, and other vegetables. *Cryptomeria japonica* is a main plantation tree in Japanese forest. The leaves and bark of *C. japonica* are regard as woody wastes. The developments of new utilization methods of the wastes have been required in Japan. The leaves of *C. japonica* defy the vegetable spider mites in natural forest. It is expected that the leaf components of *C. japonica* have acaricidal or repellent activity against spider mites. In this study, the acaricidal activities of extracts obtained from *C. japonica* leaves were investigated against two spider mites of *Tetranychus kanzawai* and *Tetranychus urticae*. The *C. japonica* leaves were extracted by hexane, ethyl acetate and methanol successively. The activities were tested by leaf disc and slide methods. Hexane extract of the *C. japonica* leaves showed strong acaricidal activity compared with other solvent extracts. The hexane extract was fractionated by silica gel chromatography and then the fractions were examined acaricidal activities for the two mites. Potent acaricidal activities were observed on ent-kaurene of diterpene hydrocarbon fraction. ent-Kaurene has been known as a major component of diterpene part in *C. japonica* leaves. There are also known other chemo-types of *C. japonica* that contained phyllocladene or ent-sclarene instead of ent-kaurene as a major diterpene in leaves. Therefore we examined acaricidal activity of phyllocladene and ent-sclarene against *T. kanzawai* and *T. uticae*. ent-Scralene showed acaricidal activities similar as ent-kaurene. The acaricidal activities of phyllocladene were weak compared with ent-kaurene and ent-sclarene.

DIURNAL AND NOCTURNAL HERBIVORE INDUCTION ON MAIZE ELICIT DIFFERENT INNATE RESPONSE OF THE FALL ARMYWORM PARASITOID, *CAMPOLETIS FLAVICINCTA*

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Herbivore-attacked plants produce specific volatile substances that represent important cues for host finding by natural enemies. The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a voracious herbivore and usually feeds on maize in all periods of the day. Given that plants need light to synthesize de novo herbivore-induced volatiles, volatile blends may be changed depending on time of the day the plant is induced, and could interfere in natural enemy foraging. In this sense, the current study aimed to investigate differential attractiveness of maize elicited by fall armyworm regurgitant under light and dark conditions to its specialist larval parasitoid *Campoletis flavicincta* (Ashmead) (Hymenoptera: Ichneumonidae). All bioassays were conducted in Y-tube olfactometer to assess parasitoid response to odors from undamaged maize, mechanical damage, and regurgitant-treated plants at 0–1, 5–6, and 24–25 h after induction. The results showed that naıve wasps were attracted to volatiles emitted by nocturnal regurgitant-treated maize at 5–6 h, but not to odors from diurnal regurgitant-treated plants. The differential attractiveness is likely due to blend composition as nocturnal regurgitanttreated plants emit aromatic compounds and the homoterpene (3E)-4,8-dimethyl-1,3,7-nonatriene in larger amounts than diurnal-treated plants.
Financial Support: FAPESP

BEHAVIOURAL RESPONSE OF THE GRAPE WEEVIL (COLEOPTERA: CURCULIONIDAE) TO HOST PLANT VOLATILES

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The grape weevil *Naupactus xanthographus* is a polyphagous insect, that causes significant damage to economically important crops in countries of southern South America. It considered a primary pest due the damage caused by larvae and adults. Furthermore, it has quarantine status in countries like Japan and The United States. Its main host species include economically important species such as grapes, avocados and apples. In the present work, we studied the behavioral responses of males and females to different volatiles of two host plants, grape (*Vitis vinifera*) and avocado (*Persea americana*). We collected the constitutive and herbivore-induced host plant volatiles in headspace entrainment equipment. The olfactory response to the plant volatiles and to synthetic compounds was determined using a Y-tube olfactometer. Finally, we identified some volatiles compounds of both host plants by GC/MS. Our results indicate that males are attracted to: i) constitutive volatiles over control, ii) herbivore-induced volatiles over control and iii) herbivore-induced volatiles over constitutive volatiles for both host plants. Males showed no preference when presented simultaneously to volatiles of both host plants. Females showed no significant preference to constitutive volatiles of both host plants compared to a control. The GC/MS analysis identified some volatiles, mainly GLVs, monoterpenes and sesquiterpenes. We hypothesize that the difference in the behavioral responses is associated with the variability of volatiles emitted. Statistical analyses of volatiles as well as olfactometer bioassays are being performed to test this hypothesis. Preliminary tests with synthetic compounds showed that males are attracted to limonene and linalool.

IMPACT OF THE VARIATIONS IN TEMPERATURE ON POLYMER INCORPORATED WITH COFFEE STEM BORER PHEROMONE

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Coffee stem borer, *Xylotrechus quadripes* Chevrolet (Coleoptera: Cerambycidae) is a serious pest of arabica coffee in India. Among the various options for the management of *X. quadripes* use of the aggregation pheromone (*S*)-2-hydroxy-3-decanone at 75mg per lure is supported at 10 traps per acre with cross wind sticky traps. Slow release formulations are preferred for sustained release and longer shelf life. A formulation of polymer incorporated pheromone was developed at this institute with a view to substitute the presently used dispensers. The present investigation was carried out to understand the effect of different temperatures on the polymers incorporated with the pheromone. Thermal analysis revealed that there was closely packed arrangements of the coffee stem borer pheromone (CSBP) with the polymer. The pheromone being a volatile organic compound showed faster release in comparison to the polymer embedded with CSB pheromone, thus showing the slow release effects. The studies revealed that formulation 1 at 17°C, 28°C and 38°C, showed constant 1.5 times slower rate release of pheromone in comparison to control i.e. liquid CSBP (28.73µl). Similarly formulation 2 showed 1.4 times slower release rate at 17°C, 1.5 times at 28°C and 1.2 times at 38°C in comparison to control pheromone (57.46 µl). The EAG studies of the pheromone incorporated with the polymer showed the percentage over honey and net amplitude data analysis was statistically non significant respectively in both formulation 1 and formulation 2, predicting that there was no change in the chemical structure when the CSBP was mixed with the polymer also indicating that the species specificity was well maintained. Thus the new formulations of CSBP embedded with the polymer showed slow release characteristic at different temperatures.

PHENOLICS IN A DEVELOPING MANGO DETER OVIPOSITION IN CASE OF *BACTROCERA DORSALIS* (HENDEL)

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Anti-herbivory compounds are the secondary metabolites present in plants which play an important role against insect infestation. Among them phenolics is an important class. A study was undertaken at Indian Institute of Horticultural Research, Bangalore ($12^0$58'N; $77^0$35'E) to know its role in five different varieties of mango against a well known polyphagous insect pest the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Tephritidae: Diptera). This species attacks mango and causes serious loss ranging from 50 to 80%. Varieties selected for the study were *cv*. Banganapalli, Alphonso, Totapuri, Langra and EC-95862. The phenol content of different stages of maturity of the selected varieties was assayed following standard procedure. The data were subjected to correlation analysis with the field infestation at harvest with LSD (P=0.05 as test criterion). It was found there was a significant negative correlation between the phenolic content and infestation levels at different maturity stages (50%, 60%, 70%, 80%, 90% and harvest). This clearly showed that increase in phenols deterred the fruit fly from ovipositing paving way for its application in breeding resistant mango varieties.

DEVELOPMENT OF SYNTHETIC ATTRACTANTS FOR MOTHS IN ORCHARDS

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Behavioral bioassays, GC-EAD (gas chromatograph-linked electroantennographic detection) and chemical analysis were carried out to develop synthetic attractants for moths in orchard. After initial evaluation of the attractiveness of various fruit-based and rice wine-based fermenting baits, EAD-active compounds were identified in the headspace samples of the fermenting baits that were attractive to moths. In field trapping experiments, the fermenting baits were attractive only to specific groups of moths. GC-EAD experiments were carried out in various species including *Epiphyas postvittana*, *Planotortrix octo*, *Cydia pomonella* and *Grapholita molesta*. Some compounds were EAD-active across all four species, while some other EAD-active compounds were species-specific. Chemical structures of the EAD-active compounds were subsequently identified by using GC-MS. Single sensillum recording was also carried out to identify olfactory active compounds on individual olfactory receptor neurons (ORNs) in moths. Based on the findings, synthetic blends with various combinations of the EAD-active compounds and ORN-active compounds were tested for their attractiveness to moths in apple orchards and pine forest in Korea and New Zealand. Our results indicate that moths have species-specific sets of ORNs and species-specific attraction to blends of volatiles.

A SMELL OF SPICE: OLFACTORY RESPONSES OF *DEPRESSARIA DAUCELLA* (LEPIDOPTERA:OECOPHORIDAE) LARVAE TO CARAWAY (*CARUM CARVI* L.) VOLATILES

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The caraway moth, *Depressaria daucella* (Denis & Schiffermuller), is a common pest of caraway (*Carum carvi* L.) in Finland and in the Czech Republic. This pest can feed on various Apiaceae such as parsnip (*Pastinaca sativa*) and carrot (*Daucus carota*) but also on wild species (e.g. water dropworts, *Oenanthe* spp.). *D. daucella* larvae hatch from eggs laid on the plant during the spring. Earliest instars feed as stem miners within the growing umbel, while older larvae build a web around the umbel, similarly to the closely related species, the parsnip moth *Depressaria pastinacella*. Florivory is thus known but no study has reported to which extent this behaviour could affect the plant, and which olfactory cues could guide growing larvae to suitable tissues. Research on *D. pastinacella* sixth instar larvae has shown that one of the two esters (octyl acetate), which is typically produced by reproductive parts of wild parsnip, is a feeding deterrent but can serve as an olfactory attractant (kairomone). However, the other ester, octyl butyrate is an olfactory repellent. In this study, we recorded the behaviour of larvae of *D. daucella* and measured larval olfactory responses towards caraway plant, flower and fruit volatiles. Because carvone is one of the main components of caraway fruits, we also tested different combinations of carvone and (R) (+) limonene using a Y tube glass olfactometer. Our study permits understanding for the first time which chemical cues influence the larval behaviour and enable *D. daucella* to locate feeding sites.

DEVELOPING A CHLORFLUAZURON BASED INSECTICIDE BAIT FOR CONTROLLING MULTIPLE TERMITE SPECIES

Partho DHANG[1]

[1]*Independent Consultant*

The pressure for better environmental stewardship forced the chemical industry to look for safer formulations and much safer application techniques. Insect baits are unique formulations which have combined information from insect sensory physiology, gustatory chemistry, semiochemicals and many more. Baits are target specific and allow easy application, thus being recognized as safer alternative to liquid sprays. This article presents a cholrfluazuron based termite bait and a baiting system which have addressed a number of behavioral aspects of termites feeding such as palatability of bait, moisture control, choice of the active ingredient, large food source and no disturbance. This resulted in a wider acceptance of the bait to a number of termite species across Asia Pacific.

SYNTHESIS AND EVALUATION OF OXYGENATED A-IONONE DERIVATIVES AS MALE ATTRACTANTS FOR THE SOLANACEOUS FRUIT FLY, *BACTROCERA LATIFRONS* (DIPTERA: TEPHRITIDAE)

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3-Oxo-α-ionone and its analogs have been characterized as highly specific male lures for the solanaceous fruit fly, *Bactrocera latifrons* (Hendel), although attractiveness of these compounds is not as strong as that of other well known tephritid male lures, such as methyl eugenol for the oriental fruit fly, *Bactrocera dorsalis* (Hendel). We found that 6-hydroxy-3-oxo-α-ionol (vomifoliol, V2) exhibits extremely potent phagostimulant activity to *B. latifrons* males, which spent over 1 hr at the chemical source. However, long distance attractiveness was very low, likely due to its low volatility. In order to develop a male lure for use as a practical monitoring agent, we synthesized a series of analogs particularly to increase the volatility of the molecules by modifying the oxygen functions and chain length. The attractant and phagostimulant activities were evaluated in an indoor bioassay.
(1) Modification of 3-oxo-function of V2: 6-Hydroxy-α-ionone (U1) was synthesized by epoxidation of β-ionone followed by a catalytic cleavage of the epoxide. 6-Hydroxy-α-ionone (U2) was prepared by a partial reduction of U1 using $NaBH_4$. Both U1 and U2 showed slightly higher attractiveness and similar phagostimulant activity when compared with V2. (2) Modification of the side chain of V2: Oxo-butenyl side chain in V2 was replaced with one of the following - H, methyl, ethyl, ethenyl, etynyl, allyl, and butyl moiety. 4-Hydroxy-3,5,5-trimethyl-2-cyclohexenone (substituted by H) and 4-hydroxy-3,4,5,5-tetramethyl-2-cyclohexenone (substituted by methyl) exhibited strong phagostimulant activity. In contrast, highly volatile compounds such as isophorone (3,5,5-trimethyl-2-cyclohexen-1-one) showed high attractiveness but with low phagostimulant activity.

COLD HARDINESS OF ASIAN LONGHORNED BEETLE *(ANOPLOPHORA GLABRIPENNIS*) LARVAE IN DIFFERENT GEOGRAPHICAL POPULATIONS

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Asian Longhorned beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae) is widely distributed in China and other Asian countries. It's also an important quarantine pest all over the world. In order to explore the geographical adaptability to low temperature of ALB, the cold hardiness of larvae from five different geographical populations (Yili in Xinjiang Province, Yanchi in Ningxia Province, Wulateqianqi in Inner Mongolia, DaXing in Beijing City and De Zhou in Shan Dong Province) were compared by measuring the supercooling point (SCP), moisture content (MC), fat content (FC) and glycogen content (GC). All indices of cold hardiness were significantly different among five geographical populations ($P<0.05$). SCP of larvae from Wulateqianqi was the lowest with a mean value of -17.47℃±3.73(SD), while the highest was from DaXing with a mean value of -5.97℃±2.07(SD). The highest moisture content was that of larvae from DaXing. Moisture content of larvae from Wulateqianqi and that from Yili were similar and lower than the others. The fat contents (FC) of larvae from different geographic populations were calculated by variance analysis and shown in the following order: FC (Wulateqianqi)> FC (DeZhou) = FC (Yili)= FC (Yanchi) > FC (DaXing). The comparison results of the glycogen content (GC) were as follows: GC (Yili) > GC (DaXing)> GC (DeZhou) = GC (Yanchi) = GC (Wulateqianqi). Those results indicated that freeze avoidance was an overwintering strategy of ALB larvae to survive from winters. The abilities of cold hardiness of larvae from different regions were associated with the circumstantial temperature of each region. However, there was no obviously correlation between cold hardiness abilities and the contents of their intracorporal physiological substances.

THE ROLE OF JASMONOYL-L-ISOLEUCINE SYNTHASE OSJAR1 IN RICE DEFENSE AGAINST HERBIVORES

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Plants produce jasmonic acid (JA) and its bioactive amino acid conjugate, jasmonoyl-L-isoleucine (JA-Ile) as major defense signals during herbivory. The biosynthesis of JA-Ile in plants, and subsequent activation of defense signaling, is mediated by the GH3 class enzymes known as *JASMONATE RESISTANT* (*JAR*) genes. Out of three *JAR*-like genes in the rice genome, at least two of them previously showed the JA conjugating activity in vitro (*OsJAR1*, *OsJAR2*). However, the biological function of these genes has not been investigated. Here we obtained OsJAR1 retrotransposon TOS17-tagged rice plants (*Osjar1*) and investigated the accumulation of JA-Ile in this mutant. In support of the wound-inducible and simulated herbivory-inducible nature of the *OsJAR1* gene, the *Osjar1* plants contained severely reduced levels of JA-Ile after simulated herbivory. Consequently, the performance of generalist herbivore (*Mythimna loreyi*) larvae was examined using *Osjar1* and wild type rice plants. In addition to the herbivory-associated phenotype, the *Osjar1* plants were male sterile and fertility could be partially restored by direct application of synthetic JA-Ile to the developing *Osjar1* panicles. In summary, we show that OsJAR1 plays an essential role in defense against herbivores, while OsJAR2 is likely to play other developmentally-related functions in rice plants.

RICE DEFENSE AGAINST HERBIVORES: WHAT ARE THE METABOLITES?

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The treatment of rice (*Oryza sativa*) leaves with oral secretions (OS) isolated from the rice generalist herbivores *Spodoptera mauritia* (lawn armyworm) and *Mythimna loreyi* stimulated the accumulation of the major defense hormone, jasmonic acid (JA), and its bioactive form, jasmonoyl-L-isoleucine (JA-Ile), similar to effects of *Manduca sexta* OS on wild tobacco (*Nicotiana attenuata*). It suggests that, as for wild tobacco, rice plants have strong ability to resist herbivore attack and to mount effective defense responses in response to insect feeding. This, most likely, is mediated by active insect elicitors, fatty acid-amino acid conjugates (FACs) in the regurgitate of the feeding larvae. Indeed, the presence of *N*-linolenoyl glutamine and *N*-hydroxylinolenoyl glutamine was confirmed by LC-MS analysis in the regurgitate of both rice herbivores used in the present study. To further monitor the rice responses and gain insights into molecular mechanisms of rice defense against chewing herbivores, we conducted a metabolomics study using control rice leaves and leaves attacked by the adapted "rice strain" of *S. frugiperda* (fall armyworm) herbivore. After LC-TOF/MS analysis, we identified several potentially novel defense-associated metabolic patterns (ions) in rice leaves and the accumulation of these ions, corresponding to unknown compounds, was further verified in time course experiments with *S. mauritia* and *M. loreyi*. Current functional identification of these metabolites is expected to contribute to the understanding of molecular mechanisms of rice defense against herbivores.

BEHAVIORAL EFFECT OF *LOBULARIA MARITIMA* VOLATILES ON *AEDES AEGYPTI*

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Aedes aegypti is considered the main vector of yellow and dengue fever, due to its ample distribution around tropical and subtropical areas, matched with its anthropophilic habitat and blood feeding choices. Hematophagous behaviour of the female *Ae. aegypti* is imperative in order to complete its reproductive cycle. Besides that, both genders consume external sugar sources in order to maintain their energetic balance. To achieve this, they will have to rely on carbohydrate sources present in the domestic environment, mostly in the way of garden and ornamental plants. The goal of this work was to identify possible feeding attracting plants and to determine the behavioral response of their volatile compounds. The plants evaluated in a two-choice feeding assay were: *T. patula, P. neochilus and L. maritima*. The experiment was designed to evaluate feeding preference by tying the plant to a toxic bait against a sucrose solution as a control. Preference was determined by recording mortality at 24h. From the plants tested, only *L. maritima* showed a positive response. Volatiles from *L. maritima* were collected by SPME (DVB/CAR/PDMS) fiber and analysed afterwards in a GC-MS. Volatile compounds were identified by injection of the standard, determination of the Kovats index and/or by comparing the mass spectrum against a library. The identified headspace volatile compounds of *L. maritima* were: Allyl isothiocyanate, 3-butenyl isothiocyanate, benzeneacetaldehyde, acetophenone, 1-octanol, 4-pentenyl isothiocyanate, benzeneethanol, 4,5-epithiovaleronitrile, benzeneacetonitrile, 4-methylpentyl isothiocyanate, hexyl isothiocyanate, 5,6-epithiohexylnitrile, 4-vinyl 2-methoxy phenol and benzyl isothiocyanate. Available compounds were tested for behavioral effect on *Ae. aegypti* in a dual choice Y-olfactometer; females had a statistical significant response to acephenone and 1-octanol, while males responded only to acetophenone.

MONOLITHIC MATRIX DISPENSERS FOR *MEGAPLATYPUS MUTATUS* PHEROMONES BASED ON NATURAL AND BIODEGRADABLE WAXES AND THEIR EVALUATION IN A FIELD TRIAL

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We have developed monolithic matrix devices in a half sphere shape for controlled release of the *Megaplatypus mutatus* known pheromones sulcatone, sulcatol and 3-pentanol. The devices were made with different mixtures of mainly biodegradable or natural waxes or lipids and inert compounds. The bases were paraffins of different melting points, paraffin oil, polyethylene glycols, pentaerythritol ester, lanolin and carnauba wax. The inert sustances employed were: kaolin, glass spheres, molecular sieves and activated charcoal. The release rates of these monolithic systems were measured in the lab in a wind tunnel. Pheromone release rates were plotted and they followed first-order kinetics according to the ecuation $y = ae^{-b\sqrt{t}}$. We determined solubility coefficients of matrices in toluene and 2-pentanol to obtain a quotient between the solubilities of the waxes for posterior correlations. We found positive correlations between the release rates with physicochemical parameters as viscosity, log P and solubility and different amounts of inert substances. For mating disruption field trials in a poplar plantation field, we tested half sphere shape monolithic dispensers made of waxes: carnauba wax for sulcatol and 3-pentanol, and paraffin wax m.p.70-80ºC for sulcatone, using kaolin as inert in an amount of 30% in all the cases.

ZEOLITES AS A DISPENSER FOR SUSTAINED RELEASE RHYNCHOPHOROL

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Rhynchophorol has been used in Eppendorf type baits with a hole in the lid to control the beetle population. This device facilitates the slow release when compared to the direct liquid. However, the validity these baits depends on the evaporation rate of the pheromone through the device, which can release excessive amount of pheromone. Aiming to prolong the release of the pheromone, different zeolites were synthesized, characterized and evaluated for use as an adsorbent for rhynchophorol. For this, the influence of variables such as: the structure of the zeolite, Si/Al ratio, nature of the compensation cation, pore size and acidity was observed for the adsorption process. Recovery studies showed that there was interaction between the pheromone and the zeolite ZSM-5 and MCM-22 at different ratios Si/Al, resulting in degradation of the pheromone, precluding its use as support for prolonged release. The materials silicalite-1, zeolite Y and zeolite L had good recovery results. Promising results have been observed when the rate of release from the zeolites obtained was compared with the commercial pheromone. Thus, the studies in this work allowed selection of zeolites with great commercial potential as a device releasing pheromone for prolonged periods.

OPTIMISATION OF A PHEROMONES SLOW-RELEASE CHITOSAN FORMULATION IN INTEGRATED PEST MANAGEMENT STRATEGIES

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Increasing restrictions in the use of conventional insecticides for pest control in developed countries further emphasize the need for new, environmentally safe methods of pest control and the control of insect population through species-specific manipulation of communication is the driving force for the use of pheromone. For more widespread use of pheromones, application techniques must become more reliable and more economic. The present research consists of verifying the efficiency of chitosan gel beads towards incorporated pheromones. The work deals with the optimization of chitosan gel beads formulation in terms of encapsulation capacity. The chitosan formulations were tested for pheromone release in laboratory and in field in order to verify their efficiency as slow release devices. The capacity of semiochemical bead to attract *Rynchophorus palmarum L.* was evaluated. Different pheromone formulations were then investigated in order to deliver these molecules on crop fields for a long period of time as biological control devices. Formulated beads showed different structural and encapsulation properties depending on various formulation factors. Chitosan formulations were characterized by confocal microscopy in order to observe the distribution of semiochemicals in chitosan network.

INVESTIGATION ON THE BIOSYNTHETIC PATHWAY OF FELININE, A PRECURSOR OF CAT SPECIFIC ODORANT, IN THE DOMESTIC CAT

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A sulphur containing amino acid, 2-amino-7-hydroxy-5, 5-dimethyl-4-thiaheptanoic acid (felinine) is excreted only in urine of the domestic cat and its near members in felidae species. Felinine is a precursor of a cat specific odorant, 3-mercapto-3-methyl-1-butanol that is a chemical signal in them. It has been suggested that 3-methylbutanol-glutathione (MBG), a felinine precursor, is formed in the liver via a glutathione S-conjugation reaction between glutathione and isopentenyl pyrophosphate (IPP) that commonly presents in various mammals as an intermediate of cholesterol biosynthesis. However, little is known about the biosynthetic pathway of MBG *in vivo*. To answer the question why MBG presents only in the felidae species in species-specific manner, here we investigated which organs produce MBG and whether IPP is a natural substrate for MBG. We firstly developed an optimized LC-ESI-MS protocol which allowed precise quantification of MBG and felinine. LC-MS detected MBG in not only serum but also several organs such as liver, lung, heart, kidney, and brain. In addition to MBG, felinine was also detected in the serum and several organs, although its level in the organs is much lower than urine. Next enzyme assays were carried out using glutathione and IPP as substrates. Glutathione S-transferases purified from cat livers did not catalyze the conjugation reaction between glutathione and IPP. These results indicate that MBG is produced in several organs in cats and IPP is not a natural substrate of MBG in cats.

WHY DO SILKWORMS ACCUMULATE IMINOSUGARS FROM MULBERRY LEAVES?

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1-Deoxynojirimycin (DNJ) is a mulberry iminosugar and potent α-glucosidase inhibitor that could prevent diabetes. Silkworms (*Bombyx mori*) accumulate DNJ by feeding on mulberry leaves. However, DNJ is toxic to many other insects. We analysed the concentrations of 3 major mulberry iminosugars, DNJ, 2-*O*-α-D-galactopyranosyl-DNJ (GAL-DNJ), and fagomine, in the larvae of mulberry-feeding insect species to clarify the specificity of iminosugar accumulations in the silkworms. Larvae (*Bombyx mori*, *Bombyx mandarina*, *Adoxophyes orana fasciata*, *Hyphantria cunea*, *Lemyra imparilis*, *Agrotis segetum*, *Sarcopolia illoba*, and *Psacothea hilaris*) and mulberry leaves were lyophilized and pulverized, and their iminosugar concentrations were analysed using hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS). DNJ and fagomine concentrations in the larvae of 2 *Bombyx* species were much higher than those in the other insect larvae. GAL-DNJ concentrations were low in all species. DNJ and fagomine concentrations in the excrement of *A. segetum* and *S. illoba* larvae were lower than those in the other larvae. Thus, *A. segetum* and *S. illoba* larvae might metabolize iminosugars, whereas the other insects excrete them. In the silkworms, *B. mandarina*, and *S. illoba*, iminosugar concentrations in hemolymph were also analysed. DNJ and fagomine concentrations in the hemolymph of the 2 *Bombyx* species larvae were much higher than that in the *S. illoba* larvae. Thus, *B. mandarina* may sequester iminosugars from mulberry leaves in their hemolymph for protection from predation. Further, iminosugar accumulation in silkworms might be an inherited character of *B. mandarina*, which is considered as the original species of *B. mori*.

A SALIVARY PROTEIN NL10256 IS ESSENTIAL IN THE FEEDING OF RICE BROWN PLANTHOPPER, *NILAPARVATA LUGENS*, ON RICE

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The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is a destructive rice pest in Asia. During the process of feeding, BPH inject saliva into rice to help digest plant materials and suppress plant responses. Using short read sequencing technology (Illumina), we performed *de novo* assembly of the salivary-glands transcriptome of BPH, and 43,312 unigenes were generated. A total of 352 genes were predicted to encode secretory proteins. To investigate the role of these putative secretory proteins in BPH feeding, one gene *Nl10256*, which shows high homology to cellulases, was chosen. By combining molecular, RNAi and bioassay approaches, we found that the protein Nl10256 is injected into rice during BPH feeding. Knockdown of this gene caused BPH a short feeding time in phloem and less amount of honeydew secreted, which subsequently decreased the survival rate and fecundity of BPH on rice. These data suggest that Nl10256 is crucial in the feeding of BPH.

REAL TIME MONITORING OF A MIGRATORY INSECT, *MYTHIMNA SEPARATA* USING A REMOTE SENSING SEX PHEROMONE TRAP

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Mythimna separata (Lepidoptera: Noctuidae) is a migratory insect invading into Korea every year, and attacks pasture grasses, maize, rice, etc. In order to estimate damage time by larvae and control time, the exact timing of adult migration is necessary. In this study, a remote-sensing system for real-time monitoring of adult occurrence was designed and tested in fields, and the efficiency of system was evaluated. The system consisted of an insect-capture trap of a modified cone-trap ('Goggal trap'), a photo-interrupter sensor in trap entrance, a signal transmission module with code division multiple access, a main board, a solar battery, and a signal collection and display part with an internet web page. Known sex pheromones of *M. separata* and *Helicoverpa armigera* were tested as lure for *M. separata* in the trap, and finally the sex pheromone of *M. separata* was selected. The similarity between the actual number of insect and the signal number in the remote-sensing trap was improved through limited sensing within a night time depending on insect's circadian rhythmic behavior, control of signal sensitivity in sensing program, and adjustment of interval and program in signal transmission. The final signal occurrence pattern in the trap was similar to the adult capture pattern in the trap, and the method provided an actual migration pattern with sharp curves in early season. The result indicated that the remote-sensing trap with sex pheromone for *M. separata* is enough to use in the future.

INSECTICIDAL ACTIVITY IN SEEDS OF A *VIGNA RADIATA* CULTIVAR

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Stink bugs and weevils are serious insect pests to pods and seeds of *Vigna* and *Glycine* plant species in fields and storehouses. To find out genetic sources resistant to the insects, ca. 900 germplams in Leguminosae were screened through injury and survivorship tests with the bean bug, *Riptortus pedestris* (Hemiptera: Alydidae). All of about 600 germplams in *Glycine max* and *G. soja* received serious damage by the insect, and most nymphs developed well on the plants. Of genus *Vigna* plants, however, about 120 germplasms of *V. nakashimae* and one cultivar of *V. radiata* showed strong resistance to the bug, while ca. 60 germplasms in other 3 *Vigna* species didn't show any resistance. A series of tests including antibiosis and preference were conducted to know the resistant mechanism in the cultivar of *V. radiata*, and it was presumed that the resistance of the cultivar is depened on chemical factors in seed, and it interferes a feeding process of the insect. In a survivorship test with artificial seeds, the resistanct factor showed a dose-dependent manner in its activity. After seed flour of the cultivar was extracted with organic solvents, the crude extract was fractionated through solvent/solvent partition and column chromatography. Several fractions in the process showed clear insecticidal acitivities to the bean bug. The active fractions had strong insecticidal activity to the bean weevil, *Callosobruchus chinensis* (Coleoptera: Chrysomelidae), too.

IDENTIFICATION OF OVIPOSITIONAL STIMULANTS FOR *LASIODERMA SERRICORNE* IN ROASTED COFFEE BEANS

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The cigarette beetle *Lasioderma serricorne* is a serious global stored-product pest that damages a wide range of stored food products by larval feeding and reproduction. However, effective control methods have not been established. If the adults can be stimulated to lay eggs in places where the larvae cannot develop, then beetle infestation of the stored commodities can be reduced. In our previous study, high oviposition activity of the beetles was observed in roasted coffee beans, dried black tea, and green tea leaves; the beetle larvae could not survive on the beans. We investigated the effect of methanol extracts of these 3 food materials on the oviposition activity of the beetles. The 3 extracts stimulated beetle oviposition in a concentration-dependent manner, proving that chemical factors stimulate the oviposition behaviour of the beetles. Especially, the ovipositional response to coffee bean extract was high at a lower dose (0.1 g bean equivalent/ml) than that for the other extracts. Therefore, we sequentially isolated the oviposition stimulants from roasted coffee beans by using hexane, chloroform, butanol (BuOH), methanol (MeOH), and 20% MeOH in water. The number of eggs was significantly higher on extracts of chloroform, 1-BuOH, MeOH, and 20% MeOH in water than that on the control. The chloroform extract was fractionated using silica-gel column chromatography, and 9 major components were identified from an active fraction by using GC-MS. Among these, significant oviposition response to catechol was observed. Thus, catechol is considered as an oviposition stimulant for the beetles in roasted coffee beans.

DETOXIFICATION OF GOSSYPOL, A COTTON SECONDARY METABOLITE, BY HELIOTHINE MOTHS

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Polyphagous insects, such as the cotton bollworm *Helicoverpa armigera* and the tobacco budworm *Heliothis virescens* lead to high yield losses in agriculture, in both the Old and the New World. Most generalist herbivores have to deal with a large variety of plant secondary metabolites that they need to detoxify to ensure successful growth and development. The major defence compound of one of their host plants, cotton, is gossypol, a sesquiterpene dimer that is toxic to most organisms. Although gossypol has been extensively studied in mammals for its antifertility, antitumor, and antiviral activities, very little is known about gossypol detoxification mechanisms in cotton-feeding insects. We wanted to investigate the mechanisms that enable Heliothine moths to feed on cotton plants containing this toxic secondary metabolite and, furthermore, to identify gossypol metabolites. Feeding studies with artificial diet containing defined concentrations of gossypol show species-specific differences in larval development. Microarray hybridizations showed the potential involvement of several enzyme families in gossypol detoxification/modification. To detect metabolites of gossypol we are expressing candidate genes in insect cell lines and performing metabolism studies. Additionally, in the frass of larvae fed on artificial diet containing gossypol, we are trying to identify metabolites using methods such as HPLC and LC/MS. Getting a better understanding of detoxification mechanisms to the natural insecticide gossypol, could eventually help to understand one of the mechanisms allowing the evolution of resistance to both toxic secondary metabolites and pesticides in insects.

GC CAPILLARY COLUMNS AS VOLATILE COLLECTORS; EFFICIENT AND ROBUST

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Collections of volatiles such as insect pheromones are typically performed with solid adsorbents rather than open tubes which, to many, appear inherently flawed due their open nature. Our studies employing open tube glass capillaries and DB-1 columns demonstrated that these open tube collectors can collect all volatile compounds entering them at room temperature. However, glass capillaries were less efficient traps for lower molecular weight and non-polar compounds than the GC column tested. Desorption of compounds collected on DB-1 columns was quickly and efficiently performed with small volumes of solvent, allowing rapid and repeatable analyses of the volatile ratios. Volatile release ratios from rubber septa (a common pheromone formulation substrate) were quite variable even though they were loaded with equal volumes and quantities of pheromone compounds. Quantification of volatile ratios from pheromone glands and host plant materials should benefit from this approach due to rapid assessments and ability to collect volatiles from point source locations.

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TARSAL GUSTATORY SENSE IN CHRYSOMELID INSECTS

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In previous studies, we showed that some coleopteran insects use tarsi as chemosensory organs in feeding, similar to orthopteran, dipteran, and lepidopteran insects. However, morphological studies by using scanning electron microscope (SEM) showed that the existence of gustatory sensilla (sensilla chaetica) on tarsi was limited to Chrysomelidae and several species belonging to other taxa. Therefore, we observed the tarsal chemosensilla of 35 species of Chrysomelidae with SEM to clarify the role of tarsi as chemosensory organs in chrysomelid beetles. All genera (species) observed possessed gustatory sensilla (sensilla chaetica) on their tarsi. Then, we investigated behavioral responses of tarsal gustation using *Galerucella grisescens*, which possess bumpy-walled tarsal chemosensilla, and *Cassida piperata*, which possess smooth-walled tarsal chemosensilla. Both *G. grisescens* and *C. piperata* with no maxillary palpi, labial palpi, and antennae could discriminate a feeding stimulant, sucrose, in choice tests. Thus, chrysomelid insects could discriminate sucrose by using only their tarsi, irrespective of the chemosensilla wall type. Further, *G. grisescens* could discriminate an antifeedant, brucine, in a choice test by using only their tarsi. Additionally, *G. grisescens* discriminated leaf disks treated with brucine. Finally, we investigated the electrophysiological responses of tarsal chemosensilla to salt and sugar by using the sensillum tip-recording technique on *G. grisescens*. Both NaCl and sucrose evoked spikes. The findings are as follows: (1) In Coleoptera, tarsal gustatory sensilla may develop in only a few families such as Chrysomelidae and (2) Chrysomelid beetles may use their tarsi as gustatory organs to discriminate their host plants.

AN ANALYSIS OF BANANA-SPOTTING BUG ACTIVITY IN AVOCADO CROPS FROM FRUIT-SET TO HARVEST

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The banana-spotting bug, *Amblypelta lutescens lutescens* (Hemiptera: Coreidae), is a serious pest of a wide range of tropical and sub-tropical crops in Australia. Avocado crops are particularly vulnerable to this pest, as bugs feed and cause damage to fruit for 5 months of the year, from fruit set through to harvest. Previously, regular application of endosulfan was the preferred control method but its environmental toxicity lead to its recent deregistration. The ban has prompted new research into areas such as the chemical ecology of *A. l. lutescens*. Recently, a synthetic analogue of the aggregation pheromone produced by male adult *A. l. lutescens* was successfully produced and tested. The overall aim of this research is to better understand bug activity and behaviour in avocado orchards using the synthetic aggregation pheromone and a newly designed pheromone trap. Differences between adult and nymph feeding behaviour on avocado fruit throughout the avocado season will be investigated. Both adult and nymph stages respond to the aggregation pheromone and the trap will be used to investigate *A. l. lutescens* population structure, population densities, and movement patterns throughout an avocado season. Finally, the interaction between Hymenopteran egg parasitoids and the aggregation pheromone will be investigated to see if foraging behaviour and/or egg parasitism rates are affected.

SEX PHEROMONE OF *ISOCERAS SIBIRICA* ALPHERAKY (LEPIDOPTERA, COSSIDAE)

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The carpenterworm, *Isoceras sibirica* Alpheraky (Lepidoptera, Cossidae), is a destructive pest affecting Asparagus Officinalis Linn, in order to develop a new and effective method for forecasting and controlling the pest, the sex pheromone was studied. Gas chromatographic (GC) analyses of pheromone gland extracts with electroantennographic (EAG) detection revealed five major compounds that stimulated male antennae. These compounds were identified by means of gas chromatography–mass spectrometry (GC-MS) as (Z)-7-tetradecen-1-ol (Z7–14:OH), (Z)-9-tetradecen-1-ol (Z9–14:OH), (Z)-7-tetradecenyl acetate (Z7–14:Ac), (Z)-9-tetradecenyl acetate (Z9–14:Ac) and (Z)-9- hexadecadecenyl acetate (Z9–16:Ac). The average levels of the chemicals in a single sex pheromone gland of a calling moth were (0.71 ± 0.24) ng, (1.42 ± 0.44) ng, (4.36 ± 0.32) ng, (8.71 ± 0.26) ng and (0.82 ± 0.38) ng, respectively. Field tests showed that Z9–14:Ac on its own was much attractive to *I. sibirica* males, neither Z7–14:Ac nor Z9–16:Ac alone caught any moths. The catches markedly increased by adding Z7–14:Ac to Z9–14:Ac. The optimum ratio of Z9–14:Ac and Z7–14:Ac was 500:250. This attractiveness was apparently enhanced when 5 % to 20 % of Z9–16:Ac was added. The best field activity was in the lure baited with a 10:5:1 ratio at a dosage of 800 μg /septum. Our study could lead to a safer and more environmentally friendly management of *I. sibirica*.

HOST PLANT VOLATILE ATTRACT THE ORIENTAL FRUIT FLY*, BACTROCERA DORSALIS*

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The oriental fruit fly*, Bactrocera dorsalis*, is a serious pest in China and has developed resistance. To control *B. dorsalis* with friendly methods, the ultrastructure of sensilla on antennae, maxillary palpi, tarsi, ovipositors was investigated with scanning electron microcope. The antennae possesse sensilla basiconica, sensilla chaetica and sensilla trichodea. The maxillary palpi bears sensilla chaetica, sensilla basiconica, and sensilla microtrichia. The tarsi carries sensilla trichoid, sensilla chaetica and sensilla basiconic. Sensilla trichoid and sensilla campaniform are found on ovipositors. With the electroantennogram, 13 host plant volatiles were identified and the effect on *B. dorsalis* behaviour was studied with four-arm olfactometer. Results showed that hexanol, 1-octen-3-ol, trans-2-hexenal, methyl eugenol, ethyl acetate, acetic acid were attractive to both male and female *B. dorsalis*.

BIOLOGICAL RESPONSE OF ADULT *TRIBOLIUM CASTANEUM* TO ENANTIOMERS OF ITS AGGREGATIVE PHEROMONE

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The red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), is a cosmopolitan pest of stored agricultural products worldwide. The pheromone 4,8-dimethyldecanal (DMD) released by feeding male contained four enantiomers and the relative ratio of four isomers (4*R*,8*R*): (4*R*,8*S*): (4*S*,8*R*): (4*S*,8*S*) at approximately 4:4:1:1. This paper reports the biological response of adult *T. castaneum* to different blends of four enantiomers by a walking bioassay in a wind tunnel and electroantennograms(EAG) with the four synthetic enantiomers. Compared with the commercial lure mixture, which is 1:1 (4*R*,8*R*) : (4*R*,8*S*), the blend that mimics the ratio produced naturally by male, which is 4:4:1:1 (4*R*,8*R*): (4*R*,8*S*): (4*S*,8*R*): (4*S*,8*S*) –DMD elicited the highest walking response of mix sex of *T. castaneum* in wind tunnel bioassay in each dose from 0.01ng to 100ng. The individual four enantiomers (4*R*,8*R*)-,(4*R*,8*S*)-, (4*S*,8*R*)-,: (4*S*,8*S*)- DMD induced significant different behaviour response compared with hexane solvent control and (4*R*, 8*R*) -DMD and commercial lure mixture had no significant difference in behaviour response at dose 100ng. The result of dose responses of *T. castaneum* indicated that the threshold of commercial lure was 10 ng. Compared with the mixture 4:1of (4R,8R)- and (4R.8S)- DMD, the blends that mimicked the commercial lure could elicit the significant difference walking response only at dose 50ng and the results confirmed that relative ratio of four enantiomers (4*R*,8*R*)-I: (4*R*,8*S*)-I: (4*S*,8*R*)-I: (4*S*,8*S*)-I of natural pheromone components at approximately 4:4:1:1 had highest bioassay response. EAG response to four individual enantiomers and their different blends confirmed that the blends mimicked natural pheromone components (mixture of 4:4:1:1 (4*R*,8*R*)-I: (4*R*,8*S*)-I: (4*S*,8*R*)-I: (4*S*,8*S*)-I) had no significantly different with that of eluate from Porapak-Q-collected volatiles from feeding males either at 0.1ng or at 1.0ng. The lab. strain GA-1, originally field-collected in Georgia, USA and the KS strain, originally field-collected in Kansas, USA had no significant differences in response to the 4:4:1:1 (4*R*,8*R*)-I: (4*R*,8*S*)-I: (4*S*,8*R*)-I: (4*S*,8*S*)-I synthetic blend and the natural volatiles collected on Porapak-Q from feeding males at 0.1ng.

RESPONSE OF RED-STRIPED GOLDEN STINK-BUG, *POECILOCORIS LEWISI* DISTANT(HEMIPTERA: SCUETELLERIDAE) TO THE ODORS OF FRUITS OF *SCHISANDRA CHINESIS* BAILLON IN SOUTH KOREA

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Poecilocoris lewisi Distant (Hemiptera: Scuetelleridae) is one of the problematic pests to harvest *Schisandra chinesis* Baillon. This experiment was done to provide the information about the pest control by understanding feeding behaviour of this pest. This study was performed to examine how much red-striped golden stink-bug, *Poecilocoris lewisi* Distant(Hemiptera: Scuetelleridae) responds to the odors of fruits of host plant (*Schisandra chinesis* Baillon). Volatile organic compounds of host plants were bioassayed after they were analysed using GC, GC-MS. Major monoterpene compounds of the fruits were analysed as: α-pinene, camphene, limonene, γ-terpinene, ρ-cymene, sesquiterpene, etc. In an olfactometer test, *Poecilocoris lewisi* was attracted to analysed terpene compounds.

SECONDARY METABOLITES OF SOYBEAN INDUCED BY GUT CONTENTS OF *SPODOPTERA LITURA*

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It is well-known that insect herbivory commonly elicits rapid plant responses. Similar to many plants, soybean (*Glycine max*) leaves emit volatile terpenes when treated with fatty acid-amino acid conjugate (FAC) elicitors such as volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] and *N*-linolenoyl-L-glutamine present in the oral secretions and gut contents of *Spodoptera litura*. However, outside of few investigations, insect-inducible metabolites in soybean remain poorly understood. In this study, we analysed the metabolic responses of two different soybean varieties, Tamahomare and Peking, to the gut content extracts of *S. litura*. Soybean leaves were mechanically damaged and treated with the gut content extracts for different lengths of time. Afterwards, leaves were extracted by MeOH/H_2O (v/v, 4/1), and analysed by LC/MS. Principal component analysis (PCA) was conducted on the resulting analytes to elucidate which compounds were significantly associated with the treatments. Daidzein and its glycosides increased in both varieties leaves, but formononetin which is monomethylated daidzein and its glycosides increased only in Tamahomare leaves. Consistent with the up-regulation of the isoflavonoid biosynthetic pathway, phenylalanine was also found to increase. In Tamahomare, formononetin was also induced after the accumulation of daidzein and its glycosides. These results suggest that a subset of isoflavonoids are induced in soybean plants treated with gut contents of *S. litura*, and methyltransferase(s) to produce formononetin are further activated in specific varieties.

CHENOPODIUM ALBUN VAR. *CENTRORUBRUM* CAUSES THE TORTOISE BEETLE, *CASSIDA NEBULOSA* L., TO DEVELOP A FEEDING DETERRENT RESPONSE TO SPINACH

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Newly emerged adults of the tortoise beetle, *Cassida nebulosa*, can feed on spinach. However, they have been observed to reject spinach after being reared on *Chenopodium album* var. *centrorubrum* (hereafter referred to as CAC). Therefore, we investigated why CAC deters the feeding response of *C. nebulosa* to spinach. Adults reared on *Gomphrena globosa* leaves ate spinach. However, adults reared on *G. globosa* leaves treated with the methanol extract of CAC did not consume spinach. These results indicate that the chemical factors of CAC may prevent *C. nebulosa* from feeding on spinach. The feeding responses to spinach were not different between adults reared on *G. globosa* extract-treated leaves (CAC or spinach) and those reared on control leaves. These results indicate that *G. globosa* does not affect the feeding behaviour of *C. nebulosa*. Therefore, we used *G. globosa* leaves for rearing the adults on CAC extract. The methanol extract of CAC was fractionated with chloroform, 1-butanol, and water. Adults reared on *G. globosa* leaves treated with all the fractions were deterred from feeding on spinach. The 1-butanol-soluble fraction produced the strongest effect and was fractionated into 5 fractions by using octadecyl silica gel column chromatography. The adults reared on *G. globosa* leaves treated with fraction 4 were slightly deterred from feeding on spinach. However, all 5 fractions did not produce a clear effect. Our results show that the feeding deterrent response may be caused by a combination of multiple chemical components.

THE VOLATILES RELEASED FROM THE OLIVE WEEVIL, *DYSCERUS PERFORATUS*

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The olive weevil (*Dyscerus perforatus*, Coleoptera, Curculionidae) is a native species in Japan. When the olive, *Olea europaea*, was introduced to Japan and planted on large scale, the weevil preferred the olive trees to the former hosts, *Ligustrum japonicum* and *L. obtusifolium*, both of which belong to the oleacea family, where the weevils live in low density. The population density, however, is extraordinary high in the case of the olive unlike in the former hosts and the weevil causes tremendous damage to the olive trees. This insect is now the most serious pest injurious to the olive in Japan. Insecticides have been used for controlling the weevils so far. However, there is a trend to reduce insecticides from food safety and ecological point of view, and the use of insect behaviour regulators seems to be one of promising ways to protect the olive against such insect pests. During the course of our study on the relationship between the olive tree and the olive weevil, we found that farnesene from the olive tree showed attractant activity toward the weevil. In this study, the volatile compounds released from the olive weevils themselves were detected by using GCMS and SPME. Here, the characterization and the activity of such volatile compounds will be discussed.

DOES OVICIDAL ACTIVE COMPOUND, BENZYL BENZOATE, KILL THE EGGS OF ONLY WHITEBACKED PLANTHOPPER?

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Some *Japonica* rice plant varieties show a resistance against only whitebacked planthopper (*Sogatella furcifera*), which is characterized by the production of an ovicidal active compound, benzyl benzoate (BB), when she lays her eggs in the rice varieties. We have reported that the production of BB was induced by elicitors (phospholipids) in female of *S. furucifera*. We have attempted to elucidate the reason why the varieties kill only the eggs of *S. furucifera* in this paper. When the stems of rice plant were perforated by needles and the methanol extract of *S. furucifera* (20 females equivalent) was injected to the wounds, the extract significantly caused the rice plant to induce the production of BB. In the case of injection of the methanol extracts of brown plant hopper (*Nilaparvate lugens*) and green rice leafhopper (*Nephotettix cincticeps*), the production of BB were similarly induced. These results clearly indicated that *N. lugens* and *N. cincticeps* also had the elicitors. LC-MS analyses revealed that all eggs of three species contained the same elicitors. 10^{-3} M of BB solution killed the eggs of the three species. In contrast, 10^{-6} M of the solution killed the egg of only *S. furucifera* and 10^{-4} M of the solution did not kill the eggs of *N. lugens* and *N. cincticeps* at all. In conclusion, *S. furucifera*'s eggs are 1000 times as weak as the other against BB. This is the reason why *Japonica* rice plant varieties show resistance against only *S. furucifera* and kill only the eggs of *S. furucifera*.

PRECOPULATORY BEHAVIOR OF *PAPILIO XUTHUS* MALES: CONTACT BY FORELEGS

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Male adults of *Papilio xuthus* (Lepidoptera: Papilionidae) primarily use visual cues such as specific color patterns for mate recognition. The artificial model with a yellow and black striped pattern is found to stimulate male approaching behaviour. The males, when approaching this model or mating partners, contact its surface by their forelegs and then attempt to copulate. The purpose of this behaviour is interpreted as the mating assessment based on contact chemical cues. However, chemoreception in the male forelegs has never been examined yet. We found that *P. xuthus* males have two different types of sensilla trichodea on the foreleg tarsi. The large type is densely packed on the medial surface, while the small type is scattered on the lateral surface. Both sensilla have a terminal pore on the tip, suggesting that these sensilla might respond to the surface substances during the precopulatory contact behaviour of males. GC-MS analyses revealed that the cuticular lipids of *P. xuthus* consisted mainly of aliphatic hydrocarbons: linear alkanes with odd number of carbon atoms from 23 to 31 were the major components, while several alkenes and branched alkanes were also contained. The two types of tarsal sensilla trichodea showed electrophysiological responses to the cuticular lipid extracts, in which the small type responded at a higher rate than the large type did. These results indicate that *P. xuthus* males can perceive the cuticular lipids of mating partners using the foretarsal chemosensilla and their precopulatory contact behaviour is probably one of the assessment for mating.

SPME COLLECTION AND GC-MS ANALYSIS OF EMITTED COMPOUND WHEN *POLYGRAPHUS POLIGRAPHUS* MALES ATTACKS NORWAY SPRUCE

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Polygraphus poligraphus is a species of bark beetle that is one of the most serious physiological pests of spruce. This species attacks mainly young weakened trees. It infests the whole stem and branches of standing trees and upper and middle parts of the freshly cut trees. Solid Phase Microextraction (SPME) is an innovative, solvent-free sample technology that is fast, economical, and versatile. SPME uses a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fiber coating removes the compounds from the sample by absorption in the case of liquid coatings or adsorption in the case of solid coatings. In this work we used SPME for sampling of volatile compounds emitted when *P. poligraphus* males attacks Norway spruce. The identity of collected compounds was determined by GC-MS and by comparison with authentic reference samples. The beetles were put in eppendorf tubes nailed to spruce logs in the lab and volatiles were collected with a SPME fiber. After sampling, the sample was injected to GC-MS for identification of the collected compounds. The emission was followed for several days and the amount emitted of the known aggregation pheromone terpinenen-4-ol was followed including its enantiomeric purity.

CHEMICAL HOST PLANT RESISTANCE TO THRIPS IN CARROT

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Excessive use of pesticides has led to insecticide resistance, human health and environmental risks. In view of an integrated pest management approach we studied chemical host plant resistance of carrot using western flower thrips (*Frankliniella occidentalis*), a key agricultural pest worldwide, as a model. Thrips resistance in carrot is chemically rather than morphologically based. We exposed 6 replicates of 30 accessions, including cultivated and wild carrots, which had been part of a carrot gene mapping population, to a non-choice thrips bioassay and to a reproduction test. Thrips leaf silver damage was significantly different between carrots, as was thrips reproduction. Number of larvae was positively correlated to thrips damage. However, no differences in thrips damage or reproduction were observed between wild and cultivated carrots. Leaf-nitrogen content was positively correlated to thrips damage while carbon, hydrogen and sulfur did not play any role, nor did leaf water contents. Preliminary SNPs associated with thrips resistance were identified. Subsequently, we established the metabolomic profiles of all samples using NMR to identify metabolites related to thrips resistance. OPLS multivariate analysis showed a clear classification of metabolite profiles related to a thrips damage gradient. Glucose, succinate and alanine were related to resistant and malic acid to susceptible carrots. SNPs associations of these metabolites are currently under investigation. Our results show that the natural variation of metabolites present in cultivated carrots may be used for improvement of herbivore resistance and thus have potential implications for plant breeders.
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FUNCTIONALLY SIGNIFICANT POLYMORPHISMS IN TUNICATE XENOBIOTIC RECEPTOR GENES

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The vertebrate xenobiotic receptor, pregnane-X- receptor (PXR), is a ligand activated nuclear receptor that regulates multiple detoxification genes. Vertebrate PXR orthologues display considerable inter-taxa variation in ligand binding domain (LBD) sequences suggesting adaptive evolution to enhane the binding of xenobiotics typically encountered by the corresponding organism. Tunicates occupy an intriguing evolutionary position, with adult tunicates being both marine filter-feeders and members of the phylum Chordata. Genomes of the solitary tunicate *Ciona intestinalis* and the colonial tunicate *Botryllus schlosseri* both encode at least two PXR orthologues, designated PXR/VDRα and β. Using PCR, PXR/VDRα and β transcripts were detected throughout the *C. intestinalis* branchial sac, gut, and ovaries. We examined three tunicate PXR/VDR LBD coding sequences for intra-species allelic variation. PXR/VDR LBD coding sequences were amplified from *C. intestinalis* (*Ci,* dissected gut, n = 30) and *Botryllus schlosseri* (*Bs,* complete colonies, n = 30) and the PCR products were sequenced using Illumina MiSeq. Single nucleotide polymorphisms (SNPs) were common in all three amplicons with a mean of 2.6 SNPs / 100 bp. The majority of the SNPs found were synonymous (*Ci*PXR/VDRα 86%; *Ci*PXR/VDRβ 70 % and *Bs*PXR/VDRα 87%) while sliding window calculations of dN/dS ratios found no evidence of positive selection. Single base deletion (i.e. frameshift) containing allelic variants were found in both the *Ci*PXR/VDRα and β genes - corresponding to predicted proteins having a DNA-binding domain but lacking a LBD. We suggest that the persistence of such frameshift mutations may reflect constitutive detoxification gene expression conferring a selective advantage in some contexts.

***COTESIA FLAVIPES* IS ATTRACTED TO *A* SESQUITERPENE EMITTED BY SUGARCANE IN RESPONSE TO SUGARCANE BORER INFESTATION**

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The sugarcane borer, *Diatraea saccharalis*, is a key pest of sugarcane in the Americas. Plants often release a blend of volatile organic compounds (VOCs) in response to damage by herbivorous insects that may serve as location cues to their natural enemies. In trying to elucidate the defense mechanisms of sugarcane response to herbivory by *D. saccharalis*, we have performed herbivore damage assays either by infesting 40-45-day old sugarcane plants with sugarcane borer larvae and treating mechanically wounded plants with oral secretion of *D. saccharalis.* The VOCs were collected and bioassays were performed in a four-arm olfactometer by using adult females of *Cotesia flavipes*, a wasp successfully used in biological control programs in sugarcane fields. Gas chromatography/mass spectrometry analysis revealed a main compound differentially emitted by infested-plants, which was identified as (*E*)-β-caryophyllene both by comparison with a mass spectrum standard library and by co-injection with an authentic (*E*)-β-caryophyllene standard. In the olfactometer bioassays, the wasps were significantly more attracted to both VOCs from infested plants and non-infested plants containing the (*E*)-β-caryophyllene standard in comparison to VOCs from non-infested plants. Interestingly, this sesquiterpene has been previously demonstrated to be emitted by maize leaves and roots in response to attack by herbivores and to act as a signal to attract natural enemies of maize herbivores. These results will pave the way for increasing the efficiency of the *C. flavipes* as a biological control, developing new biological control strategies and might be used as a foundation for the development of sugarcane borer resistant cultivars.

A GENE CODING FOR A PUTATIVE TERPENE SYNTHASE FROM SUGARCANE IS INDUCED DURING *DIATRAEA SACCHARALIS* INFESTATION

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Many plant species emit a blend of volatile compounds upon herbivore attack, especially terpenes. We have demonstrated (E)-caryophyllene emission by sugarcane plants in response to *Diatraea saccharalis* herbivory (shown in a companion poster). In order to find whether the gene expression of a terpene synthase (tps) is concomitantly induced during *D. saccharalis* herbivory, we have aligned gene sequences of tps from different Poaceae species, designed degenerate primers based on conserved regions, and evaluated by real time quantitative RT-PCR (qRT-PCR) the expression of a tps gene in sugarcane plants upon herbivory. Thirty-five-day-old plants were separated into three groups: the real herbivory (RH), in which the plants were infested with three 2nd-instar caterpillars each plant; the simulated herbivory (SH), in which plants were mechanically wounded and treated with caterpillar oral secretion; and the control plants (C), without any treatment. Total RNA was extracted from leaf tissue and converted to cDNA using a high capacity cDNA reverse transcription kit. The amplicon sequence obtained by RT-PCR with the degenerate primers was confirmed by sequencing to pertain to a gene coding for a sugarcane terpene synthase. Analysis by qPCR using sugarcane specific primers showed that the expression of tps gene was three fold higher in plants from RH group in comparison to plants from C group. Furthermore, the higher expression remained over a time course of 48hr. In plants from SH group the expression of tps was also higher than in the plants from C group, but lower than in plants from RH group.

MORPHOLOGY OF ANTENNAL SENSILLA IN TORTRICID MOTHS, *EPIPHYAS POSTVITTANA* AND *PLANOTORTRIX OCTO*

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The morphology of the antennal sensilla in the males and females of *Epiphyas postvittana* (Lepidoptera: Tortricidae) and *Planotortrix octo* (Lepidoptera: Tortricidae) was investigated by scanning electron microscopy. The number and overall length of flagellomeres was greater in females than in males in both species. The flagellomeres of the antennae in these two species contained six morphological types of sensilla: sensilla trichodea, s. basiconica, s. coeloconica, s. auricilica, s. chaetica and s. styloconica, in varying numbers and distribution along the antennae. Among these sensilla, four types (trichodea, basiconica, coeloconica and auricilica) of sensilla demonstrated multi-porous surface structures, indicating that the primary function of these four types of sensilla is olfactory. The number of each type of sensilla was similar between males and females in *E. postvittana*. In contrast, *P. octo* showed sexual dimorphism in the number of olfactory sensilla. Sensilla chaetica and s. styloconica distributed evenly along the antenna. All four types of olfactory sensilla could be further classified into subtypes according to their size, shape and surface structure. Our observation indicates that the morphology and distribution of antennal sensilla is species-specific and sex-specific. Since the male antennae of these moths contain all four types of olfactory sensilla in similar numbers to those of females and male-specific population of s. trichodea is usually responsible for female sex pheromone detection in moths, it is suggested that the males, as well as females, have well developed olfactory sensory system for detecting host-related volatile compounds.

PHEROMONE BLEND VARIATION OF *MARUCA VITRATA* AND INVESTIGATIONS ON PHEROMONE STABILITY FOR REFINING LURES IN SOUTHEAST ASIA

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The legume pod borer, *Maruca vitrata* (Lepidoptera: Crambidae), is a pantropical pest on leguminous crops and is controlled mainly by insecticides. Pheromone traps are a valuable tool in crop protection for species-specific pest monitoring and can help to reduce the use of pesticides. (*E,E*)-10,12-hexadecadienal was identified as the major sex pheromone component, and (*E,E*)-10,12-hexadecadienol and (*E*)-10-hexadecenal were described as minor pheromone components of *M. vitrata*. A blend of these components in a ratio of 100:5:5 attracted moths in field bioassays in Benin. However, this blend was not attractive in field trapping experiments in Southeast Asia. This finding indicates differences between sex pheromone blends of geographically different *M. vitrata*-populations. Our own GC-FID analyses of pheromone gland extractions of single *M. vitrata* females from Thailand and Taiwan confirmed the presence of the previously identified pheromone components, but the ratio differs from the one attracting the Benin population. (*E,E*)-10,12-hexadecadienol, described as a minor compound before, accounted for around 50% of the pheromone blend in both Asian populations. We found that (*E,E*)-10,12-hexadecadienol in particular was unstable in pheromone gland extracts and is detectable only in traces after one week. Since the ratio of the pheromone components is crucial to design efficient lures, a pheromone stability test using different pheromone dispensers was conducted under artificial field and storage conditions. According to our results, the pheromone blend ratio was similarly stable on rubber septa and polyethylene vials. Field experiments testing the attraction of different synthetic pheromone blends in Southeast Asia are ongoing.

BROWNING ON THE ROOTS OF RICE PLANT INFESTED BY RICE ROOT APHID, *RHOPALOSIPHUM RUFIABDOMINALIS*

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A browning on plants caused by attacking pathogens is one of important defensive systems. The browning on rice plant leaf acts as a defense against pathogens and the browning material would be accumulated by polymerization of serotonin. However the role and the mechanism of a browning on a rice plant root attacked by insects is unclear. A change of colour on roots after infesting of a rice root aphid, *Rhopalosiphum rufiabdominalis*, was measured separately five stages by using color swatches. A browning was observed from the 3rd day after infection. The result indicated that an aphid infestation caused a browning and aphids could not infest the deep browning area. Thus it is considered that a browning on roots of rice plant will be acting as one of the defense system against an insect pest. The metabolome analysis of the methanol extract from the roots infested by aphids or the intact roots was conducted by a CE-TOF-MS. An accumulation of serotonin (ca. 10 fold) was observed but not dopamine. It was assumed that the browning on roots attacked by aphids was caused by the activation of tryptophan pathway and polymerization of serotonin.
Ishihara et al. 2008. *The Plant Journal*, 54, 481-495.

EFFECT OF MANGO HOPPER INDUCED CHANGES IN AMINO ACID AND SUGAR LEVELS AFFECTING FLOWER AND FRUIT DEVELOPMENT

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Mango hoppers are considered as one of the major blossom pests of mango. There are about 22 species of mango hoppers reported. Among them, five are considered as major pests of mango inflorescences. Nymphs and adults suck the sap and result in withering of inflorescences mostly due to depletion of sap such as amino acids and sugars which are rich in nutrition, thereby resulting in low fruit yield. The damage caused is about 60-80%. Therefore, as a first step to assess the depletion of sugars and amino acids of mango inflorescence, a study was carried out in the mango orchards *cv*. Raspuri of Indian Institute of Horticultural Research, Bangalore, (12^0 8'N; 77^0 35'E), India, during 2010. For the present study, mango inflorescence of similar phenology and length ± 2 cm were labelled. Inflorescences without mango hopper infestation were considered as control (uninfested) and inflorescences with mango hopper nymphs were treated as experimental (infested). Simultaneously length of the panicle was also measured for two weeks. After two weeks the labeled panicles were cut, washed and dried. From this 5 gms were weighed, used for the estimation of amino acids, total and reducing sugars. It was found that there was 7.14 % reduction in the amino acid. In case of sugars, there was increase of about 8.48% in reducing sugars but in total sugars there was a reduction of 8.36%. The implications of these chemical changes in subsequent flower and fruit developments are discussed.

EVIDENCE OF A FEMALE-PRODUCED SEX PHEROMONE IN THE HUHU BEETLE, *PRIONOPLUS RETICULARIS*

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The iconic huhu beetle *Prionoplus reticularis* (Cerambycidae), a New Zealand endemic species, is widespread throughout the country associated with both native and exotic forests. Fallen logs are the favored oviposition site of the beetle, with the larvae feeding on dead wood. The huhu beetle has been classified as a quarantine pest for timber exports, which currently rely on methyl bromide treatment to meet export regulations. Methyl bromide use is being phased out in New Zealand so alternative control tactics are required for these pest insects. Initial work on this beetle suggested an olfactory assembling mechanism. Here we report long-range attraction to virgin females, volatile entrainment, solvent washes and GC/EAD work as part of an ongoing pheromone identification project. The virgin trapping trials show the presence of a powerful female-produced sex pheromone, with 368 males caught (n= 8 traps) while only 1 female beetle was captured over all traps. The volatile entrainments and solvent washes of beetles and beetle parts were analyzed by GC/EAD using male antenna. The EAD results showed that two compounds are active on the male insect antennae, but both compounds are below detection thresholds of the GC/MS for identification at this stage. Work is underway to obtain larger female extracts for GC/MS analysis and identification of the two candidate pheromone compounds.
Edwards.1961. *Transactions of the Royal Society of New Zealand,* 88, 733-741.

IMPACT OF ECOLOGICAL FACTORS ON CHEMICAL DISPENSATION OF METHYL EUGENOL WITH REFERENCE TO ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS*

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Management for oriental fruit fly, *Bactrocera dorsalis* (Hendel) mainly revolves around male annihilation using the para pheromone, Methyl Eugenol as lure to attract and kill the males. However, the lure dispensation may be influenced by ecological variables that may sustain or reduce the lure efficacy in the field. Under higher temperature (>30^{o}C) there may be an excess release leading to quick drain of lure in the dispenser resulting in low catch. A three years study (2010-2012) was conducted at IIHR, Bangalore, India to ascertain the influence of abiotic factors on *B. dorsalis* trap catch. The influence of weather variables viz., minimum temperature (r = 0.38**) and evening relative humidity (r =0.34**) on *B. dorsalis* trap catch was significantly positive. The contribution of minimum temperature (<30^{o}C) to trap catch was explained to the tune of 18%, 26%, 9% and 15% respectively during 2010, 2011, and 2012 and in pooled analysis. Similarly, evening relative humidity also exhibited significant influence on trap catch and explained the variability in the trap catch up to 19%, 43%, 5% and 12% during 2010, 2011, 2012 and in pooled analysis respectively. A multiple regression model accounted overall for 19% of observed variation in trap captures (F = 32.09, P<0.0001). So it is important to have lower rate of lure release, sustainable over a period which is facilitated under minimum temperature and high humidity conditions. The implications of chemical dispensation due to abiotic factors will be discussed in detail.

CHARACTERISATION OF MANGO TRUNK BORER (*MANGIFERA INDICA*), ITS CHEMICAL DETERRENCE THROUGH ASPHYXIATION AND REJUVENATION OF DAMAGED TRUNK THROUGH TRUNK FEEDING OF NUTRIENTS USING SEALER CUM HEALER

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India ranks #1 in the world in terms of area and production but not in productivity. Trunk borer is considered to be a major pest in major tree crops. Objectives of this study were to evaluate the preference of variety, age, mode of action of sealer cum healer and the extent of rejuvenation of damaged trunk. The trials were conducted during 2009 to 2012 in south Indian states (Karnataka, Andhra Pradesh, Tamil Nadu and Kerala) in collaboration with department of horticulture. The results indicated that mango cultivar Alphonso is the most preferred host #1 (78%); followed by Bangalora (Totapuri) (14%); followed by Neelum (8%). However if choice of variety is not available, any mango variety was susceptible to the trunk borer. Age of plant was evaluated and found that trees aged more than 6 years were highly susceptible (86%) for attack of borers. The product sealer cum healer developed by IIHR was evaluated in four Indian states and found that the control of trunk borer was nearly 100%. The mode of action of sealer cum healer was evaluated and found that the sealer cum healer kills the larvae within the trunk by asphyxiation. The rejuvenation of damaged tissues was mostly by reunion of damaged trunk. The nutrients were available to the tree through trunk absorption and enhanced photosynthetic activity to alleviate deficiency symptoms.

STEROLS FROM THE WEAVER ANT *OECOPHYLLA LONGINODA*.

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The objective of the study was to investigate the non-volatile chemistry related to weaver ants. Whole body methanol extracts of larvae, males, large and small workers of an *O. longinoda* laboratory colony were prepared. The extracts were analysed by GC-MS after derivatization and results revealed several sterols. Cholesterol, sitosterol, 5-alpha-cholestan-3-beta-ol, ergosterol and lathosterol were identified in addition to four sterols yet to be identified (unknown A-D). Furthermore variations in the sterol content of the different casts were seen. Unknown A was only found in the worker casts and was present in significantly larger amounts in the small workers compared to the large. Ergosterol was not found in the males and was present in higher amounts in the small workers compared to the large. Since it is known that insects are incapable of performing *de novo* synthesis of sterols and rely on sequestering from food sources the ants diet consisting of American cockroaches (*Periplaneta Americana*) were analysed. In these extracts unknown A, C and D, in addition to ergosterol were not found. This suggests that the sterols sequestered are transformed in *O. longinoda*. Previous studies of the sterolic profile of ants in comparison to their food sources are scarce hence this study may contribute new knowledge of transformation of sterols in ants especially in relation to zoophagous ants. In on-going studies the chemical profile of *O. longinoda* will be compared to the profile of *Oecophylla smaragdina*.

BIOLOGICAL ACTIVITY OF MOMORDICIN Ⅱ ON *OSTRINIA FURNACALIS* (GÜENÉE) AND *SPODOPTERA LITURA* (FABRICIUS)

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Bitter gourd, *Momordica charantia* L. belongs to the Cucurbitaceae family and is widely cultivated in China as a vegetable crop. Among cucurbitaceous plants, bitter gourd is rarely attacked by phytophagous insects during the growing period. Momordicins from the extracts of *M. charantia* leaves, the cucurbitane triterpenes, have been isolated and characterized. Our studies have evaluated momordicins for oviposition deterrence, antifeedant effects and inhibition of development against many phytophagous insects, such as *Plutella xylostella* and *Liriomyza sativae*. *Ostrinia furnacalis* (Güenée) and *Spodoptera litura* (Fabricius) is the most economically important corn and vegetables pest in China. We aimed at evaluating the biological activity of momordicin Ⅱ on *O. furnacalis* and *S. litura*, and providing scientific basis for the exploration of the anti-insect mode and mechanism and providing scientific basis for field applications of momordicins against the pest. The second instar larvae of *O. furnacalis* and *S. litura* were fed on the artificial diet contained momordicin Ⅱ for 3d. The results showed that momordicin Ⅱ had significant inhibition to the development of the larvae of *O. furnacalis* and *S. litura*, mainly by inhibiting their weight gains and developmental duration in a dose-dependent manner. The larval mortality was also increased with higher momordicin II concentration. Notably, in our study, momordicin II showed not only deterrent activity but also ovicidal activity against *O. furnacalis*. Eggs of *O. furnacalis* were dipped into the momordicin II solutions with different concentrations for 2 s. Compared with control, the hatching rates of *O. furnacalis* were reduced by 53.05% and 81.70% with the treatments of momordicin II at the concentrations of 0.4% and 0.6%, respectively. However, for the hatching rates *S. litura* has no significant effect. This study was carried out with the support of program of National Natural Science Foundation of China (Project No.:31171849).

POSTER LISTING

Poster Board Number	Poster Presenter	Poster Title
Symposium Topic 1: Interactions Between Plants and Animals in the Australia		
1	Andrew Gherlenda	Interactive Effects of Elevated Co2 and Temperature Effects on Plant-Insect Interactions: Comparison between Two Host Plant Species, Eucalyptus Tereticornis and Eucalyptus Robusta
2	Leslie Ann Weston	Metabolic Profiling in Echium Plantagineum (Paterson's Curse) - A Study of Bioactive Naphthoquinones and Pyrrolizidine Alkaloids Across Australia
Symposium Topic 2: Aquatic Chemical Ecology		
3	Jacqueline Fries	Chemical Sequestration and Resilience of an Antarctic Amphipod: The First of its Kind
Symposium Topic 3: Rhizosphere Ecology		
4	Elisabeth J. Eilers	Development and Application of a Method Studying the Chemical Composition of Volatile and Non-Volatile Root Exudates
Symposium Topic 4: Plasticity of Constitutive Plant Defences: Microbes to Climate		
5	Norihisa Kusumoto	Transitions of the Terpene Components and the Antifungal Properties by the Mild Heat Treatments of Conifer Leaf Oils
6	Tianxue Liu	(Z)-3-HEXEN-OL May Act As an Eicitor in Defense Response of Disease- Sensitive Maize Genotypes by Inter-Cropping With Resistance Maize Genotypes
Symposium Topic 5: Chemical Ecology of Pollination		
7	Wachiraporn Phoonan	Constituents from Labial Gland Extract of Thai Stingless Bee
Symposium Topic 6: Evolution of Chemical Communication in the Era of Genomics and Transcriptomics		
8	Jian-Cheng Chang	Transcriptome Analysis of the Chemosensory Gene Families of the Legume Pod Borer, Maruca Vitrata (Lepidoptera: Crambidae)
9	Emmanuelle Jacquin-Joly	Molecular Evolution of Sex Pheromone Receptors in Noctuid Moths of the Genus Spodoptera
10	Alexie Papanicolaou	Genomic and Informatic Capability for the Chemical Molecular Ecologist
Symposium Topic 7: The Chemical Stimulus – Its Analysis and Synthesis		
11	Maria Bergström	Cost-Efficient Synthesis of Ceralure B1; An Efficient Attractant for the Mediteranean Fruitfly (Ceratitis Capitata), A Devastating Agricultural Pest
12	Hsiao-Yung Ho	Identification of the Sex Pheromone of the Scale Insect Aulacaspis Murrayae Takahashi
13	Akiko Kanegae	Structure Determination of the Sex Pheromone Secreted by Loepa Sakaei, a Giant Silkworm Moth Inhabiting in Okinawa Island
14	Hector Masuh	Aedes Aegypti Larvae Respond to Natural and Synthetic Odorants

15	Hector Masuh	Electrophysiological and Behavioral Response of N-Heneicosane, Oviposition Pheromone of Aedes Aegypti, on Aedes Albopictus
16	Takuya Morikawa	Antitermitic Activities of Branch Heartwood Extracts of Chamaecyparis Obtusa
17	Yuki Sakamoto	Characterisation of Posticlure and the Structure-Related Sex Pheromone Candidates Prepared By Epoxidation of (6z,9z,11e)-6,9,11-Trienes and (3z,6z,9z,11e)-3,6,9,11-Tetraenes
18	Shamima Sultana	Evaluation of Size Asymmetric Competition of Barley Seedlings Against Annual Ryegrass
19	Radhika Venkatesan	Towards Elucidating the Role of FAS Genes in Plant Pathogenesis
20	Qi Yan	Identification of the Sex Pheromone Secreted by Palpita Nigropunctalis (Lepidoptera: Crambidae)
Symposium Topic 8: Microbial-Chemical Ecological Interactions among Micro-organisms and their Environments		
21	Pragatsawat Chanprapai	Antifungal Activity of Essential Oil and its Compositions from Polygonum Odoratum Lour. Against Rice Pathogenic Fungi
22	Kazuhiro Nagahama	The Role of Ewingella sp. in the Gut of the Large Pine Weevil, Hylobius Abietis
23	Edson Ncube	The Effect of Fusarium Spp. on the Growth and Development of Busseola Fusca Larvae
24	Janpen Tangjitjaroenkun	Isolation and Screening of Actinomycetes from Mangrove Soils in Thailand for Antibacterial Activity
Symposium Topic 9: Multimodal Communication (Integration of Olfaction, Taste, Vision, Acoustics, Mechanoreception)		
25	Stanislav Korenko	Communication by Chemical Cues of Agrobiont Spider Pardosa Agrestis (Westring)
26	Umar Lubanga	The Role of Vibrational and Olfactory Signals in Courtship and Mate Selection of Aacanthocnema Dobsoni
27	Lili Ren	Antennal Morphology and Sensillar Ultrastructure of Dastarcus Helophoroides (Fairmaire) (Coleoptera: Bothrideridae)
28	Zhen Zhang	Chiral Discrimination of Aggregation Pheromone and Population Divergence of Ips Subelongatus in Northeastern China
29	Hui-Jie Zhang	Sugar Receptors Identified from Helicoverpa Armigera and Bombyx Mori
Symposium Topic 10: New Studies in Chemical Ecology Involving Vertebrates and Opportunities for Solving Practical Problems of Livestock Production and Human Health		
30	Ponnirul Ponmanickam	Identification of Volatiles in the Urine of Little Indian Field Mouse, Mus Booduga
31	Devaraj Sankar Ganesh	Assessment of Estrus-Specific Faecal Volatiles in Estrus Induced Goat, Capra Hircus: An Inventive Step in Livestock Improvement
Symposium Topic 12: Molecular Mechanisms in Perception of Semiochemicals		
32	Yang Liu	Antennal Transcriptome Analysis and Functional Characteration of Pheromone Receptors in the Cotton Bollworm Helicoverpa Armigera
33	Rebecca Turner	Involvement of a Cysteine-Rich Intracellular Loop in the Activation of Drosophila Melanogaster Odorant Co-Receptor (Orco)

34	Bin Yang	Identification of Candidate Odorant Receptors in the Asian Corn Borer Ostrinia Furnacalis
Symposium Topic 14: Chemical Ecology of Invading Species		
35	Eric Jang	Tracking Individual Fly Responses to Semiochemical Lures: Radio-Frequency Identification of Bactrocera Dorsalis and Bactrocera Cucurbitae
36	Hisashi Kato-Noguchi	Allelopathy and Allelopathic Active Substance in Invasive Aquatic Plant Water Hyacinth
37	Yongyue Lu	Competition between Inasive Ants and Native Ants - Chemicals Jetted By the Ghost Ant Tapinoma Melanocephalum Fabricius Suppressed the Activity of Red Imported Fire Ant Solenopsis Invicta Buren
38	Maryia Mishyna	Seed Volatile Allelochemicals of Invasive Heracleum Sosnowskyi Manden
39	Matthew Siderhurst	Kairomone Attractants for the Coconut Rhinoceros Beetle, Oryctes Rhinoceros
40	Lloyd Stringer	Eradication with Finesse: Use of Multiple Tactics for Improved Eradication Success
Symposium Topic 15: Applied Chemical Ecology: Newest Research and Development		
41	Lin Cui	Attraction of Braconid Apanteles Wasps, Parasitoids of the Tea Geometrid Larvae, to Combinations of Volatile and Colors Related to Tea Plants
42	Chung Gyoo Park	Attraction of Turnip Sawfly, Athalia Rosae (Hymenoptera: Tenthredinidae) to Methyl Salicylate
43	Alessandro Riffel	The Interaction between Diatraea Saccharalis (Lepidoptera, Crambidae) and Sugarcane: Changes in Plant's Proteome
44	Rikiya Sasaki	Practical Application of a Sex Pheromone for Monitoring the Japanese Mealybug, Planococcus Kraunhiae (Homoptera: Pseudococcidae)
45	Abraham Verghese	From Chemical Ecology to Application: A Flashback of Olfactory Response Leading to Area-Wide Management of the Fruit Fly, Bactrocera Dorsalis in India
46	Hiroe Yasui	Male Responses to Chemical Mating Cues in Different Populations of White-Spotted Longicorn Beetles, Anoplophora Malasiaca - Hypothesis of Mate Location and Recognition System -
Symposium Topic 16: Student Award Presentations		
47	Toulassi Atiama-Nurbel	Response of the Female of Bactrocera Cucurbitae to Host Fruit Odours and to Selected Semiochemicals From Cucurbit Fruits
48	Masayuki Hayashi	Defensive Behaviour of Larvae of Green Lacewing Against Aphid-Tending Ants: Aphid Carcasses on their Backs Function as Chemical Camouflage
49	Suyog Kuwar	Molecular Evolution of Serine Protease Superfamily and Adaptive Response to Soybean Kunitz Trypsin Inhibitor in Helicoverpa Armigera
50	Naiyong Liu	Functional Differentiation of Pheromone-Binding Proteins in the Common Cutworm Spodoptera Litura
51	Zhixin LUO	Functional Analysis of Host Plant Volatiles in the Regulation of Ovipositional Behavior in the Yellow Peach Moth, Conogethes Punctiferalis

Symposium Topic 17: Chemical Ecology (general topics)		
52	Fauziah Abdullah	Toxicty Effect of Weed Chromolaena odorata Against Termites Macrotermes carbonarius and Globertermes sulphureus
53	Seung-Joon Ahn	Oviposition Preference and Larval Performance of Helicoverpa Assulta in Host and Non-Host Plants
54	Jeremy Allison	Effect of Intertrap Distance on the Capture of Large Woodborers (Coleoptera: Cerambycidae)
55	Tatsuya Ashitani	Acaricidal Activity of Cryptomeria Japonica Leave Components Against Spider Mites
56	Jose Mauricio Bento	Diurnal and Nocturnal Herbivore Induction on Maize Elicit Different Innate Response of the Fall Armyworm Parasitoid, Campoletis Flavicincta
57	Jan Bergmann	Behavioural Response of the Grape Weevil (Coleoptera: Curculionidae) to Host Plant Volatiles
58	Deepa Bhagat	Impact of the Variations in Temperature on Polymer Incorporated With Coffee Stem Borer Pheromone
59	Soumya C.B	Phenolics in a Developing Mango Deter Oviposition in Case of Bactrocera Dorsalis (Hendel)
60	Kyung-Hee Choi	Development of Synthetic Attractants for Moths in Orchards
61	Arnaud Costa	A Smell of Spice: Olfactory Responses of Depressaria Daucella (Lepidoptera: Oecophoridae) Larvae to Caraway (Carum Carvi L.) Volatiles
62	Partho Dhang	Developing a Chlorfluazuron Based Insecticide Bait for Controlling Multiple Termite Species
63	Takashi Eguchi	Synthesis and Evaluation of Oxygenated A-Ionone Derivatives as Male Attractants for the Solanaceous Fruit Fly, Bactrocera Latifrons (Diptera: Tephritidae)
64	Yuqian Feng	Cold Hardiness of Asian Longhorned Beetle (Anoplophora Glabripennis) Larvae in Different Geographical Populations
65	Kaori Fukumoto	The Role of Jasmonoyl-L-Isoleucine Synthase Osjar1 in Rice Defense Against Herbivores
66	Ivan Galis	Rice Defense Against Herbivores: What Are the Metabolites?
67	Paola Gonzalez Audino	Behavioral Effect of Lobularia Maritima's Volatiles on Ae. Aegypti
68	Paola Gonzalez Audino	Monolithic Matrix Dispensers for Megaplatypus Mutatus Pheromones Based on Natural and Biodegradable Waxes and Their Evaluation in a Field Trial
69	Euzebio Goulart	Zeolites as a Dispenser for Sustained Release Rhynchophorol
70	Euzebio Goulart	Optimisation of a Pheromones Slow-Release Chitosan Formulation in Integrated Pest Management Strategies
71	Wataru Hojo	Investigation on the Biosynthetic Pathway of Felinine, a Precursor of Cat Specific Odorant, in the Domestic Cat
72	Masatoshi Hori	Why Do Silkworms Accumulate Iminosugars From Mulberry Leaves?
73	Rui Ji	A Salivary Protein Nl10256 is Essential in the Feeding of Rice Brown Planthopper, Nilaparvata Lugens, on Rice
74	Jin Kyo Jung	Real Time Monitoring of a Migratory Insect, Mythimna Separata Using a Remote Sensing Sex Pheromone Trap
75	Jin Kyo Jung	Insecticidal Activity in Seeds of a Vigna Radiata Cultivar

76	Yuji Kamada	Identification of Ovipositional Stimulants for Lasioderma Serricorne in Roasted Coffee Beans
77	Corinna Krempl	Detoxification of Gossypol, a Cotton Secondary Metabolite, by Heliothine Moths
78	Lodewyk Kuenen	GC Capillary Columns as Volatile Collectors; Efficient and Robust
79	Yasuhiko Kutsuwada	Tarsal Gustatory Sense in Chrysomelid Insects
80	Karel Lindsay	An Analysis of Banana-Spotting Bug Activity in Avocado Crops from Fruit-Set to Harvest
81	Hongxia Liu	Sex Pheromone of Isoceras Sibirica Alpheraky (Lepidoptera, Cossidae)
82	Zhao Liu	Host Plant Volatile Attract the Orient Fruit Fly, Bactrocera Dorsalis
83	Yujie Lu	Biological Response of Adult Tribolium castaneum to Enantiomers of its Aggregative Pheromone
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