International Society of Chemical Ecology

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Nature Research Centre

Dear Colleagues,

It is my great pleasure to welcome you at the 28th Annual Meeting of International Society of Chemical Ecology in Vilnius, the beautiful capital of Lithuania, one of the new EU member states with a 1 000 year history. Lithuania is famous for its amber where ancient insects are often embedded.

Each year a different country welcomes researchers who gather aiming to present and discuss the most recent results in the area of chemical ecology. I believe that this meeting will stimulate further collaboration and warm relationship between biologists, entomologists, ethologists, chemists and researchers of many other disciplines related to interdisciplinary science of chemical ecology.

Topics of the scientific programme cover nearly the whole very broad field of chemical ecology from animal-animal and plant-animal interactions by means of chemicals to evolutionary and molecular biology, chemistry of natural products, chemical ecology of various groups of organisms, applied aspects of chemical ecology.

Let me wish you a lot of useful bring-back-home ideas in promoting advance in your research and best impressions of staying together in Vilnius, the capital of Lithuania.

Chair of the host team of the meeting
Professor Vincas Būda

ISCE Silver and Silverstein-Simeone Medal Lectures

Moderator: Wendell Roelofs

EVERYTHING'S IN FLUX: A NEUROETHOLOGICAL JOURNEY

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I have been presented with many fascinating opportunities to learn about insect olfaction related to behavior over the several decades I have been allowed to be one of the fortunate, paid explorers in the field of Chemical Ecology. One of the earliest opportunities I had was to puzzle over the relationship between the time-courses and amplitudes of electroantennogram responses to puffs of pheromone component odorants and the resulting upwind flight responses of moths. Trying to figure out how the timing of male moths' contacts with individual strands of pheromone altered their in-flight maneuverings through the air was another challenge. More recently, an opportunity to try to explain one of the most puzzling and perplexing issues in moth pheromone olfaction presented itself: why, in nearly every species, are the majority of olfactory receptor neurons (ORNs) tuned to the most abundant pheromone component in a species' sex pheromone blend? A related question concerned ORNs that are co-compartmentalized in the same sensillum: why does the ORN tuned to the most abundant pheromone component in the blend have a larger diameter dendrite than the ORN tuned to the minor component and produce a larger amplitude action potential than the minor component ORN? It had seemingly been counterintuitive that the accepted explanation touting increased sensitivity should be valid, because in that case the greatest number of ORNs should be tuned to the least abundant pheromone components, not the most, because these trace components should be the most difficult to detect. It turns out that it is no accident that time kept emerging as a factor that we paid attention to over several decades of studies. Indeed, timing is everything; the resulting hypothesis we have come up with involves molecular abundance-related flux, not concentration. This idea applies nicely not only to the evolution of peripheral sex pheromone olfactory systems, but also to the peripheral olfactory systems that detect general odorants involved in host-finding.

Moderator: Ring Carde

WAR IN THE PLANKTON: SUBLETHAL AND RECIPROCAL IMPACTS OF RED TIDE ALGAE ON COMPETING PHYTOPLANKTON

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How individual species come to be dominant members of marine planktonic communities is not deeply understood; however, it is thought that chemistry plays a substantial role. For example, some red tide-forming dinoflagellates produce toxic secondary metabolites that are hypothesized to enhance dinoflagellate fitness by acting as grazer deterrents, allelopathic agents, or antimicrobial defenses. In field and lab experiments we have shown that the red tide dinoflagellate *Karenia brevis* is allelopathic, inhibiting the growth of several co-occurring phytoplankton species, but that *K. brevis* natural products other than well-known brevetoxins are responsible for suppressing most of these species. At least one phytoplankton competitor, *Skeletonema costatum*, retaliates against *K. brevis*, reducing its allelopathic effects and degrading waterborne brevetoxins. Several other phytoplankton species also metabolize brevetoxins, removing these toxins from the water column and mitigating the negative effects on invertebrates. Death is a rare outcome of *K. brevis* allelopathy, with more subtle responses predominating, such as reduced photosynthetic output and increased cell permeability. These changes in cellular metabolism and physiology may be more readily characterized and measured by a systems biology approach than by growth or cell lysis assays. NMR metabolomics has provided preliminary evidence for sub-lethal impacts of exposure to *K. brevis* allelopathy on the metabolism of neighboring phytoplankton. Future work will expand upon these initial results with mass spectrometry-based metabolomics and proteomics methods, as well as experiments with other vulnerable competing phytoplankton species, with the goal of identifying cellular targets and understanding the molecular mechanisms of red tide allelopathy. Our results indicate that chemically-mediated interactions are reciprocal, and that ecosystem-level consequences of red tides (such as fish kills caused by waterborne toxins) may depend upon which other phytoplankton species are present.

THE PHEROMONE PHENOMENON

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Molecular recognition is prerequisite to the beginning of life, and thus "chemical signalling" is the oldest means for the transmission of information.

Principles of chemical signalling may have evolved several times and for different reasons, typical elements being made up of a broad spectrum of secondary metabolites originating from the ubiquituous pathways of classical catabolism or metabolism (1).

As for their biogenetic origin, most of the relevant compounds are acetogenins, polyketides, and terpenoids, the biosynthesis of which is not restricted to animals, but is also valid in plants and microorganisms, in terrestrial as well as in aquatic ecosystems. The role of (endo)symbionts in the production and transformation of animal associated chemical signals as well as in evolutionary processes (e. g. insect plant-relationships) needs to be investigated.

- Acetogenins show straight carbon skeletons, frequently forming rows of *bis*-homologues.
- Polyketides involving propanoate units will typically show (n,n+2x)-dimethyl-branching.
- Terpenes show isoprene-units as substructures, diversified by derivatization.

Due to past co-evolutionary processes, structures of semiochemicals from plants, insects, and microorganisms can be identical, a phenomenon based on *de novo*-synthesis as well as on sequestration. The same compound may be used as a chemical messenger by quite different organisms and in an entirely different ecological context. The information linked to a specific chemical structure is not necessarily constant and may change during evolution. This becomes particularly evident in compounds causing a high status of alertness in the receiver, i. e. signals that cause attraction, aggression, defense or stampede. Compounds evolved in the context of defense, may even change into attractants.

This scenario may not only indicate similar biogenetic pathways but also common roots including aspects of coevolution. It points to general concepts in the establishment of "chemical languages" and the principle development of appropriate receptor systems. Consequently, questions concerning an "etymology" of semiochemicals may be raised.

Any chemical signal needs a receptor at the receiver site – and it may be asked whether semiochemicals may also be recognized by proteins that are not primarily involved in chemical communication. As a result, would it make sense to generally screen semiochemicals for biological activities other than their presently known function? Methyl *p*-hydroxybenzoate, a component of the honey bee queen pheromone, exhibits strong antibiotic properties – and there are quite some more examples proving the physiological versatility of semiochemicals. Consequently, it may also be asked whether two organisms reacting to the same volatile compound (e. g. bark beetles and hornets or bark beetles and elephants or elephants and moths or frogs and cucujid beetles etc.) share the same receptor.

References

1. Francke W., Schulz S. 2010. Pheromones of terrestrial invertebrates. In: L. Mander, H.-W. Liu (Eds.) K. Mori, guest editor, Comprehensive Natural Products Chemistry II. Elsevier, Oxford. Vol. 4: 153–224.

A NEW DAWN FOR CHEMICAL ECOLOGY IN PEST MANAGEMENT: DELIVERING CHEMICAL ECOLOGICAL PEST MANAGEMENT TOOLS VIA THE SEED USING MOLECULAR BREEDING AND GENETIC MANIPULATION IN CROP PLANTS

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The study of the chemical ecology of plant / insect interactions has defined not only the many types of secondary plant metabolites involved, but has also elucidated sophisticated mechanisms by which plants can exploit intervention from the higher trophic level and benefit from plant / plant interactions. The evolutionary role of these phenomena is still a matter for investigation, but the semiochemicals involved are already proving to be valuable tools in pest management. These semiochemicals can be developed by production in crop plants, utilising molecular breeding or genetic manipulation (1). An important aspect will be delivery through the seed, which is more sustainable than application of formulated chemicals to the crop and can utilise non-toxic modes of action together with non-constitutive expression (2, 3), in contrast to the first generation of crops genetically modified for pest management.

Acknowledgements

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References

1. Physiological Entomology. 2012. Vol. 37: 2–9.
2. Proceedings of the National Academy of Sciences USA. 2008. Vol. 105: 4553–4558.
3. Ecology Letters. 2011. Vol. 14: 1075–1083.

NIGHT AND DAY: MOTH AND BUTTERFLY PHEROMONES NOT SO DIFFERENT AFTER ALL

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The Lepidoptera (moths and butterflies) are the second largest group of insects with >180,000 described species. In moths (ca 165,000 species), mate communication is mediated by the use of female-produced long-range sex pheromones that are chemically conserved. The vast majority of moth sex pheromones are fatty-acid derived long-chain unsaturated alcohols, acetates or aldehydes. More or less species-specific blends have been selected for efficient mate finding and reproductive isolation. On the other hand, butterflies (ca 15,000 species) appear to have lost female-produced pheromones as p art of their mate communication and typically rely on visual cues to mediate long-range attraction. Though they have not completely abandoned chemical communication: they use chemically diverse, male-produced courtship pheromones, which have been selected as sexual ornaments, aphrodisiacs or anti-aphrodisiacs.

Members of two gene-families, namely desaturases (1) and reductases (FARs) (2, 3), have been reported to be of fundamental importance for the specific production of sex pheromones in female moths. Also, Lepidoptera-specific biosynthetic lineages appear to have evolved early in the evolution of Lepidoptera (3, 4). We previously reported that male European corn borer moths produce courtship pheromones that are chemically similar to the female-produced pheromones (5). Interestingly, investigations of pheromone communication in African Bush Brown butterflies (genus *Bicyclus*) revealed that male butterflies can produce pheromone components structurally similar to those biosynthesized by female moths (6). Recent investigations have demonstrated that males of *Bicyclus anynana* and *B. martius* not only smell like female moths but as a matter of fact make use of orthologous biosynthetic genes for pheromone production.

A conceptual model of birth-and-death evolution of multigene families (see 1 and references therein) explains not only the divergence of female moth pheromones but also the production of female pheromone components in male corn borer moths and the evolution of butterflies that smell like moths. **Moth and butterfly pheromones are not so different after all** – at least not when it comes to biosynthesis. Current investigations are underway to examine whether the parallelism extends to the reception of the pheromone signals.

Acknowledgements

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References

1. Roelofs & Rooney. 2003. PNAS 100: 9179–9184.
2. Lassance et al. 2010. Nature 466: 486–489.
3. Liénard et al. 2010. PNAS 107: 10955–10960.
4. Liénard et al. 2008. BMC Evol Biol 8: 270.
5. Lassance & Löfstedt. 2009. BMC Biol 7: 10.
6. Nieberding et al. 2008. PLoS ONE 3: e2751.

Abstracts

Symposium 1. Plant – Animal – Animal Interactions

Moderators: Monika Hilker and Anke Steppuhn

Keynote speaker

IDENTIFICATION OF TRANSPORT PROTEINS IMPLICATED IN THE SEQUESTRATION OF PLANT DERIVED METABOLITES BY USING RNAI IN JUVENILE LEAF BEETLES

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Insects have used plants as a food source for millions of years. In response to herbivores, plants developed several morphological and biochemical adaptations which allowed them to wage a kind of warfare; one strategy of this war was based on toxic secondary metabolite production. One of the adaptation mechanisms of phytophagous insects to their food source is the uptake, modification or concentration of these chemicals into specialized tissues or glands for their own benefit – a phenomenon that has been termed sequestration. It is prevalent in many orders of the Insecta. Moreover, the variety of metabolite classes which can be sequestered by insects has challenged analytical scrutiny since decades. But concerning the transport mechanisms that must facilitate the sequestration of secondary metabolites from plants into insects there is a flagrant lacuna in our knowledge. Though numerous publications postulate implication of carriers in sequestration, until today no transport protein in any insect order has been unambiguously identified.

The leaf beetle larvae of the subtribe Chrysomelina possess a sophisticated network for sequestration of plant derived compounds from their hosts. After transfer into the larval defensive glands the metabolites are converted into biological active deterrents. In order to identify putative transport proteins involved in the sequestration process we have carried out expression analyses by next-generation-sequencing and compared transcript level of putative transport proteins in different larval tissues of *Chrysomela populi*. For example, we have determined high transcript level of genes encoding ABC transporter and glucose transporter in defensive glands. Subsequent *in-vivo* silencing of an ABC transporter by RNAinterference (RNAi) revealed a defenseless phenotype incapable of secreting the defensive compounds which suggests that the protein is participating in the sequestration of plant derived precursors. The results were corroborated by immunohistological studies showing that the protein is localized in the intracellular membranes of the gland cells. *In-vitro* assays resulted in a broad substrate spectrum of the transporter. From our results we conclude that (*i*) RNAi is a convenient method to show *in-vivo* relevance of putative transport proteins and (*ii*) the uptake into the defensive glands is a highly complex process which seems to involve transporters of different selectivity.

Acknowledgements

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LOCAL DISTRIBUTION OF EPICUTICULAR WAX COMPOUNDS ON THE LEAF SURFACE OF *POPULUS TRICHOCARPA*

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The surface of terrestrial plants is covered by a cuticle consisting of cuticular waxes, which play crucial protective role in a constantly altering environment. Next to their primary function – barrier against nonstomatal water loss, they influence plant interactions with pathogens (by preventing their germination) and herbivores (by affecting insect attachment and locomotion). The chemical composition of epicuticular waxes (very long chain hydrocarbons, alcohols, acids, esters) controls also physical parameters such as surface wettability and the microstructures on the leaf surface [1].

Selective isolation of exclusively epicuticular waxes from *Populus trichocarpa* was achieved through mechanical removal (cryo-adhesive) of the wax layer [2]. This method allowed the generation of an intact imprint that preserves the location of surface waxes and allows a transfer to an target (ZnS) applicable for further spectroscopic and other analytical studies. The current method avoids the commonly used solvent extraction, which destroys spatial resolution and lacks required specificity.

Mass Spectrometry based techniques like GC-MS and MALDI-TOF-MS were applied to get information about the chemical composition of the *Populus trichocarpa* surface. A broad range of wax components was identified: saturated and unsaturated long chain alkanes, alcohols, fatty acids, dicarboxylic acids (C_{15}-C_{33}). In addition, MALDI-TOF-MS as a powerful tool in a large molecules analysis, enabled detection of a very long chain esters C_{40}-C_{54} and alkanes C_{54}, C_{56}.

Fourier transform infrared (FT-IR) microspectroscopy is another powerful technique that enabled the generation of infrared maps of the leaf surface imprints. This chemical maps reflect the spatial distribution of functional groups present in the sample with a high resolution (80 μm). After leaf damage by feeding larvae of *Chrysomela populi*, the attacked surface areas exhibited very specific patterns. On the damage border absorption bands located around 3 300 cm^{-1} (OH), 1 600 cm^{-1} (COO^{-}); 1 410 cm^{-1} (O-H); 1 240 cm^{-1} (OH); 1 070 cm^{-1} (C-O-O-C); 1 050 cm^{-1} (C-O) increased significantly compared to the undamaged area. These bands are assigned to the different wax compounds (alcohols, acids, esters) which are more abundant in the wounded area. Hence, not only regeneration of existing wax layer takes place but also accumulation of additional components. FT-IR maps combined with multivariate statistics (Hierarchical Cluster Analysis) and time dependent experiments showed clearly, that already 6 h after leaf damage, plant creates multilayer of additional epicuticular waxes, which protects from the destructive consequences of tissue damage, in particular excessive water lost.

Acknowledgements

This research is supported by the Jena School for Microbial Communication stipend.

References

1. Riederer et al. 2005. J. Chem. Ecol.
2. Jetter et al. 2001. Plant Physiol.

DEFENCE CHEMICALS IN ASPEN AND ASSOCIATED COMMUNITY STRUCTURES

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With the use of UHPLS-ESI/TOFMS techniques we have uncovered an unexpected high diversity of phenolic glycosides (PGs) in the Swasp collection of Swedish aspen genotypes (*Populus tremula*) when compared to the North American sister species *P. tremuloides*. Associations of PGs with biological activity have been reported in Salix and Populus trees, but only for a few compounds and in relation to a limited number of herbivores. We have studied the association of fungal and arthropod species in an assemblage of *P. tremula* genotypes from all over Sweden (the SwAsp collection) and found a highly complex pattern of mainly specialists, but we also see examples of relationships that link specific species to aspen genotype and defence chemical profile. We speculate that the composition of defence compounds in this naturally varying collection of trees, shape the specialist community of arthropods that we find in *P. tremula*, and that the compounds are also responsible for the lack of outbreak species we find on European aspen – opposite the generalist lepidopteran species that are typical for *P. tremuloides*. While genes have been identified for most other branches of the phenylpropanoid pathway including the lignin, the flavonoid and the tannin sub-branches, the biosynthesis of PGs has remained a mysterious black box. However the diversity of PG compounds and the way they co-occur within the Swasp population allows us to suggest principles for their biosynthesis. Consequently, the mapping of PGs in *P. tremula* not only enables us to recognize and characterize genotypes and chemotype groups in aspen, it also opens up for understanding the biosynthesis, evolution and ecological function of PGs.

ASYMMETRIC ADAPTATION TO INDOLIC- AND ALIPHATIC-GLUCOSINOLATES IN THE B AND Q SIBLING SPECIES OF *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE)

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The role glucosinolates play in defending plants against delicate phloem feeders such as aphids and whiteflies is currently not clear as these herbivores may avoid bringing glucosinolates from the phloem sap into contact with myrosinase enzymes. Here, we investigated the effects of high levels of aliphatic- and indolic-glucosinolates on life history traits and detoxification gene expression in two sibling species, B and Q, of the whitefly *Bemisia tabaci*. High levels of aliphatic-glucosinolates decreased the average oviposition rate of both species, and reduced the survival and developmental rate of Q nymphs. High levels of indolic-glucosinolates decreased the oviposition rate and survival of nymphal stages of the B species and the developmental rate of both species. Molecular analyses revealed two major asymmetries between the B and Q species. First, specific GST genes (*BtGST1* and *BtGST2*) were significantly induced during exposure to indolic-glucosinolates only in Q. This may reflect the genes putative involvement in indolic-glucosinolates detoxification and explain the specie's good performance on plants accumulating indolic-glucosinolates. Second, the constitutive expression of eight of the ten detoxification genes analyzed was higher in the Q species than in the B species. Interestingly, four of these genes were induced in B in response to high levels of glucosinolates. It seems, therefore, that the B and Q species differ in their "optimal defense strategy". B utilizes inducible defenses which are profitable if the probability of experiencing the stress is small and its severity is low, while Q invests significant resources in being always "ready" for a challenge.

HERBIVORE-INDUCED OR SUPPRESSED PLANT JASMONATE-DEFENSES AFFECT THE FORAGING STRATEGY OF A PREDATOR

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According to the central dogma in plant-herbivore ecology, herbivory elicits direct plant-defenses, i. e. increased production of toxins and feeding deterrents, as well as indirect plant-defenses, i. e. increased attractiveness to foraging predators via increased emission of volatiles. We discovered that this is not always the case: it appeared that the red spider mite *Tetranychus evansi* suppresses jasmonate (JA) and salicylate (SA) defenses. We reasoned that herbivores in isolation will benefit from defense suppression, but that in natural communities these benefits will not always be evident. In the field, *T. evansi* is often found together with *T. urticae*: a sister species that induces plant defenses similar to many herbivorous insects. We found that *T. evansi* suppressed also the defense processes otherwise induced by *T. urticae* when sharing a leaf and thereby increased *T. urticae*'s reproductive performance. This shows that induction of JA has negative effects on fecundity, whereas suppressing JA prevents this effect. However, we also observed that spider mite eggs on JA-induced plants were less vulnerable to predation. When offered a choice, predatory mites preferred eggs from *T. urticae* that had been feeding on JA-deficient plants over those from wild type plants. On the contrary, they did not discriminate between the eggs laid by *T. evansi*, because JA response is absent in JA-deficient plants and suppressed in wild type plants. Taken together, direct plant defenses negatively affect the reproduction of herbivores, but they may also reduce vulnerability to predation, so that the net effect in a natural setting may be quite different. Thus, defense suppression may backfire on *T. evansi* in nature.

Acknowledgement

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Keynote speaker

PRIVATE CHEMICAL COMMUNICATION IN THE OIL-FLOWER OIL-BEE POLLINATION SYSTEM AND HOW *CEROPEGIA* TRAP FLOWERS DECEIVE KLEPTOPARASITIC FLIES

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Many plant-pollinator interactions are mediated by flower scents, however, the specific compounds responsible for pollinator attraction are only known from a few systems. This presentation will focus on i) the chemistry and evolution of compounds involved in attracting oil collecting bees to oil secreting flowers and ii) the chemical mimicry in *Ceropegia*-fly pollination systems.

i) The highly specialized mutualistic relationship between floral oil secreting plants and oil collecting bees has evolved independently in more than ten plant and two bee (Apidae, Melittidae) families, and occurs throughout the globe. The oil is used by these bees as a constituent of the cell lining within the nest and as larval food provision. It typically consists of acylglycerols or free fatty acids and a common trait of the constituent fatty acids is an acetyl group on the beta carbon. By chemical, electrophysiological, and behavioral analyses, we identified an uncommon volatile acetylated glycerol in oil secreting plants around the world, demonstrated its attractiveness to oil collecting bees, and found that oil bees have specific olfactory adaptations in the periphery of the olfactory circuit to detect this compound.

ii) Six percent of the angiosperms are pollinated by deceit and among the most fascinating deceptive plants are those with pitfall flowers for temporarily trapping of the insect pollinators. Insects are suggested to be deceived by chemical mimicry; however, there is a big gap in our knowledge of these mimicry systems, which hampers the understanding of the evolution and ecology of these plants. One of the most speciose genera with pitfall flowers is *Ceropegia* and several species of this genus are pollinated by kleptoparasitic flies. Such flies are known to steal food from predatory arthropods (e. g. spiders), by feeding on haemolymph or other secretions released by their insect prey items. Honey bees eaten by spiders are an important food source of several kleptoparasitic flies, and here we demonstrate that some *Ceropegia* species mimic alarm and other pheromones of honey bees to attract their kleptoparasitic fly pollinators.

Acknowledgements

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EVOLUTION OF HOST-PLANT INTERACTIONS IN SPECIALIST BEES

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Although pollen foraging behavior of bees seems highly inherited in range and specificity, some rare "host-plant shifts" occurred during evolution inside bee clades of specialist species. The origin and the mechanism of these host-plant shifts remain misunderstood. Visit of novel host plant can be based on morphological or phylogenetical similarity with ancestral host-plant but host switches to unrelated plant families are also common. Because the proportions of nutrients (e. g. proteins, lipids and amino acids) can vary widely among pollens of different plant species, floral specialisations in a new host plant could be constrained by the chemical composition of its pollen, reducing the range of suitable alternative hosts. We studied herein protein content, total amino acids and sterols of host plant species of two groups of three sister species of solitary bees: the *Colletes succinctus* group (*Colletes halophilus, C. hederae* and *C. succinctus*) and the *Melitta leporina* group (*Melitta leporina, M. nigricans* and *M. tricincta*). The results show that chemical compositions of host plant pollen are quite similar, corroborating the hypothesis that host plant specialisation and evolutionnary shifts could partly be explained by chemical composition of pollen.

WHY DO ANTS COLLECT DIASPORES?

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In temperate floras ants are well known dispersers of plant diaspores. Diaspores with fleshy and nutritionally rich elaiosomes attached experience enhanced chances to get dispersed. Nevertheless, ants are choosy even among elaiosome-bearing diaspores and, moreover, also disperse diaspores lacking such appendages. Hence, so far it remained unclear which diaspore properties drive the ants' decision to remove some diaspores but reject others. Do specific diaspore compounds release transport behaviour or do ants decide for a nutritional profile optimally fitting their physiological demands?

We expected a crucial role of fatty acids in diaspores and elaiosomes as behaviour-releasing compounds, as widely suggested in the literature on ant-diaspore interactions. However, bioassay-guided chemical analyses revealed that neither fatty acids fractions nor any of the so far proposed compounds alone acts as chemical trigger for the ant specie we tested, *Myrmica ruginodis*. Instead, the amount and composition of amino acids in elaiosome-bearing diaspores were identified as better predictors of these ant species' choice, but are probably not the only crucial components. We expect a mixture of two or multiple components. However, observations of a second ant species, *Lasius fuliginosus*, revealed clearly different responses to both, elaiosome extracts and fractions of different polarities and pure substances formerly suggested as universally acting trigger compounds. Therefore, we suggest a strong species-specificity in ant-diaspore interactions, probably referring to differences in diets and demands among ant species – mirrored in their of choice of diaspores of variable chemical properties.

ETHYL VANILLATE CONFERS MATING ADVANTAGE IN MALE ORIENTAL FRUIT FLY

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Males of the Oriental fruit fly, *Bactrocera dorsalis*, have been shown to be strongly attracted to the orchid *Bulbophyllum elevatopunctatum*, by probing on the floral lip, petals and sepals of the flowers. Whilst chemical analyses have demonstrated the presence of methyl eugenol as one of the component of floral volatiles, a novel compound identified as ethyl vanillate (EV) was also detected in a particular variety to be attractive to sexually mature males. Interestingly, we show that males are attracted to feed on EV and current analyses suggest that male flies sequester EV in substantial quantities in to their rectal glands. This is probably the first record of a male attractant with a benzoic ester structure in the Oriental fruit fly. Thus, the attraction of virgin females and males of the Oriental fruit fly to conspecific males fed with EV was investigated using wind tunnel bioassays. Concomitant with decreasing light intensity, males fed with EV significantly attracted higher numbers of conspecific males and females during mating period at dusk. Large field cage studies also showed that when groups of EV-deprived and EV-fed males were released in the enclosure, significantly higher numbers of EV-fed males were observed and caught *in copula* than the EV-deprived males. This suggests that EV may possibly act as a male sex pheromone in Oriental fruit fly males in the absence of methyl eugenol. This will be further discussed in light of fruit fly-orchid coevolution.

DIFFERENTIAL RESPONSES TO LARVAL CHEMICALS AS A FUNCTION OF EXPERIMENTAL CONTEXT IN ANTS

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In ants, division of labor between workers can be explained by differences in perception and / or response to social stimulations, in particular larval ones. These differences may be explained by differential response thresholds which lead to define behavioral groups of workers. Nurses are specialized in brood care, foragers in food supplying of the colony and domestics in nest maintenance. Nurses are expected to be sensitive to larval stimulations. Foragers have no direct contact with larvae but they regulate food provision according to the number of larvae in the colony, suggesting a volatile chemical signal. On the contrary, domestics are suspected to be indifferent to larvae.

To determine if the three behavioral groups of *Ectatomma tuberculatum* workers are able to detect larval chemical compounds at distance, we compare their responses in two experimental conditions. The first one is a dynamic olfactometer, a Y-maze with air flow, where individuals are tested alone. The second one is a static olfactometer, a Petri dish with a bottom made with wired mesh and without air flow, where individuals are tested in groups. In both experiments, workers have to make the choice between an area with larvae and an empty area. We demonstrate that only nurses and foragers detect volatile chemical signals from larvae but this ability depends on the experimental context. By contrast, domestics never show this capacity whatever the device. Larvae can be used as proximal signal to regulate individual worker behavior and the differential response threshold between workers can be used as a mechanism for task allocation.

ANT-APHID MUTUALISM – IMPLICATION OF HONEYDEW MICROFLORA

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Some ant and aphid species can present a mutualistic relationship, ants using aphid honeydew as sugar source and in exchange providing the aphid colony cleaning and protection. From a behavioral point of view, this phenomenon has been well studied for decades. However, its chemistry and semiochemical mechanisms are still largely unknown. This study aims to identify semiochemicals involved in the establishment of this relation, using both chemical and behavioral approaches. Bioassays revealed that the greatest part of ant attraction toward aphid colonies is due to honeydew volatile compounds; enabling ant scouts to find more quickly aphid colonies and distantly recognize myrmecophilous species. Many of those VOCs seeming to have microbial origins, the main honeydew microorganisms have been isolated and their roles in VOCs production and ant attraction have been investigated. It appeared that honeydew microflora holds a key role in the establishment of ant-aphids mutualistic relationship.

CAN BIRCHES TREATED WITH METHYL JASMONATE ATTRACT INSECTIVOROUS BIRDS IN THE NATURE?

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In addition to invertebrate predators and parasitoids, also insectivorous birds are attracted to herbivore-damaged trees, even when they do not see or smell the defoliated leaves, herbivores or their faeces. Our earlier studies suggested that insectivorous birds, similarly to invertebrate predators and parasitoids, may also use the volatile organic compounds (VOCs) emitted by herbivore-damaged birches (1). The olfactory ability of most birds, including passerines, was long thought to be negligible. However, recent studies have shown that passerines can use their olfaction, for example, during foraging, in aromatising nests and recognising predators. The VOC production in plants is mediated, at least partly, by the jasmonic acid signalling pathway, and often, a similar VOC production than the one caused by herbivores can be induced simply by exposing plants to methyl jasmonate (MeJa) without any herbivores (2). There have basically been no MeJa treatment studies with trees in nature so far. We studied the effects of MeJa on mountain birches (*Betula pubescens* ssp. *czerepanovii*) at the Kevo Subarctic Research Institute in northern Finland. There were in total four treatments: herbivore [autumnal moth (*Epirrita autumnata*)], higher level of MeJa (30 mM), lower level of MeJa (15 mM) and control. All birches had three branches covered with mesh bags but only in the herbivore treatment there were larvae inside the bags. Bird predation rate was followed with artificial plasticine larvae (10 larvae per birch) which were checked daily. Birds were significantly more attracted to the herbivore birches, and the three other treatments did not differ between each other. Variation between individual trees in VOC emissions was so big that it masked all possible treatment effects. But the bird predation rate was higher in birches that emitted more α-pinene. Thus, that may be one cue the birds use to find herbivore-damaged birches. But we could not with MeJa mislead the birches or the birds to think that there was herbivory. However, some of the birches in the high MeJa treatment suffered from necrotic spots in their leaves. Since there was no difference between higher and lower MeJa treatments, the lower level should be used in future experiments with mountain birches in nature to avoid unwanted effects.

References

1. PLoS ONE 3: e2832.
2. Journal of Chemical Ecology 32: 725–743.

VOLATILE ORGANIC COMPOUNDS OF *EUCALYPTUS BENTHAMII* BEFORE AND AFTER HERBIVORY BY *THAUMASTOCORIS PEREGRINUS* (HETEROPTERA, THAUMASTOCORIDAE) INFLUENCE THE PREFERENCE OF MATED FEMALES

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Thaumastocoris peregrinus feeds on *Eucalyptus* plantations in South Africa and South America, and there is no effective control strategy available for this pest. Thus, understanding how plants and insects interact may be useful, in the future, as basic information for new pest management strategies. Based on that, aerations of *Eucalyptus benthamii* plants were done before and after herbivory by *Thaumastocoris peregrinus*, and after mechanical damage. The extractions were done every 24 h for five days. The chromatographic profiles of all treatments were compared and the volatile organic compounds were identified and quantified. Three major compounds α-pinene, aromadendrene and globulol were released after herbivory and detected in lower quantities in control and after mechanical damage. In total, 15 compounds were quantified for all treatments. In general, after herbivory plants released a greater quantity of the compounds when compared with other treatments. There were no differences between photophase and scotophase treatments. Bioassays performed on Y olfactometer revealed a preference of mated females to unattacked plants when compared with plants after herbivory. Virgin females did not show any preference.

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CHEMICAL ANALYSIS OF METHYL JASMONATE TREATED SCOTS PINE *PINUS SYLVESTRIS* USING GC-MS AND LC-MS

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In my PhD work I analyze conifer constituents that seedlings produce and emit into the environment. The aim is to identify chemical resistance markers in Scots pine *Pinus sylvestris* against the large pine weevil *Hylobius abietis*. This insect is one of the economically most important forest pests in Sweden and almost 90% of the seedlings could die without protective measures such as shelterwood and soil scarification (1). Insecticides add to the protection of the seedlings but the future prohibition call for replacement. The signal hormone methyl jasmonate is exogenously applied to young conifers in order to induce chemical defence by mimicking insect herbivory (2, 3). Based on biological results from field trials in Sweden and in Spain, I evaluate stress responses from seedlings using GC-MS and LC-MS. From this I have found induction of β-pinene in Scots pine, after pretreatment with methyl jasmonate, and in Maritime pine *Pinus pinaster* and Monterey pine *Pinus radiata* after pine weevil infestation. The induction is observed in both stem and needles. Further I investigate possible activation of the (-)-pinene-synthase using chiral analysis with 2D-GC-MS.

Acknowledgements

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References

1. Peterson M., Örlander G. 2003. Canadian Journal of Forest Research. Vol. 33(1): 64–73.

2. Moreira X et al. 2012. Plant Defence: Biological Control. Vol. 12: 345–362.

3. Moreira X et al. 2012. Quantitative comparison of chemical, biological and mechanical induction of secondary compounds in *Pinus pinaster* seedlings. Trees, Structure and Function. Vol. 26: 677–683.

LEARNING HOW TO ESCAPE FROM AN ALARM PHEROMONE IN INSECTS

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Behavioural plasticity might be the key for survival in a constantly changing environment full of new dangers. Alarm pheromones are generally released by individuals for alerting conspecifics from a potential danger. Non-associative cognitive processes might increase the sensitivity of individuals to a particular stimulus allowing a quicker response (sensitization), or contrarily decrease the intensity of response to a particular stimulus if it is no longer informative (habituation). Similarly, associative processes might allow individuals to anticipate the occurrence of a danger after a previous association with predictive stimuli (classical conditioning) or even guide animals to elicit safer actions as a consequence of their own experience (operant conditioning). Cognitive processes involved in the modulation of the escaping response might then dramatically increase the probability of surviving of animals in natural environments.

In the triatomine bug *Triatoma infestans*, adults release a blend from their exocrine glands which alerts conspecifics about the presence of a danger. Although the composition of the alarm pheromone of this species and its functional effect have already been described, not much is known about the effect of individual compounds of this pheromone on the behaviour of adults and larvae and its modulation by cognitive processes.

We present here evidences of the cognitive capacities of *T. infestans* in an escape context under different conditioning paradigms. In non-associative experiments, we pre-exposed larvae to the alarm pheromone or to its individual compounds and subsequently tested their escape response to the alarm pheromone over a walking olfactometer. Under a classical conditioning context, we coupled the alarm pheromone with either a negative or a positive reinforcement and then tested their escape response over the same olfactometer. Finally, using an operant paradigm, animals were trained to avoid a certain zone of an experimental arena which was associated with the presence of the alarm pheromone.

Both, non-associative and associative paradigms are being applied to reveal up to which extent cognitive plasticity can modulate relevant innate behaviours in insects.

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PHEROMONES OF CLICK BEETLES (COLEOPTERA: ELATERIDAE): SEX OR AGGREGATION PHEROMONES?

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Originally it was thought that the pheromones of *Agriotes* click beetles fall into the "classical" sex pheromone category (produced by females to attract males). When working with synthetic pheromone lures, L. Furlan (unpublished data) observed that sizeable numbers of females were also caught. We present evidence that in several *Agriotes* species, synthetic pheromones also attract females. Further indication for the response of female beetles to the pheromone is that in EAG studies, antennae of both females and males show similar response intensities to a series of pheromone structures, responding best to the major pheromone component of the species. This suggests that female beetles are also able to perceive and differentiate between pheromone signals. In the case of *A. ustulatus*, where a floral lure is already known, this floral lure presented together with the pheromone (in the same trap) will catch much more females than the floral lure, or the pheropmone *per se*. It is widespread among aggregation pheromones that the pheromonal response is greatly increased by the presence of plant-derived scents. Consequently, perhaps the categorization "sex pheromone" should be revisited, and it is possible that click beetle pheromones also show some "aggregation" traits. Aggregation pheromones attract both sexes, and are particularly widespread in some coleopteran families (e. g. Scolytidae, Curculionidae, etc.). Lures also capable of attracting also females are especially "attractive" for practical applications.

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Symposium 2. Chemical Ecology Meets Evolutionary and Molecular Biology

Moderators: Walter S. Leal and Wei Xu

Keynote speaker

MOTH SEX PHEROMONE RECEPTORS AND DECEITFUL PARAPHEROMONES

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The olfactory acuity in insects is remarkable. Equipped with thousands of finely tuned sex pheromone sensors on the antennae, male moths can detect with inordinate sensitivity scents remotely released by conspecific females when they overtly advertise their readiness to mate. The insect's olfactory system is also so selective that male moths, for example, can discriminate female-produced sex pheromones from compounds with minimal structural modifications. Yet, there is an exception for this "lock-and-key" tight selectivity. Formate analogs can be used as replacement for less chemically stable, long-chain aldehyde pheromones, because male moths respond physiologically and behaviorally to these parapheromones. However, it remained hitherto unknown how formate analogs interact with aldehyde-sensitive odorant receptors (ORs). To investigate how this "deceitful" detection of formate analogs is manifested at moth OR level, we studied odorant-OR interactions in the navel orangeworm, *Amyellois transitella*, a major pest of the multibillion dollar almond, pistachio, and walnut industries in California. Using single sensillum recordings and the *Xenopus* oocyte recording system we compared responses from olfactory receptor neurons housed on male moth antennae with those elicited by OR-expressing oocytes when challenged with the major constituent of the navel orangeworm sex pheromone, Z11Z13-16Ald, and its formate analog, Z9Z11-14OFor. The surprising results were also obtained with oocytes-expressing a *Heliothis virescens* sex pheromone receptor.

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A SUGAR GUSTATORY RECEPTOR IDENTIFIED FROM THE FOREGUT OF COTTON BOLLWORM *HELICOVERPA ARMIGERA*

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Helicoverpa armigera (Hubner) is one of the most polyphagous and cosmopolitan pest species, the larvae of which feed on numerous important crops such as cotton, peanuts, soybeans or maize. The gustatory system is critical in guiding insect feeding behaviours. Here we identify one gustatory receptor, HarmGR12, from *H. armigera*, which shows high identity to DmGR43a from *Drosophila melanogaster* and BmorGR9 from *Bombyx mori*. Reverse transcriptase PCR (RT-PCR) revealed HarmGR12 is highly expressed in larval foregut, with little or no expression in other chemosensory tissues. Membrane topology studies indicated that, like two previously studied *B. mori* GRs, HarmGR12 has an inverted topology relative to G protein-coupled receptors, an intracellular N-terminus and an extracellular C-terminus. Calcium imaging studies confirmed HarmGR12 is a sugar receptor showing a high response to D-Fructose, D-Galactose and D-Maltose but not to the other sugars tested. This highly-expressed foregut-specific gustatory receptor may contribute to the control of larval feeding behaviour.

CHARACTERIZATION OF PHEROMONE RECEPTOR GENES IN THE TURNIP MOTH, *AGROTIS SEGETUM*

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Mate recognition in moths involves the production of sex pheromones by females and their detection by specific receptors on male antennae. The female turnip moth, *Agrotis segetum* (Noctuidae), produces a mixture of chemically related pheromone components including (*Z*)-5-decenyl, (*Z*)-5-dodecenyl, (*Z*)-7-dodecenyl and (*Z*)-9-tetradecenyl acetate (*Z*5-10:OAc, *Z*5-12:OAc, *Z*7-12:OAc and *Z*9-14:OAc) (1). The pheromone components, as well as the behavioral antagonists (*Z*)-5-decenol (*Z*5-10:OH) and (*Z*)-8-dodecenyl acetate (*Z*8-12:OAc), elicit specific responses from receptor cells on the male antenna (2, 3). This set of similar ligands and corresponding specific receptors makes the turnip moth a suitable model to unravel the molecular basis of specific ligand-receptor interaction. We cloned nine candidate pheromone receptor (PR) genes and the *Orco* gene from *A. segetum* by degenerate primer based RT-PCR and RACE PCR. These genes were named *AsegOR1-9* and *Aseg/Orco*. By construction of a phylogenetic tree including these genes and functionally characterized PRs from other moth species, we found that the candidate genes clustered in different expansions of previously described PR genes from the noctuid *Heliothis virescens*. Notably, six of them cluster in the expansion of HR16 and share high amino acid sequence identities with each other (71.0 to 98.4%). This suggests a rapid evolution of PR genes in *A. segetum*. Using the *Xenopus laevis* oocyte expression system and two-electrode voltage clamp recording, we have identified the ligands of five PR candidates from the HR16 expansion. AsegOR9 shows specific and large response to *Z*5-10:OAc, the pheromone component that evokes the largest electrophysiological response *in vivo*. Compared to AsegOR9, AsegOR1 shows a significantly smaller response to *Z*5-10:OAc, and a similar response to the antagonist *Z*8-12:OAc. AsegOR6 displays a major response to the antagonist *Z*5-10:OH, and a smaller response to *Z*5-10:OAc. AsegOR7 share 97.7% sequence identity (10aa differences) and the same response spectrum with AsegOR1, but with much lower sensitivity. Similarly, AsegOR8 is significantly less sensitive than AsegOR6, although they share 98.4% sequence identity (7aa differences) and the same response spectrum. Characterization of other PR candidates is underway as part of an extensive study of the molecular basis of receptor-ligand interactions.

Acknowledgements

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References

1. Journal of Chemical Ecology. 1982. Vol. 8: 1305–1321.
2. Journal of Chemical Ecology. 1985. Vol. 11: 1209–1221.
3. Physiological Entomology. 1995. Vol. 20: 81–92.

ACUITY OF THE PERIPHERAL OLFACTORY SYSTEM IS MEDIATED BY ANTENNAL GROOMING

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In insects, pheromones and other odorants are detected by neurons housed in olfactory sensilla on the antennae. Before the molecules can reach the receptor proteins bound to the membrane of the dendrites inside the sensillum they have to penetrate the sensillar cuticle and be solubilized in the hydrophilic sensillar lymph. The latter process is mediated by pheromone and odorant binding proteins that ferry the molecules to the receptor cells. It is still unclear, however, how odorant molecules traverse the cuticle and what role, if any, cuticular lipids play in this process.

Field emission scanning electron microscopy revealed that when *Periplaneta americana* males (American cockroach) were prevented from grooming one antenna, they accumulated a substance over the entire length of the non-groomed antenna. By analyzing cuticular lipids of groomed and non-groomed antennae using gas chromatography we demonstrated significant accumulations of cuticular hydrocarbons (CHC) on the non-groomed antennae of two cockroach species (*P. americana* and *Blattella germanica*), the carpenter ant (*Camponotus pennsylvanicus*), and the house fly (*Musca domestica*). We hypothesized that the accumulation of CHC might interfere with olfaction. Indeed, in *P. americana*, non-groomed antennae of males exhibited a lower sensitivity both to the female sex pheromone, periplanone-B, and the general odorant, geranyl acetate. Therefore we demonstrate that grooming has a hitherto unknown function in insects: enhancement of the acuity of the peripheral olfactory system by removal of excess cuticular lipids. The adaptive significance of a continuous deposition of cuticular lipids on the antennae is yet to be determined.

THE CHEMICAL ECOLOGY OF THE PACIFIC BEETLE COCKROACH, *DIPLOPTERA PUNCTATA*

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The cockroach, *Diploptera punctata*, has a wide geographic distribution throughout much of Asia and Hawaii, and as is one of the few vivaparous insect species know, it has been extensively studied by to gain insight into the underlying physiology of this unusual form of reproduction. However, there is not a great deal known about the mating behaviour of this species. We will present results, using populations from Hawaii and Thailand, showing that (i) there is very little interbreeding between the two populations, (ii) short distance pheromones are involved in mating, (iii) there are marked intersexual and inter-race differences in the cuticular profiles (iv) cuticular profiles change with age, and that in a Y tube olfactometer males respond preferentially to 5-day-old virgins over air but do not discriminate between air and newly-emerged virgins.

The last observation was interesting, given that in the literature it has been reported that mating occurs just after the female has moulted, before her cuticle has even hardened. Furthermore, while newly-mated females show no overt courtship behaviour, older individuals do, including strong rejection behaviours when courted. We postulated that the idea that females normally mate immediately after emergence is an artefact of the rather simple cages used in laboratory rearings. We found that mating success declined as the complexity of the environment was increased, suggesting that in nature a female may hide during moulting and only mate once her cuticle has hardened.

Acknowledgements

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SPECIATION IN PROGRESS: PHEROMONAL DIVERGENCE BETWEEN THE TWO STRAINS OF *SPODOPTERDA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

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The noctuid moth *Spodoptera frugiperda* consists of two different strains: the corn-strain and the rice-strain. Although both strains are morphologically indistinguishable from each other they exhibit a variety of genetic and behavioral differences. The existence of pre- and postzygotic isolation mechanisms in this species indicates that both strains are in the progress of sympatric speciation. We investigated the importance of variation in sex pheromones as a prezygotic mating barrier between both strains. We found strain-specific female pheromone differences in laboratory and field populations from Florida. Genetic analysis revealed multiple genomic regions that are involved in the corn- and rice-strain specific pheromones. Currently, we are mapping candidate genes to our QTL map. Field experiments in Florida showed strain-specific attraction of males towards the important secondary sex pheromone component Z7-12:Ac. Interestingly, strain-specific attraction depended on the type of field, corn or grass field, that the experiment was conducted in, indicating that habitat-specific volatiles mediate strain-specific pheromonal communication in this species.

LOOKING FOR A SIMILAR PARTNER: HOST PLANT SPECIES RATHER THAN GEOGRAPHIC ISOLATION PROMOTES SEXUAL ISOLATION OF HERBIVOROUS INSECTS

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Speciation of herbivorous insects might be initiated by the use of different host plants if plant species affect insect mating signals and thus, trigger assortative mating. Here, we studied the effects of different host plant species on mating behavior of the mustard leaf beetle *Phaedon cochleariae* feeding upon various brassicacean plants. We used two geographically isolated populations – one feeding in the field on large bittercress and the other on watercress. We addressed the questions (a) whether mates of these populations show assortative mating behavior, and, if so, (b) whether this is due to population genetic effects or to host plant effects. Bioassays showed that males preferred to mate with females of the same host plant population compared to females of the alternative host plant population. In order to find out whether this male mating preference was due to population-specific female cues or to cues dependent on the host plant species, we reared beetles of each population for one generation on the alternative host plant of the other population. Males preferred females feeding on the same host plant to those feeding on the alternative plant, irrespective of the population. These results showed that the preference of males for "same host plant females" is not genetically fixed in a population, but phenotypically plastic and dependent of the host plant species used. Furthermore, chemical analyses showed that the host plant species affected the quantitative composition of the beetles' cuticular hydrocarbon profiles which serve as contact pheromones. A discriminant analysis clearly separated the cuticular hydrocarbon profiles according to the host plant species the beetles were feeding upon, however, population effects were also detected. Our results suggest that plant-induced phenotypic divergence in mate recognition may act as an early barrier to gene flow between populations of herbivorous insects feeding on different host plant species, thus preceding genetic divergence and initiating ecological speciation.

THE ROLE OF FEMALE MOTH SEX PHEROMONE IN SEXUAL SELECTION

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The role of female sex pheromones in natural selection, as a means for species recognition and avoiding hybrid offspring, has been widely accepted, but their significance in shaping sexual selection through mate choice has been largely ignored. As a result of natural selection variation in sex pheromone characteristics among females is expected to be low and males are not expected to choose mates. Sexual selection, however, may result in variance in pheromone traits among females and males are expected to choose females based on this variation. Here we show that significant variation in pheromone amounts exists in females within a population, that pheromone amounts in glands of large females is greater than in glands of small females, and that males choose their mates based on their pheromone characteristics. These results are puzzling in light of the widely accepted low cost to female moths in producing sex pheromone. We demonstrate possible costs of pheromone production for females and discuss the likely reasons for males to be choosy.

PHEROMONE AND MOLECULAR CHARACTERIZATION OF BRAZILIAN POPULATIONS OF THE FALL ARMYWORM *SPODOPTERA FRUGIPERDA*

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The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the major pest of maize crops worldwide. In Brazil, the use of commercial synthetic sex pheromone to monitor *S. frugiperda* has not been successful possibly due to the fact that this pheromone was originally identified from populations from another countries. Thus, the current study aimed to evaluate the genetic variability and the chemical composition of the sex pheromone from different Brazilian populations of *S. frugiperda* to obtain a new pheromone to be incorporated into the management of this pest. Preliminary results involving genetic variability within and between populations revealed the presence of at least two distinct host strains (corn and rice) differently distributed in the studied regions. We have characterized on average 10 haplotypes in each studied region. These results provide a basis for the analysis of the pheromone of *S. frugiperda* and how pheromone composition may be affected by the genetic structure of the Brazilian populations of the fall armyworm.

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SOCIAL ENVIRONMENT DETERMINES DEGREE OF CHEMICAL SIGNALLING

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Few studies have attempted to distinguish between cues and signals in the context of chemical communication. A number of chemical substances have been shown to vary with physiological state, such as stage of oestrus cycle, fertility, dominance status or nutritional condition, but little is known about whether this variation is incidental or adaptive. Here we provide evidence of a substance whose emission varies with breeding state, but is not merely an incidental by-product of physiological state, but rather, an evolved signal. Breeding females of the facultative biparental burying beetle, *Nicrophorus vespilloides*, release (*E*)-methyl geranate, a terpenoid that appears to reflect their juvenile hormone titre and helps males to identify female breeding status and to distinguish between their breeding partner and non-breeding intruders. We demonstrate that females respond flexible to their social environment and emit high amounts of methyl geranate only in the presence of a male partner, i. e. a receiver, but not in the case of uniparental care. Receiver-dependent chemical signalling is expected to evolve when costs are involved in the production or transmission of the signal. Costs can arise, for instance, if signal production depends on the availability of limited nutritional resources or if signal transmission leads to attraction of predators. We present data showing that chemical signaling is costly, and that higher investments in signaling result in reduced clutch sizes, but not a shorter life span, in the burying beetle *Nicrophorus vespilloides*.

READINESS OF TERMITE WORKERS FOR SUICIDAL CHEMICAL DEFENSE INCREASES WITH AGE

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We send our young men to war, ants send their old ladies (1). This metaphor illustrates a long-known feature of insect society ergonomics, i. e. that older workers generally assume a larger share of the riskiest tasks, such as foraging or defense (2). More precisely, it is remaining fitness rather than absolute age which determines the cost of engaging in risky activities (3, 4). Among fully sterile castes of social insects, suicidal defensive behavior may be favored, if benefits to the colony overweigh the cost of losing one of its members. Here, we show that, as workers of the termite *Neocapritermes taracua* age and their food-collecting ability declines, they develop a unique two-component suicidal chemical defense consisting of copper-containing blue protein crystals, stored in an external pouch over the metathorax, and intra-abdominal salivary gland vesicles. During fight, the dorsal abdominal wall ruptures and the crystals react with the labial gland secretion to produce a toxic and sticky droplet; both the amount of defensive substances and the readiness to explode increase with workers' age. Our findings represent an excellent example of age-dependent transition to risky tasks in social insects, corroborating the predictions of kin selection theory (4). At the same time, we reveal an intricate and spectacular mechanism of chemical suicidal defense, without any equivalent in social insects.

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References

1. The Ants, Springer. 1990.
2. Annual Review of Entomology. 1992. Vol. 37: 637–665.
3. Animal Behavior. 1994. Vol. 48: 467–469.
4. Annual Review of Ecology, Evolution, and Systematics. 2007. Vol. 38:103–128.

EVOLUTION OF CARDENOLIDE SEQUESTRATION AND RESISTANCE IN LYGAEID BUGS

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Conspicuous black and red aposematism is a pervasive feature of the hemipteran subfamily Lygaeinae distinguishing them from other members of the mostly dull colored family Lygaeidae. Indeed, the warning coloration is associated with chemical defense which is often relying on sequestered host plant toxins. The best investigated species, the large milkweed bug *Oncopeltus fasciatus*, obtains cardenolides from *Asclepias* seeds and displays intricate adaptations to these toxins. However, usage of cardenolides seems to be widespread within the Lygaeinae (1). Cardenolides are powerful toxins which specifically inhibit the ubiquitous animal enzyme Na^+K^+-ATPase. In *O. fasciatus* cardenolide resistance is mediated by a Na^+K^+-ATPase possessing reduced sensitivity to cardenolides (target site insensitivity). To reconstruct the evolution of cardenolide sequestration and metabolism as well as target site insensitivity we established a molecular phylogeny comprising 18 lygaeine species and 4 outgroups (CO I/II; 28S rRNA). Cardenolide sequestration and metabolism were assessed by feeding studies with radioactive tracers in 7 species. Target site insensitivity was evaluated by in vitro assays of Na^+K^+-ATPase and sequence analysis. We found that sequestration of cardenolides and also target site insensitivity are probably synapomorphic characters of the lygaeine subfamily. Our data suggest that European members of the genus *Arocatus* lost black and red aposematism as well as the ability to sequester cardenolides. Nevertheless target site insensitivity, was retained although these species are no longer exposed to dietary cardenolides. The extensive association of Lygaeines with apocynaceous plants and the basal origin of adaptations to cardenolides suggest that this host plant relationship is the original one yet dependence on cardenolides led the bugs on to plants from many other families (Ranunculaceae, Plantaginaceae, Brassicaceae, Asparagaceae) featuring these compounds.

References

1. Scudder G. G. E., Duffey S. S. 1972. Cardiac glycosides in the Lygaeinae (Heteroptera: Lygaeidae). Can. J. Zool. Vol. 50: 35–42.

CONVERGENT EVOLUTION OF TOLERANCE TO CARDENOLIDES – LEAF BEETLES EVOLVED DIFFERENT TRICKS

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In leaf beetles cardenolides may either be encountered as secondary compounds present in their host plants (in Eumolpinae, Chrysomelinae and Criocerinae) or may be synthesized *de novo* as anti-predator defense (in Chrysomelinae). While in non-adapted species cardenolides act as potent toxins by blocking the Na,K-ATPase which is e. g. essential for nervous function, adapted leaf beetles do not suffer ill effects. We here investigate whether these convergent adaptations to cardenolides are based on a single physiological mechanism or whether several tricks have evolved within a single insect family to avoid the noxious effects of a potent toxin.

To determine whether the Na,K-ATPase of the investigated species is tolerant or susceptible to cardenolides we obtained data from in vitro enzyme assays, gene sequences and heterologous expression studies of genetically engineered Na,K-ATPase genes in cell culture. We can show that several species have independently acquired resistance to cardenolides by substituting the identical amino acids in their Na,K-ATPase genes that are essential for cardenolide binding. This striking convergence of adaptations at the molecular level is, however, not the only solution to avoiding cardenolide toxicity. Our findings suggest that in Eumolpinae and Criocerinae blocking the uptake in the body cavity and restricting access to the nervous tissue are two alternative routes taken to avoid toxic effects of dietary cardenolides.

MOLECULAR EVIDENCE OF FACULTATIVE INTRAGUILD PREDATION BY LARVAL *MONOCHAMUS TITILLATOR* (COLEOPTERA: CERAMBYCIDAE) ON BARK BEETLE LARVAE

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Woodboring beetles from the genus *Monochamus* (Coleoptera: Cerambycidae) commonly overlap spatially and temporally with bark beetles in recently-killed pine trees. This association is facilitated by kairomonal responses by *Monochamus* spp. to bark beetle pheromones. These associates have traditionally been classified as resource competitors; however, laboratory assays suggest that larval *M. carolinensis* may be facultative intraguild predators of bark beetle larvae. Field experiments demonstrated that *M. titillator* is attracted to pheromones of the southern pine beetle guild (SPBG). PCR-based molecular gut content analyses were used to characterize subcortical interactions between *M. titillator* and members of the SPBG. Species-specific PCR primers were developed to detect DNA of members of the SPBG in the gut contents of *M. titillator* larvae. Using these primers the half-lives of SPBG DNA were estimated in the laboratory. The gut contents of a total of 271 field-collected *M. titillator* larvae were analyzed and twenty-six (9.6%) tested positive for DNA of members of the SPBG. Of these larvae, 25 (96.2%), 1 (3.8%), 0 (0%), and 0 (0%) tested positive for *Ips grandicollis*, *I. calligraphus*, *Dendroctonus terebrans*, and *D. frontalis* DNA respectively. Results from this study support the hypothesis that larval *Monochamus* spp. are facultative intraguild predators of bark beetle larvae in the field. Additionally, this study demonstrates the capabilities of PCR in elucidating the interactions of cryptic forest insects and provides a powerful tool to better understand mechanisms driving southern pine beetle guild population fluctuations.

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DOES ACUTE EXPOSURE TO DIESEL EXHAUST POLLUTION ELICIT A NEUROLOGICAL STRESS RESPONSE IN HONEYBEES AND IMPACT ON THEIR ABILITY TO LEARN AND MEMORIZE A FLORAL ODOR?

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In recent years honeybees, *Apis mellifera*, have been undergoing a global decline, which in the USA has been attributed to Colony Collapse Disorder (CCD), whilst across Europe there have been abnormally high winter losses. It is likely that these losses are a result of multiple stresses resulting from a combination of infectious agents and other stress factors, rather than from a single cause (1). Much effort has been invested in better understanding the relative contributions of pathogens, parasites and insecticides; however in comparison, the role of air pollutants has received far less attention.

Despite efforts to mitigate vehicle exhaust pollution, diesel exhaust remains a major contributor towards air pollution, principally as a result of a continuing increase in the proportion of new vehicle registrations being diesel engined cars. Diesel Exhaust Pollution (DEP) consists of a cocktail of gases and particulates which are known to have negative impacts on the mammalian Central Nervous System (CNS). However, it is not yet known whether exposure to DEP results in similar impacts on invertebrates, such as honeybees.

We will present our findings on how an acute exposure to DEP impacts upon worker honeybees learning, memory and neurology. We used molecular and histological techniques to investigate the impacts that the acute DEP exposure has on a number of stress markers in honeybee CNS tissue. In addition, we investigated whether such an acute exposure to DEP affects a honeybee's ability to tolerate a secondary stress. Finally, we utilised a well-established Pavlovian conditioning paradigm, the proboscis extension response, to investigate the effects of an acute exposure to DEP on the ability of a worker honeybee to learn and memorize a floral odour.

Acknowledgements

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References

1. PLoS One. 2009. Vol. 4: e6481.

DECIPHERING THE SIGNATURE OF CUTICULAR LIPIDS WITH CONTACT SEX PHEROMONE FUNCTION IN A PARASITIC WASP

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Cuticular hydrocarbons (CHCs) covering the insect cuticle are supposed to serve primarily as a water barrier against desiccation. But CHCs are also used by many insects as contact pheromones. However, it is often not well understood, which components of a complex CHC profile are responsible for an observed behavior. In addition, the interaction between CHCs and more polar lipids also occurring on the insect cuticle has often been neglected. Males of the parasitic wasp *Lariophagus distinguendus* respond to female CHCs by wing-fanning, a characteristic element of male courtship behavior. Also newly emerged males elicit wing fanning in older consexuals, but young males lose their attractiveness within the first days of their live (1). This process is accompanied by the loss of 3-methylheptacosane (3-MeC27) and some minor components from the male CHC profile. It was unknown, however, whether the presence of 3-MeC27 is causally involved in the pheromone function of the *L. distinguendus* CHCs. In a subtractive fractionation-recombination approach using size exclusion chromatography, we investigated the putative key function of 3-MeC27 for the male courtship behavior. Furthermore, we studied the interaction of 3-MeC27 with the other CHCs and with triacylglycerides (TAGs) which we also detected on the cuticle of *L. distinguendus* (2). We found that 3-MeC27 is in fact essential for the pheromone function but also the other CHCs and surprisingly even the TAGs were necessary for a full wing fanning response. When applied to filter paper alone, 3-MeC27 was behaviorally inactive. In combination with the other CHCs and TAGs (which were inactive without 3-MeC27), however, 3-MeC27 was perceived enantioselectively by the responding males with (*S*)-3-MeC27 acting significantly stronger than the (*R*)-enantiomer. By application of 3-MeC27 to the cadavers of 4-d old males, these became as attractive again for consexuals as cadavers of newly emerged males. We conclude that 3-MeC27 is a key component of the *L. distinguendus* courtship pheromone which, however, has to be perceived with a chemical background of other CHCs and more polar cuticular lipids.

References

1. Behavioral Ecology and Sociobiology. 2005. Vol. 58: 111–120.
2. Journal of Experimental Biology (2012) in press.

ANT ASSOCIATIONS: INTERSPECIFIC TOLERANCE AND THE EVOLUTION OF CHEMICAL CUES AND SIGNALS

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Aggression between ant colonies is the norm. However, various *Crematogaster* and *Camponotus* species tolerate each other to various degrees and some of these species even live together in a shared nest (parabiosis). In order to understand the responsible mechanism for this unusual tolerance, we studied the interspecific nestmate recognition in several ant associations of *Crematogaster* and *Camponotus* species from the Palaeo- and Neotropics and the Mediterranean region (1, 2). Additionally, the composition of the cuticular profiles of the associated *Camponotus* species was compared with the cuticular profiles of several non-associated *Camponotus* species from different continents and climate zones (3). The cuticular hydrocarbon profiles of these associated *Camponotus* species shows of unusual patterns and/or compounds compared with other *Camponotus* species. We conclude that by evolving a shift towards long-chain cuticular hydrocarbons or towards a high percentage of n-alkanes in the profile, associated ant species provides fewer recognition cues and thus permit unusual interspecific tolerance by their *Crematogaster* partner.

Acknowledgements

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References

1. Frontiers in Zoology. 2008. Vol. 5: 16.
2. Ecological Entomology. 2010. Vol. 35: 711–720.
3. Evolution. 2012. Vol. 66: 896–904.

Keynote speaker

ACTIVATION OF A TUNICATE XENOBIOTIC RECEPTOR BY MARINE MICROALGAL BIOTOXINS

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Vertebrate xenobiotic receptors, such as the pregnane-X-receptor (PXR, NR1I2), are ligand activated nuclear receptor (NR) transcription factors which regulate multiple genes involved in detoxification reactions. From an evolutionary perspective PXR orthologues represent a highly derived branch of the NR family tree – the root of which lies deep at the base of animal phylogeny. Vertebrate PXR orthologues are unusual amongst the NRs in displaying considerable inter-taxa variation in ligand binding domain (LBD) sequences with some evidence of positive selection. Consequently, it has been hypothesized that such PXR LBD differences may reflect adaptive evolution enhancing the binding of those toxins, both endogenous and exogenous, typically encountered by the corresponding organism. Tunicates (phylum Chordata) occupy an intriguing combination of ecological and evolutionary positions – with adult tunicates being both marine filter-feeders and members of a sister clade to the Vertebrata. As many as 12 tunicate genome sequencing projects are at various stages of completion / annotation with the genome of the solitary ascidian *Ciona intestinalis* (published 2002) encoding two PXR orthologues (designated PXR/VDRα and β) and that of the pelargic *Oikopleura dioica* (published 2010) encoding as many as six. Pursuing the idea that metazoan xenobiotic receptors may adaptively evolve to bind toxic chemicals commonly present in the organism's environment / diet, we investigated the activation of *C. intestinalis* VDR/PXRα LBD by 4 natural toxins, produced by microalgae and to which *C. intestinalis* may have been exposed over evolutionary time, in addition to 11 synthetic environmental toxicants. Using a luciferase reporter assay we showed that *C. intestinalis* VDR/PXRα was activated, at nanomolar concentrations, by 2 of the 4 natural marine microalgal biotoxins tested (okadaic acid, EC_{50} = 18.2 ± 0.9 nM and pectenotoxin-2, EC_{50}= 37.0 ± 3.5 nM) along with 1 of 11 synthetic toxicants (esfenvalerate, EC_{50} = 0.59 ± 0.7 μM). However, the microalgal biotoxins gymnodimine and yessotoxin were not active in the luciferase assay. Human PXR was activated by okadaic acid at similar concentrations to *C. intestinalis* VDR/PXRα (EC_{50} = 7.2 ± 1.1 nM), albeit not so strongly, but not by pectenotoxin-2. In contrast two related *C. intestinalis* NRs, orthologous to the vertebrate farnesoid X (FXR) and liver X (LXR) receptors, along with the PXR of a freshwater fish (zebrafish, *Danio rerio*), were not activated by any of the 15 chemicals tested. These results are consistent with the original hypothesis that natural toxins present in the diet of filter-feeding marine invertebrates, may have acted as selective agents in the molecular evolution of tunicate xenobiotic receptor LBDs. Given the large number of tunicate species that occupy a wide range of ecological niches and climatic zones we propose that tunicate xenobiotic receptors may represent a valuable source of 'pre-shaped' sensor elements for the detection of marine microbe associated bioactive compounds.

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THE ECOLOGICAL AND EVOLUTIONARY RELATIONSHIPS BETWEEN CONSTITUTIVE AND INDUCED DETOXIFICATION RESISTANCE IN THE GENERALIST HERBIVORE *BEMISIA TABACI*

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The ability to metabolize (detoxify) plant toxins (allelochemicals) is considered one of the major weapons insect have evolved in their coevolutionary 'arms race' with plants. Generalist insect herbivores that feed on a wide range of plant families, can utilize two different modes for regulating their detoxification genes: the constitutive mode, in which the detoxification genes are expressed independent of encountering a defended plant, and the induced mode, in which the detoxification genes are activated only after contact with the plant toxic chemistry. Despite the fact that transcriptional regulation of detoxification genes by dietary constituents should be particularly challenging for broadly generalist species, the inducible mode is assumed to be advantageous over the constitutive mode, largely because it is believed to confer an adaptive phenotypic plasticity that enables reducing the costs associated with maintenance of detoxification.

Molecular and genomic research conducted in the last decade has brought, however, evidence that this assumption may not always hold. Unexpectedly, transcriptional regulation of detoxification genes appears to be relatively unspecialized in generalist herbivores. In addition, extensive genomic surveys indicate that only a minority of detoxification genes respond to a variety of environmental inducers.

Here, we used the *Bemisia tabaci* complex of species (and particularly the B and Q species), as a model system for studying the evolution of constitutive and induced detoxification resistance and their associated tradeoffs. Quantitative real-time PCR analyses of 19 detoxification genes, belonging to the cytochrome P450 monooxygenases (P450), glutathione S-transferases (GST) and carboxylesterase (COE) families, indicated that ten of the 19 genes analyzed were significantly over-expressed in the Q species (relative to the B species) when feeding on plant hosts or sucrose diet, strongly suggesting that the Q species have adapted a constitutive detoxification resistance strategy for dealing with toxic plant chemistry. dsRNA silencing of specific over-expressed genes was associated with significant reduction in the Q species performance on various plant hosts. We therefore argue that the importance of constitutive detoxification resistance to plants toxic chemistry may have been underestimated. It may actually be more advantageous for generalist herbivores, in various ecological contexts, to move from one plant species to the other with their detoxification system turned on at all times, as these species are likely not to have the ability, anyway, to respond to specific compounds with the "right" optimal gene.

HOST SHIFTS FROM LAMIALES TO BRASSICACEAE IN THE SAWFLY GENUS *ATHALIA*

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Interactions with the plant chemistry can be a key driver of host shifts in herbivorous insects and thus of plant-insect evolution. Within the sawfly genus *Athalia*, several species are pests on Brassicaceae, whereas others are specialized on Lamiales. Plants of both taxa contain characteristic glucosides, which are sequestered by the larvae. To investigate the possible direction of host shifts within the genus *Athalia*, the sequestration specificity and feeding deterrence of iridoid glucosides (IGs) and glucosinolates (GSs) was studied in larvae of five species, which either sequester IGs from their hosts within the Plantaginaceae (Lamiales) or GSs from Brassicaceae, respectively. In addition, adults were tested for feeding stimulation by a neo-clerodane diterpenoid which is characteristic for Lamiales. Larvae of the Plantaginaceae-feeders did not sequester artificially administered GS, and were more deterred by GSs than Brassicaceae-feeders were by IGs. In contrast, larvae of the Brassicaceae-feeders were able to sequester artificially administered catalpol (IG), which points to an ancestral association with Lamiales. In line with this finding, adults of all tested species were stimulated by the neo-clerodane diterpenoid. Fundamental physiological pre-adaptations such as the formation of a glucoside transporter and ways to circumvent an activation of glucosides by glucosidases are necessary prerequisites for successful host shifts between Lamiales and Brassicaceae.

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References

1. Opitz S. E. W., Boevé J.-L., Nagy Z. T., Sonet G., Koch F., Müller C. 2012. Host shifts from Lamiales to Brassicaceae in the sawfly genus *Athalia*. PLoS ONE 7(4): e33649. doi:10.1371/journal.pone.0033649.

METABOLISM OF AROMATIC GLUCOSINOLATE-DERIVED CYANIDE IN THE SPECIALIST HERBIVORE *PIERIS RAPAE*

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Plants of the order Brassicales are defended by the formation and storage of glucosinolates and the enzymes responsible for their hydrolysis, the myrosinases. Upon herbivore feeding and tissue disruption, toxic isothiocyanates are released from this system in order to deter the threat. Nevertheless, ancient Pierinae, a subfamily of the Lepidoptera with modern-day members such as *Pieris rapae*, colonized the Brassicales. They had found a way of dealing with this by forming a nitrile specifier protein (NSP) which redirects glucosinolate hydrolysis to form nitriles instead of isothiocyanate (1). Recent research has shown that nitriles originating from phenylalanine-derived glucosinolates like phenylacetonitrile are further metabolized in the herbivores' body to α-hydroxynitriles decomposing under release of cyanide (2). Phylogenetic analysis has shown that ancestral plants of the Brassicales contain predominantly phenylalanine-derived glucosinolates such as benzylglucosinolate (3). Thus, the elimination of toxic cyanide was and still is essential for the Pierinae's survival on their food plants.

Transfer of cyanide to cysteine catalysed by β-cyanoalaninesynthase (β-CAS) is described as the most effective way for the detoxification of cyanide. In addition, formation of thiocyanate from cyanide via a sulfur transfer reaction catalysed by an enzyme common to all organisms, rhodanese, can be assumed to play a role in cyanide detoxification (4). To find out, if one of these enzymes is the main cyanide detoxification enzyme in *P. rapae*, feeding experiments with *Arabidopsis thaliana* plants with manipulated glucosinolate profiles were performed and metabolites and enzyme activities analyzed. We found increased amounts of β-cyanoalanine in larval body and faeces as a consequence of high aromatic glucosinolate content. Direct fumigation with [^{15}N]HCN resulted in a strong increase of both (M+1) β-cyanoalanine and (M+1) thiocyanate, indicating a function of both enzymes in the metabolism of toxic concentrations of cyanide. Additional experiments localised enzyme activities for both β-CAS and rhodanese to the larval guts.

Acknowledgements

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References

1. PNAS. 2007. Vol. 51: 20427–20431.
2. PloS one. 2012. Vol. 7(4): e35545.
3. Phytochemistry. 2010. Vol. 71: 2074–2086.
4. Plant Physiology. 1980. Vol. 65: 1199–1202.

STEREOSELECTIVITY IN THE PHEROMONE COMMUNICATION OF *NASONIA* WASPS

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Reliable localization and recognition of mates is a prerequisite for sexual reproduction. Hence, speciation is typically accompanied by the diversification of sexual signals and their perception. In organisms relying on olfaction, chirality of the chemical signals is one way to achieve species specificity. Males of the parasitic wasp genus *Nasonia* attract virgin conspecific females by using a substrate borne sex pheromone, which is produced in the rectal vesicle (1, 2). The major component of the pheromone is 5-hydroxy-4-decanolide (HDL) which is synergized by the minor component 4-methylquinazoline (4-MQ) (3). The HDL molecule has two chiral centres and consequently four possible stereoisomers. However, only two of these have been found in *Nasonia* wasps. Males of all *Nasonia* species produce (4*R*,5*S*)-HDL, but *N. vitripennis* males produce additionally considerable amounts of (4*R*,5*R*)-HDL. This suggests that the combination of (4*R*,5*S*)-HDL and 4-MQ is the plesiomorphic pheromone phenotype and that (4*R*,5*R*)-HDL evolved after *N. vitripennis* has split from the ancestor of all other *Nasonia* species approximately one million years ago. However, it was hitherto unclear whether the two pheromone phenotypes are discriminated by *Nasonia* females, which would enable them to avoid costly interspecific mating. We investigated this question in the two sympatric *Nasonia* species *N. vitripennis* and *N. giraulti*. We found that females of both species are attracted to homo- and heterospecific sex pheromone extracts when tested against a solvent control. However, *N. giraulti* females respond much less to the chemical signals than do females of *N. vitripennis*. When single enantiopure compounds were tested, females of both species were attracted to (4*R*,5*S*)-HDL. In contrast, (4*R*,5*R*)-HDL proved to be completely unattractive for both species when offered alone. We experimentally transformed the "*giraulti*" pheromone phenotype into the "*vitripennis*" pheromone phenotype by adding the behaviourally inactive (4*R*,5*R*)-HDL in realistic doses [60% of the existing (4*R*,5*S*)-HDL] to a natural *N. giraulti* pheromone extract. *Nasonia vitripennis* preferred the experimentally created "*vitripennis*" phenotype whereas *N. giraulti* did not discriminate between the two blends. Our data suggest that stereoselectivity in both pheromone biosynthesis and olfaction has accompanied speciation in *Nasonia vitripennis*.

References

1. Journal of Experimental Biology. 2007. Vol. 210: 2163–2169.
2. PNAS. 2008. Vol. 105: 8914–8919.
3. Journal of Chemical Ecology. 2008. Vol. 34: 99–102.

CRACKING THE OLFACTORY CODE OF A BUTTERFLY: THE SCENT OF AGEING

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Although olfaction is a primary mode of communication, its importance in sexual selection remains understudied. Here, using the butterfly *Bicyclus anynana*, we address all the parameters of importance to sexual selection for a male olfactory signal (1, 2). We show that variation in the male sex pheromone composition indicates male identity and male age. Courting males of different ages display small absolute (c. 200 ng) but large relative (100%) change of one specific pheromone component (hexadecanal) which, unlike the other components, showed no heritability. Females prefer to mate with mid-aged over younger males and the pheromone composition is sufficient to determine this preference. Surprisingly refined information is thus present in the male olfactory signal and is used for sexual selection. Our data also reveal that there may be no "lek paradox" (3) to resolve once the precise signal of importance to females is identified, as hexadecanal is, as expected, depleted in additive genetic variation.

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References

1. Nieberding C., Schneider M. V., de Vos H., Lassance J. M., Estramil N., Andersson J., Bång J., Hedenström E., Lofstedt C., Brakefield P. 2008. Male sex pheromones in the butterfly *Bicyclus anynana*: towards an evolutionary analysis. *PLoS ONE* Vol. 3(7): e2751.

2. Nieberding C., Fischer K., Saastamoinen M., Allen C., Wallen E., Hedenström E., Brakefield P. 2012. Cracking the olfactory code of a butterfly: the scent of ageing. Ecology Letters DOI: 10.1111/j.1461-0248.2012.01748.x

3. Kotiaho J. S., Lebas N. R., Puurtinen M., Tomkins J. L. 2008. On the resolution of the lek paradox. Trends Ecology Evolution. Vol. 23: 1–3.

EXPANDING ON OLFACTORY COMMUNICATION IN A BUTTERFLY: CUTICULAR CHEMICALS INDICATE SEX AND AGE IN *BICYCLUS ANYNANA*

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Chemical (olfactory or gustatory) communication is fundamental to most living organisms but widely understudied compared to other channels of communication such as vision and audition, principally in sexual selection (1).

Here we present the first extensive analysis of cuticular chemical diversity in a butterfly, and investigate whether molecules inform potential mating partners about sex and age. *Bicyclus anynana* was chosen for its well-known potentialities as a lab model in eco-evo-devo studies (2, 3). Chemical interactions have been investigated so far in this species with focus on volatile olfactory components: male sexual pheromones composition and their roles in sexual selection (4, 5), their change in ratio with male age, inbreeding coefficient, and other factors (6).

Here we aim completing the picture fully and focus on gustatory non-volatile cuticular chemical diversity in this model species. Indeed, as for Drosophila, the courtship of this butterfly is composed of a series of steps that include short-range interactions during which various chemicals may be involved in mate-choice, through olfactory but also gustatory channels of communication (Nieberding et al., data not published).

More than hundred cuticular chemicals were identified and quantified by GC-MS analyses on different parts (abdomen, antennae, head, legs and wings) of *B. anynana* individuals (n = 42, 210 GC-MS analyses) of each sex and at different ages (from 1 to 21 days old). The analysis of the chemical distribution was realised by multivariate statistical analyses (PERMANOVA). The results led to the conclusion that some cuticular chemicals are indicative for the body parts and can inform about sex and age.

References

1. Coleman S. W. 2009. Taxonomic and sensory biases in the mate-choice literature: there are far too few studies of chemical and multimodal communication. Acta Ethol. Vol. 12: 45–48.

2. Beldade P., Brakefield P. M. 2002. The genetics and evo-devo of butterfly wing patterns. Nature. Vol. 3: 442–452.

3. Saenko S. V., French V., Brakefield P. M., Beldade P. 2008. Conserved developmental processes and the formation of evolutionary novelties: examples from butterfly wings. Phil. Trans. R. Soc. B. Vol. 363: 1549–1555.

4. Costanzo K., Monteiro A. 2006. The use of chemical and visual cues in female choice in the butterfly *Bicyclus anynana*. Proc. R. Soc. B. doi:10.1098/rspb.2006.3729.

5. Nieberding C. **M., de Vos H., Schneider M. V., Lassance J.-M., Estramil N., An**dersson J., Hedenström E., Löfstedt C., Brakefield P. M. 2008. The male sex pheromone of the butterfly *Bicyclus anynana*: towards an evolutionary analysis. Plos One. 3:e2751: 1–12.

6. Nieberding C. M., Fischer K., Saastamoinen M., Allen C. E., Wallin E. A., Hedenström E., Brakefield P. M. 2012. Cracking the olfactory code of a butterfly: the scent of ageing. Ecology Letters. Vol. 15: 415–424

THE CHEMISTRY OF WING ANDROCONIA OF *HELICONIUS* BUTTERFLIES

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While sex-pheromones of moth are well understood, the pheromones of butterflies are less well explored. Until now, just a few pheromones have been identified in male butterflies (1). The South American *Heliconius* butterflies are of special interest because of their complex mimicry rings, varying wing patterns and co-occurrence of species. Males use antiaphrodisiacs that are transferred during mating to females (2). In addition, males also possess androconia on the wings. Several observations indicate that these organs are used to attract females. We investigated here the chemistry of the androconia of several species to identify pheromones probably released from them.

Pentane and dichloromethane extracts of androconia from several *Heliconius* species were analyzed by GC-MS. The major compounds, not found on female wings, were aldehydes such as octadecanal, 9-octadecenal and their higher homologues. While *Heliconius melpomene* subspecies vary just in the ratio and concentration of these components, extracts of *Heliconius cydno* also contain fatty acid derivatives like esters and lactones. The males are known to produce lactones like (*S*)-coriolide (1) in their abdominal glands containing antiaphrodisiacs (3, 4). Here we show that the compounds occur also in the wing androconia. Individual analyses of the composition and amount of the androconial compounds will bring insight into speciation, function, and individual mating success. They data will be related to genetic analysis to elucidate processes of speciation and evolution within the complex *Heliconius* mimicry patterns.

References

1. Ando T., Inomata S-I., Yamamoto M. 2004. Top. Curr. Chem. Vol. 239: 51–96.
2. Schulz S., Estrada C., Yildizhan S., Gilbert L. E. 2008. J. Chem. Ecol. Vol. 34: 82–93.
3. Schulz S., Yildizhan S., Stritzke K., Estrada C., Gilbert L. E. 2007. Org. Biomolec. Chem. Vol. 5: 3434–3441.
4. Estrada C., Schulz S., Yildizhan S., Gilbert L. E. 2011. Evolution. Vol. 65: 2843–2854.

DIVERGENCE OF TWO SIBLING *GRAPHOLITA* SPECIES IN SEX PHEROMONE BIOSYNTHETIC MACHINERY ANALYSED BY A DEEP SEQUENCING TRANSCRIPTOME ANALYSIS

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Two internal apple feeders of *Grapholita molesta* and *G. dimorpha* are highly similar in morphological characters and feeding behaviors. Moreover, their sex pheromones share two major components of *Z*8-dodecenyl acetate (*Z*8-12Ac) and *E*8-dodecenyl acetate (*E*8-12Ac), but their relative compositions are different. Dichotomous molecular markers were developed in this study. Using these markers, these two species female adults were identified and their abdominal tips were used for RNA extractions. Extracted RNAs were read by a short read deep sequencing technology using and Illumina HiSeq 2000. Almost 3–4 Gb reads were de novo assembled and resulted in 76,361 contigs of *G. dimorpha* and 104,463 contigs of *G. molesta*. More than 70% of these contigs were annotated and classified by a typical GO analysis. Transcriptomes related with sex pheromone biosynthesis were selected and grouped into fatty acid synthase, fatty acid oxidation, desaturase, reductase, and isomerase. These analyses identified sex pheromone biosynthesis machineries, which showed significant differential expressions between two sibling species. Field monitoring assays indicated that minor components resulted from fatty acid reductases were crucial in isolating two sibling species.

A CONTROL FROM THE OENOCYTES IS NECESSARY TO WATERPROOF THE RESPIRATORY SYSTEM IN DROSOPHILA LARVAE

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Oenocytes are involved in fatty acid (FA) metabolism in drosophila larvae and adults and in pheromone biosynthesis in adults. Their ablation in adults is viable and leads to adults without hydrocarbons. In larvae, oenocyte ablation results in lethality.

Inhibition of FA synthesis in oenocytes through acetyl-coenzymeA-carboxylase (ACC) RNAi knock-down led also to lethality, whereas disruption of ACC in the larval fat body was viable.

We found that this lethality was associated to a water-tightness failure of the spiracles, the organs controlling the entry of air into the trachea. This phenotype depends on the synthesis of very-long-chain FA (VLCFA) within the oenocytes and lately results in a lethal anoxic issue. Using flies knock-down for specific elongases, we have determined the elongase required in this process. In summary, ACC and an elongase fulfill an essential function of the oenocytes through VLCFA synthesis by inducing a remote-control necessary to maintain the integrity of the respiratory system.

BIOSYNTHESIS OF PLANT VOLATILE ELICITING FACS IN LEPIDOPTERAN CATERPILLARS, FRUIT FLIES AND CRICKETS: CONVERGENT EVOLUTION OR PHYLOGENETIC INHERITANCE?

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Fatty acid amino acid conjugates (FACs), first identified in lepidopteran caterpillar spit as elicitors of volatile organic compounds for several plants, also have been reported as one of major compontents of uncertain function in gut tracts of *Drosophila melanogaster* and *Teleogryllus* crickets (1). The profile of FAC analogs in these two insects were similar to that of tobacco hornworm *Manduca sexta*, showing glutamic acid conjugates predominantly over glutamine conjugates. On the other hand, large number of the lepidopteran species so far screened for FACs have the glutamine conjugates as main components, although one quarter of the species did not have any trace of FACs (2).

The physiological function of the glutamine conjugates in *Spodoptera litura* (Noctuidae, Lepidoptera) larvae was presumably to enhance nitrogen assimilation (3). But in other insects the physiological background of FACs, especially for the glutamic acid conjugates, is totally unknown except that the FACs have so far been identified only from the gut tracts in all insects.

In order to gain more detailed information, in vitro biosynthesis of the FACs was examined in three different lepidopteran caterpillars (*M. sexta*, *S. litura* and silkworm), and the results were compared with that of *D. melanogaster* and *Teleogryllus emma*. The gut tissues of any of the tested insects, including silkworm which have no FACs, showed the ability to synthesize glutamine conjugates when incubated with glutamine and sodium linolenate. However, the same experiment with glutamic acid revealed that the synthetic pathway of the glutamic acid conjugates differed among the insect family. Lepidopteran caterpillars and *D. melanogaster* seem to conjugate glutamic acid with fatty acids just like when they synthesize glutamine conjugates. However, this direct conjugation was not observed in the case of *T. emma*. Instead, they first synthesize glutamine conjugates and hydrolyze the side chain of the glutamine moiety to yield glutamic acid conjugates.

If the FACs found in these insects are the result of a convergent evolution, this could be a remarkable case that in such evolutionarily-distant species in different food habitats have independently developed the same set of 8 FAC analogs (including hydroxylated FACs). The other possibility could be that the ancestral species originally had the glutamine conjugates, and some insects lost the ability while some other species independently developed those glutamic acid conjugates or hydroxylated FACs as the variations, converged into the same patterns.

References

1. Journal of Chemical Ecology. 2007. Vol. 33: 1376–1381.
2. Journal of Chemical Ecology. 2010. Vol. 36: 319–325.
3. PNAS. 2008. Vol. 105: 18058–18063.

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HOW TO BECOME A SUCCESSFUL ROOT PEST IN THREE SIMPLE STEPS – A BEGINNER'S GUIDE

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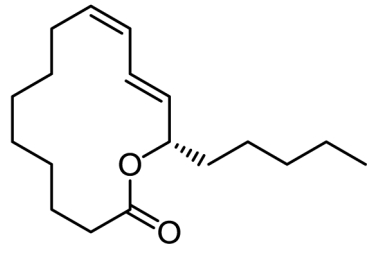
Root herbivores are among the most harmful agricultural pests. Yet, how they interact with their host plants is a vastly understudied area of chemical ecology. Over the last years, we have investigated the behavioral and chemical interplay between maize (*Zea mays*) and the western corn rootworm (*Diabrotica virgifera virgifera*), a highly specialized root herbivore, and our research highlights why *D. virgifera* is such a successful and damaging pest. First *D. virgifera* does not only use CO_2 as a host location cue below ground, it can integrate root-herbivore-induced (*E*)-β-caryophyllene and leaf-herbivore-suppressed ethylene as additional signals to select suitable host plants (1, 2). Second, once *D. virgifera* has located a host plant,



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it can tolerate and exploit benzoxazinoids, toxic secondary metabolites that are exuded by maize roots, to orient itself within the root system and feed on the most nutritious tissues (3). Third, although *D. virgifera* attack does induce a defense response in maize roots, the plants do not become more resistant, but more susceptible against subsequent attack (4). Evidently, *D. virgifera* is very well equipped to deal with constitutive and induced maize defenses, and we propose that breeding for tolerance rather than resistance may help to reduce its negative impact on agricultural productivity.

References

1. Erb M., Robert C. A. M., Hibbard B. E., Turlings T. C. J. 2011. Sequence of arrival determines plant-mediated interactions between herbivores. Journal of Ecology. Vol. 99: 7–15.

2. Robert C. A. M.*, Erb M.*, Duployer M., Zwahlen C., Doyen G. R., Turlings T. C. J. 2012. Herbivore-induced plant volatiles mediate host selection by a root herbivore. New Phytologist. DOI: 10.1111/j.1469-8137.2012.04127.x *Equal contribution.

3. Robert C. A. M., Veyrat N., Glauser G., Marti G., Doyen G. R., Villard N., Gaillard M. D. P., Köllner T. G., Giron D., Body M., Babst B. A., Ferrieri R. A., Turlings T. C. J., Erb M. 2012. A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. Ecology Letters. Vol. 15: 55–64.

4. Robert C. A. M., Erb M., Hibbard B., French W., Zwahlen C., Turlings T. C. J. (submitted). A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density dependent manner.

HOW PARASITOIDS PRODUCE SEXY SONS – DIETARY LIPIDS CONTROL MATE CHOICE AND FERTILITY IN *NASONIA*

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The nutritional state of parasitoids depends on the oviposition decision of their mother and often well fed males have better mating chances. The preference performance hypothesis predicts that female insects oviposit preferentially on hosts on which their offspring performs best. A causal connection, however, between the host choice of the mother and the mating chances of her offspring has never been shown. We demonstrate that females of the parasitic wasp *Nasonia vitripennis* prefer those hosts for oviposition that have been experimentally enriched in linoleic acid (LA) (1). We show by ^{13}C-labelling that LA from the host diet functions as a precursor of the male sex pheromone consisting of a mixture of (4*R*,5*S*)- and (4*R*,5*R*)-5-hydroxy-4-decanolides. Males from LA rich hosts produce and release higher amounts of the pheromone and consequently are more attractive for virgin females than those from LA poor hosts. Additionally, males from LA rich hosts possess three times as many spermatozoa. Hence, a high pheromone titer reflects honestly male mate quality.and virgin females orienting towards concentration gradients of the male sex pheromone increase their chance of mating with a male of sufficient fertility. Hence, parasitoid females making the right oviposition decisions may increase both the fertility and the sexual attractiveness of their sons.

References

1. Proc R Soc Lond B. 2011. Vol. 278: 3286–3293.

COMPARATIVE CUTICULAR LIPID ANALYSIS OF *DROSOPHILA* USING ULTRAVIOLET LASER DESORPTION / IONIZATION MASS SPECTROMETRY

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Cuticular lipids have been proposed as a marker for identifying species and mapping evolutionary relationships (1). However, in order for the profile to be a reliable chemical fingerprint of a species, it is essential that all components are identified. Gas chromatography mass spectrometry, the conventional methods of analysis, may provide only a partial chemical profile consisting of apolar and smaller molecular weight compounds. One complementary analytical method that may be able to offer a more complete chemical picture is ultraviolet-laser desorption ionization (UV-LDI) MS. UV-LDI MS is able to detect larger, polar compounds and allow spatially-resolved profiling of single insects (2).

I analyzed the cuticular lipid profile of 12 different *Drosophila* species using UV-LDI MS. The lipid profiles of different *Drosophila* species show distinct differences with respect to lipid classes and relative quantities. Among the different kinds of lipids identified from the cuticular surface were C20–C37 unsaturated hydrocarbons (including alkenes, dienes, trienes), oxygen-containing hydrocarbons, and triglycerides. Closely-related species and populations show quantitative rather than qualitative differences. In contrast, distantly-related species show striking differences in lipid profiles, particularly in the components of male sex glands. Males and females of all species exhibited distinct sexually dimorphic lipid compositions. The cuticular profiles of recently mated females also show changes in lipid composition, indicating that some male-specific compounds were transferred to females during copulation. These results indicate that cuticular lipids of insects can be used as biomarkers specifying species, sex, and mating status. Such information is important for species identification and may be used to trace evolutionary relationships.

Acknowledgements

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References

1. Kather R. A., Martin S. J. 2012. Cuticular hydrocarbon profiles as a taxonomic tool: advantages, limitations and technical aspects. Phys. Entomol. Vol. 37: 25–32.

2. Yew J. Y., Dreisewerd K., Luftmann H., Müthing J., Pohlentz G., Kravitz E. A. 2009. A new male sex-pheromone and novel cuticular cues for chemical communication in *Drosophila*. Curr Biol. Vol. 19(15): 1245–1254.

A GEOGRAPHICAL POLYMORPHISM WITH MALE HYDROCARBONS LEADS TO SEXUAL ISOLATION IN DROSOPHILA

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In *Drosophila*, female hydrocarbons are known to be involved in premating isolation between different species and pheromonal races. However, the role of male-specific hydrocarbon polymorphism is not as well documented. The dominant cuticular hydrocarbon (HC) in male *D. melanogaster* is usually 7-tricosene (7-T; C23:1), with the exception of central African populations, in which 7-pentacosene (7-P; C25:1) is dominant. Here, we describe a novel population from Comoro Island (Com) that includes males with sex pheromone profiles ranging from high 7-T to high 7-P. We maintained Com flies at different temperatures, without selection. After 18 months, flies reared at 21 °C had a 7-T hydrocarbon profile and flies reared at 25 °C had an intermediary to 7-P profile.

We also specifically selected for high 7-T, high 7-P, or intermediate hydrocarbon profiles. There was a direct relationship between chain length and resistance to desiccation in both temperature- and phenotypically-selected Com lines. We also show that the 7-P/7-T ratio depended on temperature in Com and the other strains tested, with generally more 7-P at higher temperatures. There was partial reproductive isolation between flies with clear-cut phenotypes (7-T and 7-P). These results show that the dominant male pheromones are under environmental selection due to heat / desiccation stress.

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(-)-IRIDOMYRMECIN IS THE MAJOR COMPONENT OF THE FEMALE SEX PHEROMONE IN THE *DROSOPHILA* PARASITOID *LEPTOPILINA HETEROTOMA*

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(-)-Iridomyrmecin and (+)-isoiridomyrmecin have recently been identified as components of the defensive secretions of the parasitoid wasp *Leptopilina heterotoma* (1). While (-)-iridomyrmecin occurred only in females, males had the more stable (+)-isoiridomyrmecin. This sex-based difference suggests that (-)-iridomyrmecin might also function as a female sex pheromone in *L. heterotoma*. After extracts of females were demonstrated to attract males in a Y-tube olfactometer, we used a subtractive fractionation approach involving adsorption chromatography and preparative gas chromatography to elucidate the essential pheromone components. The attractive volatile components were found in the dichloromethane fraction of the extraction protocol. This fraction contained (-)-iridomyrmecin as the major component (85%), as well as some hitherto unidentified iridoids. Removal of (-)-iridomyrmecin from the attractive fraction resulted in a complete loss of bioactivity, which could be restored by adding synthetic (-)-iridomyrmecin, but not by its enantiomer (+)-iridomyrmecin. When offered alone, however, synthetic (-)-iridomyrmecin was not attractive for male wasps suggesting that the unidentified iridoids in the attractive fraction are also essential for bioactivity. We therefore conclude that, in addition to its defensive effect, (-)-iridomyrmecin is the major component of the multi-component female sex pheromone in *L. heterotoma*, thus constituting an excellent example of semiochemical parsimony.

References

1. Journal of Chemical Ecology. 2012. Vol. 38: 331–339.

SEMIOCHEMICAL PARSIMONY IN *LEPTOPILINA HETEROTOMA* (FIGITIDEA, HYMENOPTERA), A PARASITOID OF *DROSOPHILA*

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Wasps of the genus *Leptopilina* (Figitidae, Hymenoptera) are solitary larval parasitoids of *Drosophila* flies.

In this study we show, that female *Leptopilina heterotoma* produce (-)-iridomyrmecin in a cephalic gland and release it upon encounter with potential predators. Bioassays show that iridomyrmecin has a strong repellent effect on ants and that stereoisomers of iridomyrmecin differ in their repellent properties. Specifically, (-)-iridomyrmecin repelled the ants significantly longer from potential food items than (+)-iridomyrmecin, (+)-isoiridomyrmecin or (-)-isoiridomyrmecin. Using headspace analyses we also show, that the wasps adjust the amount of iridomyrmecin released depending on the size of the predator.

Males of *L. heterotoma* produce only the less effective (+)-isoiridomyrmecin for defense. This suggests a second possible function of iridomyrmecin in the sexual communication of *Leptopilina*. In support of this hypothesis, we have now demonstrated that (-)-iridomyrmecin, in combination with some minor iridoid compounds, is an essential component of the female sex pheromone blend that attracts males and triggers courtship behavior.

Furthermore, preliminary data suggest that (-)-iridomyrmecin is also used by *L. heterotoma* females as a spacing pheromone to detect and avoid host patches which are already exploited by conspecific females.

The threefold function of (-)-iridomyrmecin in defence, sexual communication, and host patch selection is an excellent example demonstrating the economic use of costly chemical messengers by insects, commonly referred to as 'semiochemical parsimony'. The fact that pure (-)-iridomyrmecin is sufficient for chemical defence but additional minor components are essential for the sex pheromone function indicates *L. heterotoma* may be a prime model organism for the study of cue signal evolution.

References

1. Journal of Chemical Ecology. 2012. Vol. 38: 331–339.

Symposium 3. Biosynthesis and Chemistry of Natural Products

Moderators: Anna-Karin Borg-Karlson and Irena Valterová

Keynote speaker

LACTONES USED IN CHEMICAL COMMUNICATION: AN OVERVIEW ON THEIR FORMATION AND EXAMPLES OF NEW SEMIOCHEMICALS

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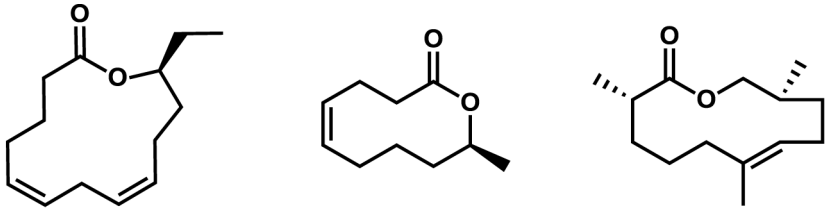
Lactones are one of the most successful structural motifs found in organic semiochemicals. They are formed by diverse organisms such as insects, bacteria, plants, amphibian and even marine organisms. The reason for their use as volatile signal might be the change from a reservoir polar precursor, soluble or transferable in the aqueous environment of the cell, into a compound of markedly increased volatility and decreased hydrophilicity. This transformation can be performed enzymatically in one step from the appropriate hydroxy acid precursor.

Volatile lactones are found in the major classes of natural products. Nevertheless, their abundance varies. While lactones derived from fatty acids are quite common, those of terpenoid or amino acid origin are rare. In the lecture the formation of lactones will be discussed form a biosynthetic viewpoint. Although only few studies on lactone biosynthesis were performed, the general pathways of natural product biosynthesis can be used to propose their formation in many cases. In the lecture the occurrence and biosynthesis will be discussed under this perspective. Furthermore, new lactones involved or likely to be involved in chemical interactions will be presented. The striking co-occurrence of defined compounds in unrelated animal lineages will highlight convergent chemical evolution. Some examples found in beetles and frogs will be presented.



IRIDOID BIOSYNTHESIS IN LEAF BEETLE LARVAE – METAL COFACTORS CONTROL CHAIN LENGTH OF AN ISOPRENYL DIPHOSPHATE SYNTHASE FROM *PHAEDON COCHLEARIAE*

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Phaedon cochleariae larvae produce the cyclopentanoid monoterpene Chrysomelidial to defend themselves against predators. This compound is stored in dorsal exocrine glands and is released as droplets when the larvae become attacked.

Isoprenyl diphosphate synthases (IDS) are the key enzymes in isoprenoid biosynthesis and catalyze the chain length determining steps. They could act as a regulatory branch point between the biosynthesis of Chrysomelidial and other important terpenoid metabolites such as juvenile hormones in insects.

Here we report about the biochemical characterization of a novel IDS from *P. cochleariae* which shows an absolute requirement for divalent cations. Both Mg^{2+} and Co^{2+} stimulate activity of the enzyme but result in different products. Mg^{2+} as cofactor enhances the formation of farnesyl diphosphate (FPP), while Co^{2+} as a cofactor forces the enzyme to produce geranyl diphosphate (GPP). When Co^{2+} was used, the enzyme utilized IPP (K_m = 0.8 μM) and DMAPP (K_m = 11.6 μM) as substrate. In contrast, with Mg^{2+} the enzyme preferred IDP (K_m = 11.79 μM) and GPP (K_m = 3.4 μM) as substrates and not DMAPP (K_m = 1 103 μM).

These observations are consistent with a concept that the metal cofactors determine not only the affinity to substrates, but also control the chain length of the product. The results offer the opportunity to regulate iridoid biosynthesis and other terpenoids independently via the local concentrations of metal cofactors and only a single enzyme.

CHARACTERIZATION OF PROTEINS INVOLVED IN THE IRIDOID BIOSYNTHESIS IN THE DEFENSIVE GLANDS OF *PHAEDON COCHLEARIAE*

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The larvae of the mustard leaf beetle, *Phaedon cochleariae*, developed a sophisticated chemical defense mechanism. They are capable of releasing the iridoid chrysomelidial (monoterpenoids) which deters predominantly invertebrate predators. Chrysomelidial is stored in nine pairs of exocrine glands on the back of the larvae. In case of disturbance, droplets of secretions are presented from the tip of the glands. The main challenge for the larvae is to develop a functional defense system without auto-intoxication. To achieve this, the nontoxic precursor 8-hydroxygeraniol-beta-D-glucoside derived from the mevalonate pathway, is transported into the defensive glands for further conversion into the bioactive active end product. In the secretions, a enzyme machinery releases the aglycon and proceeds via oxogeranial to chrysomelidial (1, 2). Especially the cyclyzation reaction is not fully understood.

Proteome analyses of the larval secretions revealed several candidate sequences – including genes which encode members of the juvenile hormone binding protein (JHBP) superfamily. By RNAi-mediated silencing of one gene out of this family we observed a shift in the composition of the larval secretions. Despite chrysomelidial, oxogeranial accumulated after the RNAi-treatment. This led to the conclusion that the protein which is responsible for oxogeranial cyclysation was down-regulated. In order to corroborate the *in-vivo*-results, currently *in-vitro* analyses with the cloned and heterologously expressed candidate protein are under investigation.

References

1. Veith M., Boland W. 1996. Biosynthesis of Iridoid Monoterpenes in Insects: Stereochemistry of an Alcohol Oxidase from Defensive Secretions of the Larvae of the Leaf Beetle *Phaedon armoraciae* (Coleoptera: Chrysomelidae). Tetrahedron. Vol. 52: 6601–6612.
2. Dissertation M. Veith.

THE RELATIONSHIP BETWEEN THE TIME COURSES OF THE B-THUJAPLICIN PRODUCTION AND OF THE ACTIVITIES OF TERPINOLENE OXYGENASES IN *CUPRESSUS LUSITANICA* CULTURED CELLS

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β-Thujaplicin (hinokitiol) is one of the extracts of Cupressaceae heartwood and is a tropolonoid. This compound has strong and broad antimicrobial spectrum and an important role in conservation of Cupressaceae woods (1). The biosynthesis pathway of β-thujaplicin has not been known yet, hence we have been trying to reveal it in *Cupressus lusitanica* (Mexican Cypress). *C. lusitanica* cultured cells derived from cambium of *C. lusitanica* tree were used for the experiments and maintained on modified Gamborg B5 solid medium for more than 20 years in our laboratory. When cells are incubated with fungal elicitor (partially purified yeast extracts), some volatile and non-volatile monoterpenes including β-thujaplicin are produced (2).

From our previous study, among ten volatile monoterpenes emitted from elicitor treated *C. lusitanica* cells, only terpinolene was converted to 5-isopropylidene-2-methylcyclohex-2-enol (IME) and 1,6-epoxy-4(8)-*p*-menthen-2-ol (EMO) and metabolized by cytochrome P450 monoxygenases prepared from *C. lusitanica* cells. EMO was a substance that was produced and reserved in cells and medium, only when the cells were exposed to elicitor. The reaction proceeded via two oxidation steps, the first reaction was from terpinolene to IME and the second reaction was from IME to EMO (3). The reactions were strictly stereo- and regio-regulated, because any isomers of IME and EMO were never formed.

These reactions were thought to be the first two steps of β-thujaplicin biosynthesis pathway. To reinforce this hypothesis, the time courses of the activities of the enzymes and the level of β-thujaplicin production were examined.

The enzymes showed the highest activities at 3 days after the treatment with elicitor and kept the same activity levels for 2 days. The behaviors of the two enzyme activities were similar. The highest amount of β-thujaplicin was observed at 5 days after the addition of elicitor, and the CYP enzyme activities increased prior to β-thujaplicin production. From these results, the two enzymes are thought to be related to β-thujaplicin biosynthesis.

As mentioned above, the CYP activities did not changed during the day 3 to 5, however, the rate of β-thujaplicin production from the 3rd to 5th day after elicitation was quite high. Considering obvious accumulation of EMO after elicitation, EMO may also have an antimicrobial activity, similar to β-thujaplicin.

Acknowledgements

The research was supported by Research Fellow of Japan Society for the Promotion of Science (23001520).

References

1. Current Medicinal Chemistry. 2007. Vol. 14: 2597–2621.
2. Journal of Plant Physiology. 2009. Vol. 166: 720–728.
3. American Journal of Plant Sciences. 2012. Vol. 3: 268–275.

A SINGLE POINT MUTATION IN THE TRANSMEMBRANE DOMAIN CHANGES SPECIFICITIES OF ACYL-COA DESATURASES AND DRIVES INSECT SEX PHEROMONE DIVERSITY

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Manduca sexta, a model moth organism, uses an unusually complex pheromone blend, which is composed of twelve 16-carbon aldehydes with various degrees of unsaturation and double bond geometry (1). The biosynthesis of hexadecatrienals, which comprise a substantial part of the pheromone blend, is poorly understood, and numerous fatty acyl-CoA desaturase (2) orthologs are expected to coordinately produce the *M. sexta* pheromone blend. Understanding of the specificity determinants of membrane desaturases is very limited because their three-dimensional structures are not known.

The membrane acyl-CoA desaturases play a prominent role in the biosynthesis of unsaturated fatty acid-derived sex pheromones that moths utilize for sexual communication and species reproductive isolation. The results presented here demonstrate that a single amino acid substitution in the transmembrane domain of acyl CoA desaturases is sufficient to dramatically shift the sex pheromone precursor composition and potentially may result in mating isolation and speciation.

Acknowledgements

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References

1. Arch. Insect Biochem. Phys. 1989. Vol. 10: 255–271.
2. Insect Biochem. Mol. Biol. 2007. Vol. 37: 601–10.

THE USE OF A STABLE ISOTOPE TRACER METHOD TO DETERMINE SYNTHETIC RATES OF SEMIOCHEMICALS IN INSECTS

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Over the last 50 years, there has been considerable research characterizing semiochemical production in insects. Most of this work has focused on identifying semiochemicals and characterizing the behavioural responses of insects to these chemicals. The identification of these semiochemicals has also facilitated study on their biosynthesis, particularly in regard to how their production varies in relation to various endogenous and exogenous signals. In spite of the effort on describing semiochemical production, there have been few actual direct *in vivo* kinetic studies to quantify actual rates of production of these chemicals. Part of the problem is that, in insects, these chemicals are often produced in relatively small amounts, and introducing natural levels of labeled precursors into the insect for kinetic study is difficult. Our recent demonstration that ingested sugar is rapidly converted to sex pheromone in the moth, *Heliothis virescens*, presented us with a tool to introduce labelled precursor in natural amounts into an insect for studying semiochemical (sex pheromone) production. We discuss use of a stable isotope tracer-tracee method, mass isotope distribution analysis (MIDA), commonly used in biomedical studies, for determining synthetic rates of sex pheromone and pheromone fatty acid precursors in *H. virescens*. As well as its application for determining synthetic rates of sex pheromone in moths, MIDA could have wider general applicability for determining synthetic rates of other polymeric semiochemicals in other insect taxa.

GC-FT-IR ANALYSES OF SEX PHEROMONES SECRETED BY NETTLE MOTHS

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While an IR spectrometer is useful to determine functional groups of an unknown compound, it has been rarely used for pheromone researches because of low sensitivity. Recently, a new type of GC-FT-IR (Discover IR, Spectra Analysis) has been developed by applying a system with a cooled and turned disk, where compounds eluted from a capillary GC column are fixed and irradiated with an IR beam. The FT-IR measurement is carried out sensitively as well as GC-MS.

Several nettle moth species in the family of Limacodidae secrete sex pheromone components with a terminal conjugated dienyl structure. Separation of two geometrical isomers of the terminal dienes is insufficient even on a high polar GC column and it is not easy to determine configuration of the double bond by the chromatographic behaviour (1). However, GC-FT-IR measurement of a crude pheromone extract of *Parasa lepida lepida* could confirm *Z*-configuration of 7,9-decadien-1-ol, which showed no absorption at 960 cm^{-1}. On the other hand, the dienyl component of another nettle moth species, *Monema flavescens,* showed a band at 960 cm^{-1} indicating *E*-configuration.

References

1. Biosci. Biotechnol. Biochem. 2009. Vol. 73: 1156–1162.

NATURE'S CON ARTISTS EXPOSED – THE CHEMISTRY OF *D. GLYPTODON* REVEALED

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Orchids are well known for their extraordinary species diversity and for their specialised pollination systems. *Drakaea* orchids, endemic to Western Australia, are pollinated by the sexual deceit of male thynnine wasps through mimicry of the insect pheromones. An investigation into chemical communication between these orchids and their pollinators has found that pyrazine based compounds are used as semiochemicals by *Drakaea* orchids. Following the successful identification, synthesis and field testing of four compounds in *D. glyptodon* the study has been expanded to include five additional species. We have strong indications that all species are utilising related compounds, including novel oxygenated pyrazines.

This talk will outline the methods utilised in the isolation, identification and synthesis of the identified semiochemicals, including solid phase microextraction (SPME), GC/electroantennographic detection (GC/EAD), GC/MS and GC/HRMS. Finally, the implications for our understanding of the role of chemical changes in orchid speciation will be briefly explored.

IDENTIFICATION AND SYNTHESIS OF THE MALE-PRODUCED SEX PHEROMONE OF THE STINK BUG, *PALLANTIA MACUNAIMA* (HETEROPTERA: PENTATOMIDAE)

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Pallantia macunaima is a soybean pest found in southern Brazil, and pheromones are potentially useful for monitoring stink bugs. Aiming the identification of the sex pheromone in the species, the volatiles released by males and females adults were collected by aeration techniques, and the GC analysis of these extracts revealed the presence of a male-produced compound. Bioassays performed with Y-tube olfactometer showed that the males' extracts are very attractive for females. GC-EAD experiments indicated that the antennae of females are highly sensitive to this male-produced compound, supporting the behavioral data. GC-MS and GC-FTIR analysis of the natural compound, and the respective alcohol, trimethylsilyl ether and hydrocarbon derivatives, suggested a methyl branched ketone for this male-specific compound. After the synthesis of three different proposed structures, the natural product was identified as 6,10,13-trimethyltetradecan-2-one. This synthetic ketone co-eluted with the natural product on three different GC stationary phases. Y-tube olfactometer showed that females are attracted to the synthetic ketone, making it possible to confirm the biological activity of this compound. We are working now in a stereoselective synthesis of all the four possible sterereoisomers, in order to verify the influence of chirality on insect behaviour, as well as the determination of the absolute configuration of the natural compound.

Acknowledgements

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IDENTIFICATION OF A NEW TERMITE TRAIL-FOLLOWING PHEROMONE, NONADECADIENONE, IN *GLOSSOTERMES OCULATUS* (SERRITERMITIDAE)

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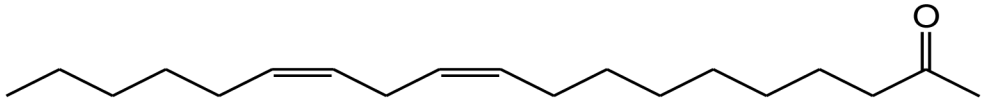
Societies of termites, living in permanent darkness, communicate mainly by chemical signals. Among them, the trail-following pheromones are ubiquitous and their chemistry and biology have been studied in more than 60 species from all families except for the Neotropical family Serritermitidae. Surprisingly, the chemical variability of the trail-following pheromones is quite low, with only 8 compounds described up to now.

Using a combination of two-dimensional gas chromatography, preparative GC, electrophysiology and behavioural bioassays, we identified (10*Z*,13*Z*)-nonadeca-10,13-dien-2-one as the trail-following pheromone produced by the sternal glands of pseudergates in the serritermitid *Glossotermes oculatus* (1).



The structure was proposed based on EI mass spectrum and fragmentation pattern similar to linoleic acid and its derivatives. This made us hypothesize that the pheromone is likely biosynthesized as an acetyl derivative of naturally occurring linoleic acid. Indeed, the corresponding position of the two double bonds was confirmed using DMDS derivatization. The compound was synthesized and its biological activity verified in bioassays. The activity threshold was estimated to be 10^{-2} ng/cm and the quantity of pheromone in the sternal gland of one pseudergate was determined using three independent quantification approaches to range from hundreds of picograms to units of nanograms.

We succeeded in identifying a new trail-following pheromone with unexpected chemical structure contrasting with the universal presence of C12 unsaturated alcohols in other advanced families. Our results underline the outstanding position of the family Serritermitidae among advanced termites.

Acknowledgements

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References

1. Chemical Senses. 2012. Vol. 37: 55–63.

TENDON GLAND – A NEW MEMBER OF EXOCRINE ORGAN SET IN BUMBLEBEE: STRUCTURE, SECRETION CHEMISTRY AND POSSIBLE FUNCTIONS

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Tendon glands are newly discovered exocrine glands in the bumblebee species *Bombus terrestris*. These glands were previously recorded in stingless bee *Melipona seminigra*, and the effect of their secretion on food source marking was proven (1). The secretory cells occur in three independent regions in femur, tibia and basitarsus, separated from each other by unmodified epidermal cells. They are attached to the hollow pretarsal tendons, into which the secretion is produced, and released at the unguitractor plate base through a narrow opening. The tendon glands are well-developed in all castes, similarly to the tarsal glands (2), which are completely independent from the tendon glands. The most abundant organelles of the tendon gland cells comprise smooth endoplasmic reticulum and numerous mitochondria; the differences among particular bumblebee age categories are only insignificant.

The tendon gland secretion is made of blend of several hydrocarbons from C21 to C30 and wax esters. While the secretion is nearly identical in females (both, workers and queens), males' secretion differs in having considerably larger proportions of wax esters with aliphatic or terpenic alcohol parts. Another difference between males and females consist in the distribution of double bond positions in the unsaturated hydrocarbons: while the double bonds are only in position 9 in males, they are also in other positions in females. On the other hand, the secretion of tarsal glands is very similar to body washes, and both are different from the tendon gland secretion. These differences indicate a special function of the male-specific compounds, which is most likely linked to bumblebee mating behaviour.

Acknowledgements

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References

1. Journal of Chemical Ecology. 2004. Vol. 30: 793–804.
2. Chemoecology. 1991. Vol. 2: 35–40.

IDENTIFICATION OF A NEW AGGREGATION PHEROMONE COMPONENT OF THE MOROCCAN LOCUST *DOCIOSTAURUS MAROCCANUS*

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Gregarious locusts are mass migrating insects that produce great economic losses in agriculture representing a serious threat to the livelihood of a tenth of the world population. Control of these pests against outbreaks and plagues have been relied solely upon application of sprays of conventional pesticides. However, these treatments have been highly controversial for environmental, human safety and economic reasons and therefore, new alternative, environmentally-friendly strategies for locust control are urgently needed.

The Moroccan locust, *Dociostaurus maroccanus* (Orthoptera: Acrididae), is a polyphagous pest of crops and pastures, particularly in Southern Europe, North Africa and the Middle East, but devastating outbreaks have also been noticed in Iran, Afghanistan and adjacent countries of the former USSR. As other locust species, if environmental and climatic conditions are favorable, the Moroccan locust can shift from a solitary to a gregarious phase, increasing dramatically in number and leading to the formation of large, landscape-devouring swarms. We have found that phytal, a novel natural product, promotes aggregation in the Moroccan locust. Both isomers of phytal are produced by sexually mature adult males, elicit electroantennographic responses and induce aggregation behaviour in adults of both sexes in laboratory bioassays. Phytal is mainly produced in male legs and is biosynthesized by an oxidative process in the gut after insect feeding on phytol-containing food. Phytol is a diterpene alcohol present in the chlorophyll structure of all plants. Our results point out that phytal may play an important role in several biological functions of the Moroccan locust.

MULTIMODAL SIGNALS IN GALLERIINAE – A COMPARATIVE STUDY

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The subfamily Galleriinae is noted for a number of nocturnal species that coevolved with Hymenoptera, namely Apoidea (bees and relatives). Especially notable among Galleriinae are the waxworms (*Achroia grisella* and *Galleria mellonella* larvae) which are both significant as beekeepers' pests and as commercial items, as well as *Aphomia sociella*, a parasite of bumblebee nests.

The Galleriinae moths exhibit aberrant mating system compared to great majority of Lepidoptera, where females attract males by sex pheromone. Mating in Galleriinae is regulated chemically and acoustically. Based on the hierarchy of the respective signal modality, two distinct groups have been recognized. Males of one group (*Achroia, Corcyra*) use ultrasonic signals to attract females, while chemical communication is supposed to mediate close-range interactions. Males of the second group (*Galleria, Aphomia*) initiate mating chemically and ultrasonic communication mediates close range interactions.

To understand the adaptive forces shaping this aberrant pair forming systems, we performed a comparative study of two species that coevolved with Hymenoptera. Specifically, we studied complexities of premating behavior in *Aphomia sociella* and *Galleria mellonella*. Males of both species attract females using sex pheromone released from male-specific wing glands and dispersed during specific behavior characterized by stationary-wing-fanning (calling behavior). In both species, the pheromone released is a multicomponent blend consisting of chemically diverse compounds. In both species, females guided by the male pheromone fly to calling males. Female proximity terminates male calling and triggers male courtship behavior in both species. In both species, male courtship behavior is associated with walking-wing-fanning and ultrasonic production. In spite of these remarkable similarities, multimodal signals that govern communication between sexes differ in both species: i) male pheromones are quite different (they share only one minor compound), ii) *A. sociella*, but not *G. mellonella* females advertise their presence by female-specific courtship pheromone, iii) ultrasound courtship songs affect mating efficiency in *A. sociella*, but not in *G. mellonella*.

Ecological significance of these remarkable differences in the closely related species will be discussed.

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FATE OF CHLOROPHYLL IN THE GUT OF LEPIDOPTERAN LARVAE

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Chlorophylls (Chls), the green pigments responsible for photosynthesis in plants, algae and bacteria, are also part of the daily diet of herbivorous feeders (1). Biochemistry and regulation of Chl biosynthesis and degradation are intensively studied in plants. Chl degradation occurs during leaf senescence and fruit ripening, but is also noticed as a response to biotic and abiotic stresses. For many years, Chl degradation was considered a biological enigma. Only the identification and structure determination of several key Chl catabolites as natural breakdown products allowed the stepwise elucidation of a programmed Chl degradation pathway which is common to higher plants and is known as senescence (2). Chls are very stable in their natural environment inside the chloroplast, but whenever this organ becomes damaged the Chls become susceptible to chemical transformations leading to degradation. First reactions take place in the periphery of the tetrapyrrol macrocycle, later transformations lead to oxidative ring opening resulting in linear tetrapyrroles (3). Different factors such as high temperature, extreme pH values, enzymatic actions, molecular oxygen and light contribute or initiate the degradation of Chl. To gain more information on Chl degradation in the gut of plant-feeding insects, regurgitate and frass of five Lepidopteran caterpillars were analysed for early Chl catabolites. The major metabolites were determined and quantified by using LC-MS, UV, Fluorescence and LC-NMR spectroscopy. Except of pheophytin *a*, none of the degradation compounds were detected in fresh leaves of the food plants (*Phaseolus lunatus*, *Nicotiana attenuata*). The observed spectrum of metabolites can be attributed to the combined action of esterolytic gut enzymes and the strongly alkaline milieu in the digestive tract. Interestingly, intact Chl was not detected in the gut of the larvae of *Spodoptera littoralis*. Substantial amounts of Chls were found to be strongly complexed in the mid-gut, but not in the frass and not in heat-inactivated (microwave) digestive fluids suggesting non-covalent binding of Chl. We studied a lipocalin-type protein from the gut juice of the Egyptian cotton leafworm *S. littoralis* previously identified as the chlorophyllide-binding protein from the silkworm *Bombyx mori* by LC-MS/MS-sequencing. Binding assays demonstrated that Chl-binding was pH dependent.

Acknowledgements

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References

1. Phytochemistry. 1999. Vol. 50: 195–202.
2. Biochimica Et Biophysica Acta-Bioenergetics. 2011. Vol. 1807: 977–988.
3. Journal of Chromatography A. 1995. Vol. 690: 161–176.

EFFECT OF EXTRACTS AND COMPOUNDS IN CONIFERS ON PLANT SEEDS

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Sugi (*Cryptomeria japonica* D. Don) and Hinoki (*Chamaecyparis obtusa* Endl.) are classified as evergreen conifer and endemic to Japan. They are suitable for lumber in order to build a house and a construction, and are planted as main species in Japan. It is well known that heartwood of these trees contain norlignans and terpenoids. The norlignan is C_{17} phenolic compound. The structure of the compound is characterized by a loss of one carbon from a lignan (C_{18} phenolic compound). The heartwood has high durability owing to the various extractives. It was considered that some components of heartwood controlled growth of other plants. However, there are few reports in relation to effect of the compounds.

In this study, the extracts and the compounds contained in *C. japonica* and *Ch. obtusa* heartwoods were examined for effect on seed germination and growth (radicle and hypocotyl) against herbaceous plants and conifers, with the aim of investigation of its allelopathic potentialities. We used the following seeds of herbaceous plants (*Lactuca sativa*, *Trifolium repense*, *Lolium multiflorum*) and conifers (*C. japonica*, *Ch. obtusa*, *Pinus densiflora*, *P. thunbergii*, *Larix kaempferi*). The heartwood of *C. japonica* and *Ch. obtusa* were extracted successively with *n*-hexane, EtOAc and MeOH solvent. Norlignan samples (hinokiresinol, sugiresinol, hydroxysugiresinol, agatharesinol and sequirin-C) were isolated from *C. japonica* or *Ch. obtusa* heartwood in previous work (1–3), were used in this research.

Ethyl acetate and methanol extracts from *C. japonica* produced inhibitory effect on conifer germination (*C. japonica*, *P. densiflora* and *L. kaempferi*). The extracts affected negatively all herbaceous plants growth. All extracts from *Ch. obtusa* exhibited the strong inhibitory effects of all herbaceous plants germination as well as all plants growth. Of the norlignans, hydroxysugiresinol, agatharesinol and sequirin-C had higher inhibitory effects of all herbaceous plants growth than other norlignans. Our findings indicate that phytotoxic activity of norlignan depends upon the numbers of a hydroxyl group. In addition, norlignans had a promoting activity of hypocotyl growth of *L. kaempferi*.

We will discuss the effects of norlignans, terpenoids and other compounds contained of *C. japonica* and *Ch. obtusa* heartwood on seed germination and plant growth.

References

1. Mokuzai Gakkaishi. 1981. Vol. 27: 654–657.
2. Mokuzai Gakkaishi. 1985. Vol. 31: 28–38.
3. Abstracts of the 62nd Annual Meeting of the Japan Wood Research Society. 2012. M15-P-PM15

PHENOLIC GLYCOSIDE DIVERSITY IN THE SALICACEAE

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Phenolic glycosides (PGs) represent the signature secondary compounds of the Salicaceae (willows and poplars). This chemical class has well-documented anti-herbivore properties that appear to increase with greater molecular complexity (1). Using liquid chromatography-mass spectroscopy (LC-MS) methods, we examined the PG diversity of several species in the Salicaceae at different scales. At the broadest scale, we collected and analyzed the PGs from 24 species of *Salix* from the Västerbotten region of northern Sweden. Many of these species had no previous research regarding their PG chemistry and we found unreported compounds in most of the species with prior analyses. At the finest scale, we assessed the PG diversity of a single species, *Populus tremula*, using the Swedish Aspen (SwAsp) collection, which consists of over 100 genotypes from throughout Sweden (2). Building upon initial work (3), we discovered greater PG diversity in *P. tremula*, including more complex structures and new isomers of existing compounds in substantial amounts. We identified three PG chemotypes in the population with individuals high in either cinnamoylsalicortin, acetylsalicortin, or with only the usual background of salicortin and tremulacin. The types of compounds found and their patterns in both *Salix* and in *P. tremula* help to shed light on the biosynthesis of PGs, which is currently poorly understood.

Acknowledgements

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References

1. Canadian Journal of Botany. 2007. Vol. 85: 1111–1126.
2. Tree Genetics & Genomes. 2008. Vol. 4: 279–292.
3. Journal of Chemical Ecology. 2011. Vol. 37: 857–870.

Symposium 4. Chemical Ecology of Microorganisms

Moderator: Stefan Schulz

Keynote speaker

CHEMICAL ECOLOGY OF ACTINOMYCETES ASSOCIATED WITH LEAF CUTTING ANTS

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Leaf-cutting ants cultivate the fungus *Leucoagaricus gongylophorus*, which serves them as major food source. This mutualistic relationship is threatened by pathogens such as the fungus *Escovopsis weberi* which can overgrow *L. gongylophorus* (1). Therefore leaf cutting ants take great care of their fungus garden removing any suspicious material into waste chambers and using chemical treatment. In addition symbiotic microorganisms support leaf cutting ants to defend their fungus garden against infections (2). Recently we identified candicidin macrolides as highly active antifungals from symbiotic *Streptomyces* of the leaf cutting ants. Furthermore it became evident that the fungus garden of leaf cutting ants houses complex microbial communities (3, 4).

By combining phylogenetic data and database searches with liquid chromatography mass spectrometry (LC-MS) we quickly identified additional secondary metabolites (antimycins A_1–A_4, valinomycins, and actinomycins) from *Streptomyces* symbionts of leaf-cutting ants.

Using mass spectrometry some antibiotics were directly identified from leaf-cutting ant samples, suggesting that the compounds exert their antimicrobial and antifungal potential in the ants' nests. Strong synergistic effects of the secondary metabolites produced by ant-associated *Streptomyces* were observed. Leaf cutting ant associated microorganisms use their antimicrobial compounds apart from the protection of the ants' nests to compete out other (symbiotic) microorganisms (4).

References

1. Proc. Natl. Acad. Sci. U.S.A. 1999. Vol. 96: 7998–8002.
2. Nature. 1999. Vol. 398: 701–704.
3. Proc. Natl. Acad. Sci. U.S.A. 2009. Vol. 106: 4742–4746.
4. Proc. Natl. Acad. Sci. U.S.A. 2011. Vol. 108: 1955–1960.

THE SMELL OF BACTERIA

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Volatiles are only a small proportion of the total number of metabolites produced by living organisms. However, because of their unique properties they are predestined to act as infochemicals in intra- and interspecies communications in the atmosphere as well as in the soil.

Bacteria like other organisms emanate a wealth of volatile compounds (1). Approximately 800 compounds are presently known, and it is likely that this is just the "tip of the iceberg", considering that up to now i) volatile profiles of a large number of bacteria have yet not been obtained, ii) a huge number of microbes have yet not been identified, and iii) volatile profiles are altered when bacteria grow on different media or in different habitats. Besides the interest in the complexity of bacterial volatile profiles, individual volatile organic compounds (VOCs) are also worth to study, since compounds with structures that are new to science can be obtained. One example is 'sodorifen', which was recently isolated from *Serratia odorifera* 4R × 13 (2). Since the underlying biosynthetic pathway of this compound remains so far a mystery, several approaches are undertaken to elucidate the biosynthesis of 'sodorifen'. Furthermore, to understand the biological and ecological roles the effects of bacterial volatiles are investigated in different organisms, e. g. plants (3, 4).

References

1. Effmert U., Kalderas J., Warnke R., Piechulla B. Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol, accepted.

2. von Reuss S., Kai M., Piechulla B., Francke W. Octamethylbicyclo(3.2.1)octadienes from *Serratia odorifera*.

Angewandte Chemie 122: 2053–2054, 2010.

Angewandte Chemie Int. Ed. 49: 2009–2010, 2010.

3. Kai M., Piechulla B. 2010. *Serratia odorifera*: Analysis of volatile emission and biological impact of volatile compounds on *Arabidopisis thaliana.* Applied Microbiology and Biotechnology. Vol. 88: 965–976.

4. Wenke K., Wanke D, Kilian J., Berendzen K., Harter K., Piechulla B. 2011. Volatile-associated molecular pattens of two growth-inhibiting rhizobacteria commonly enrol AtWRKY18 function. The Plant Journal. Vol. 70: 445–459.

A SMELLY WORLD: HOW BACTERIAL VOLATILES INFLUENCE THE GROWTH OF OTHER ORGANISMS

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Increasing evidence indicates that bacteria can interact with other organisms through the emission of volatile compounds. We have recently demonstrated that plant growth promotion by bacterial volatiles is a general feature of root-associated bacteria, and especially of *Burkholderia* species. In addition to direct plant growth promotion, bacterial volatiles have been shown to inhibit the growth of phytopathogenic fungi of agronomical relevance such as *B. cinerea*, *R. solani* or *A. alternata*. Moreover, we have recently extended our investigations to volatile-mediated bacteria-bacteria interactions and observed induction of antibiotic tolerance in *Escherichia coli* when exposed to the volatiles of various *Burkholderia* strains. We are currently analysing which active molecules are responsible for these strong effects of bacterial volatiles on plants, fungi and bacteria.

VOLATILE EMISSION WITHIN THE BACTERIAL GENUS *SERRATIA*

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Bacteria developed unique strategies to adapt to different environments, for example by the production of secondary compounds (e. g., antibiotics). It was only recently recognized that bacteria also produce opulent volatile compounds. Such volatiles can function as inter- and intra-organismic infochemicals, or as attraction or defense signals. The volatile profiles appear to be different between bacterial groups (summarized in 1). Moreover, single volatiles may function as characteristic markers. The rhizobacterium *Serratia plymuthica* 4R × 13 (formerly *S. odorifera* 4R × 13) emits a large and diverse spectrum of volatiles including sulfur containing compounds, nitrogen containing compounds, alcohols and the unique compound octamethylbicyclo(3.2.1)octadiene ('sodorifen') (2, 3). 'Sodorifen' ($C_{16}H_{26}$) is composed of a new and unusual type of carbon skeleton. Each carbon atom of the bicyclic structure is methylated or methylenated. Beside unraveling the underlying biosynthetic pathway, additional information about the ecological function/s of 'sodorifen' can be deduced from its emission from different species or isolates of the genus *Serratia.* A correlation to the habitat of the species might provide further insights. Here we present volatile profiles of various *Serratia* sp. isolated from different habitats. A 16S rDNA based phylogenetic tree demonstrates the refined relationship of these species and isolates within the genus *Serratia*, which further will be correlated with the presences / absence of 'sodorifen' emission.

References

1. Effmert U., Kalderas J., Warnke R., Piechulla B. 2012. Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol, in revision.

2. von Reuß S., Kai M., Piechulla B., Francke W. 2010. Octamethylbicyclo(3.2.1)octadiene from *Serratia odorifera*. Angewandte Chemie. Vol. 49: 2009–2010.

3. Kai M., Crespo E., Cristescu S. M., Harren F. J. M., Piechulla B. 2010. *Serratia odorifera*: Analysis of volatile emission and biological impact of volatile compounds on *Arabidopsis thaliana*. Appl Microbiol Biotechnol. Vol. 88: 965–976.

INTERKINGDOM SIGNALLING – BACTERIAL QUORUM SENSING SIGNALS ARE TRANSPORTED INTO PLANTS AND INDUCE SPECIFIC RESPONSES

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The plant rhizosphere is colonized by numerous microorganisms and the bacterial densities in the rhizosphere can exceed 100 times those of the bulk soil. Over the last two decades it has become apparent that bacteria are able to communicate with each other via a process named Quorum sensing (QS) and thereby orchestrate bacterial gene expression, e. g. antibiotic production, biofilm formation, fruit body development, virulence, motility, and bioluminescence. QS is also involved in mediating the interaction between different bacterial species and between bacteria and eukaryotic organisms. Considering that QS is such an important process for bacteria, eukaryotic hosts might have evolved mechanisms to eavesdrop on this bacterial communication and to take advantage of the bacterial dependency on QS.

N-acyl-homoserine lactones (AHLs) are the major signalling molecules in QS of Gram-negative bacteria and the best studied QS signalling molecules so far. Bacterial species living in the rhizosphere are producing AHLs more frequently than species living in the bulk soil and pathogenic as well as mutualistic plant-bacteria interactions are dependent on AHLs.

It has been shown that AHLs are transported systemically into plants (1, 2) and induce specific plant responses including systemic resistence (3, 4). However the mechanism of transport and translocation into plants and their fate remains almost unknown. Using tritium-labelled AHLs as well as AHL-sensor strains and monoclonal antibodies we were able to show that the uptake and transport is an active process of *H. vulgare* plants. Furthermore we could show that AHLs induce a biphasic response in barley involving "classical" components of the plant immune response.

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References

1. Götz et al. 2007. Analytical and Bioanalytical Chemistry. Vol. 389: 1447–1457.
2. von Rad et al. 2008. Planta. Vol. 229 : 73–85.
3. Schuhegger et al. 2006. Plant, Cell and Environment. Vol. 29: 909–918.
4. Schikora et al. 2011. Plant Physiology. Vol. 157 : 1407–1418.

HUMAN SKIN MICROBIOTA AFFECTS ATTRACTIVENESS TO MALARIA MOSQUITOES

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Chemical cues are generally considered to be of overriding importance for mosquitoes seeking their hosts, from which they need to take a blood meal to be able to reproduce. The nocturnally feeding malaria mosquito *Anopheles gambiae sensu stricto* is highly anthropophilic, and mainly uses human body odours to locate its host. Humans differ in attractiveness to mosquitoes based on the volatiles they emit. Skin microbial communities play key roles in the production of body odour, and the microbial and chemical signatures of individual humans are qualitatively and quantitatively correlated. We demonstrated that volatiles released by skin bacteria attract malaria mosquitoes in a laboratory and field setup (1, 2). An experiment with 48 volunteers showed that the composition of the skin microbiota affects the attractiveness of an individual to mosquitoes. Bacterial plate counts and 16S rRNA sequencing revealed that individuals with a high abundance of bacteria on their skin were significantly more attractive to the malaria mosquito *Anopheles gambiae sensu stricto*, whereas individuals with more diverse skin microbial communities were less attractive to this mosquito (3). Bacterial genera that are associated with the relative degree of attractiveness to mosquitoes and the volatiles they emit were identified and tested in laboratory and field setups (3, 4). Future studies should indicate if volatiles released by skin microbiota also determine host specificity of mosquitoes. The discovery of the connection between skin microbial populations and attractiveness to mosquitoes may lead to the development of new mosquito attractants and personalized methods for protection against disease vectors.

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References

1. PLoS ONE. 2010. Vol. 5(12): e15829.
2. Entomologia Experimentalis et Applicata. 2011. Vol. 139(2): 170–179.
3. PLoS One. 2011. Vol. 6(12): e28991.
4. Malaria Journal. 2011. Vol. 10(1): 28.

MANIPULATION OF MICROBIAL COMMUNITY IN FLY DEVELOPMENT MEDIA TO DEVELOP NOVEL CONTROL STRATEGIES

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Filth flies are the most important pests in agricultural environment, as well as in urban settings. The infestation of filth flies can further cause food contamination and disease transmitting. The economically losses caused by the infestation of filth flies have reached over billions of dollars losses annually. The present presentation reports new progresses on the development of filth fly control via manipulating microbial community in filth fly larval and oviposition media, which includes the discovery of novel botanical-based antibacterial agents, identification of bacterial community that is important for their larval growth, and their further development natural product larvicides and oviposition deterrents for controlling the two major fly pests, stable fly, horn fly and house fly.

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Keynote speaker

CHEMICAL SURPRISES FROM AN UNCULTIVATED SPONGE SYMBIONT

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Marine sponges are a rich source of bioactive natural products, many of which play an important role in chemical defense. An impressive example is the sponge *Theonella swinhoei*, which has yielded more than 120 compounds belonging to diverse structural types. Many sponges also harbor highly complex consortia of symbiotic bacteria that are suspected to be the true source of at least some of the secondary metabolites. In previous work, our group demonstrated a bacterial origin of polyketides for two different sponges (1–3), but the taxonomic identity of sponge-associated producers remained unknown. Using a strategy consisting of single-cell analysis and metagenomic sequencing, we identified the bacterial producer of onnamide-type polyketides in *T. swinhoei*. Surprisingly, the data suggest the symbiont to be a chemically exceptionally prolific bacterium, producing not only onnamides but most other compounds reported from this sponge chemotype. Furthermore, functional characterization of one biosynthetic pathway revealed the existence of a new natural product family, termed proteusins, with several unprecedented biosynthetic transformations. These results reveal a key role of symbiotic bacteria in the chemistry of their sponge hosts and provide new strategies to study uncultivated symbionts in a more systematic fashion.

References

1. Proc. Natl. Acad. Sci. U.S.A. 2004. Vol. 101: 16222.
2. Nat. Biotechnol. 2008. Vol. 26: 225.
3. Nat. Chem. Biol. 2009. Vol. 5: 494.

BELOW GROUND CHEMICAL ECOLOGY; NEW TECHNIQUES FOR SAMPLING AND MASS SPECTROMETRIC ANALYSES OF ROOT VOLATILES

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The loss of methyl bromide as a soil fumigant has led to a need for new technologies to manage nematodes. One approach is to utilize semiochemicalls, but techniques for studying plant-insect-nematode-interactions are not well developed. In contrast to leaf volatiles release into the air, root volatiles are released into airspace where the main dispersing is by diffusion. To minimize the effects of transferring plants to an artificial environment, we designed probes for sampling of volatiles in vivo that in combination with thermal desorption GC/MS analyses allow very short sampling times and small volumes. The low impact sampling making it possible to continuously monitor release and interactions governed by volatile semiochemicalls, as well as to distinguish volatiles released in response to damage from continuously released volatiles. In addition, a thermal desorption injection system was redesigned to minimize artefacts caused by thermal degradation and that can be integrated into the injector of existing GC/MS systems without interfering with normal operations. These developments have allowed us to effectively study chemical signalling affecting the behaviour of soil dwelling nematodes.

ACTIVITY SUPPRESSION IN SECOND-STAGE JUVENILES OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA*

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The potato cyst nematode *Globodera pallida* (Stone, 1973) Berhens, 1975 is one of the major pests causing potato harvest losses worldwide. In favourable environmental conditions, second–stage juveniles (J2) of potato cyst nematodes migrate towards host plant roots (1). Paired amphids are the most important nematode chemoreceptors located in the cephalic region of nematodes. These are responsible for orienting nematodes in the soil environment (2). To date, only two host–plant released attractants are known for potato cyst nematode J2s: linalool (3) and α-solanine (4). Suppression of nematode response to an attractive chemical compound may lead to the ability of disturbing normal nematode behaviour. Hence, it could be applied for the biological control of potato cyst nematodes.

Behavioural tests were carried out to reveal the effect of zinc sulphate on the behaviour of the potato cyst nematode J2s. A watch glass was filled with 50 µL of $ZnSO_4 \cdot 7H_2O$ aqueous solution of 3 mM concentration. Twenty *G. pallida* J2s were immersed in the solution and stored for 2; 5; 15 or 30 minutes. Either $MgSO_4 \cdot 7H_2O$ aqueous solution of 3 mM concentration or distilled water was used as controls. Then nematodes were transferred to Petri dishes with the attractant, i. e. α-solanine of 1×10^{-4} M concentration and behavioural test had been carried out. The result was recorded after 15 minutes. Exposure of *G. pallida* J2s to 3 mM zinc sulphate aqueous solution suppressed nematode response to the attractive chemical compound (α-solanine). This effect of zinc sulphate solution endured for the entire exposition interval – from two up to 30 minutes and statistically significantly differed from that of distilled water ($P < 0.05$). The two minute exposure to magnesium sulphate aqueous solution of 3 mM concentration suppressed *G. pallida* response to α-solanine as well. This nematode response was significantly different ($P < 0.05$) from that to distilled water, however for a short time only (two minutes).

For the first time, it was determined that the aqueous solution of zinc sulphate suppresses *G. pallida* response to the attractive chemical compound – α-solanine. Compared to the aqueous solution of magnesium sulphate, the aqueous solution of zinc sulphate effectively disrupts normal nematode behaviour to α-solanine.

References

1. Weischer B. 1959. Experimentelle Untersuchungen uber die Wandenburg von Nematoden. Nematologica. Vol. 4: 172–186.

2. Jones J. T., Perry R. N., Johnston R. L. 1994. Changes in the ultrastructure of the amphids of the potato cyst nematode, *Globodera rostochiensis*, during development and infection. Fundamental and Applied Nematology. Vol. 17(4): 369–382.

3. Būda V., Čepulytė-Rakauskienė R. 2011. The effect of linalool on second–stage juveniles of potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida*. Journal of Nematology (USA) – in press.

4. Čepulytė-Rakauskienė R., Būda V. 2011. α-Solanine effect on potato cyst nematode *Globodera rostochiensis* and *Globodera pallida* second–stage juveniles. The 27th ISCE Annual Meeting. Meeting Overview, 24–28 July, British Columbia, Canada: 94.

Symposium 5. Chemical Ecology of Vertebrates

Moderator: Raimondas Mozūraitis

CAN THE NEONATAL RECOGNITION CUE OF SHEEP, *OVIS ARIES*, BE A PHEROMONE?

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The strong bond formed between ewe and lamb shortly after parturition is an important factor in lamb survival. Evidence exists that the ewe can distinguish her lamb by its unique smell. We have identified 133 volatile organic compounds in the cranial wool of Döhne Merino lambs (1) that are presumably constituents of the neonatal recognition cue of this sheep race. Quantitative analysis of the odour profiles of the twins of 16 ewes of a flock of 165 twin-bearing ewes revealed that the wool volatiles of twins are qualitatively as well as quantitatively practically identical, but differ from those of other twins or non-twin lambs in the flock. A *P*-value <0.0001 was calculated, indicating that the pairing of twins according to the qualitative and quantitative composition of the wool is statistically highly significant. However, in bioassays ewes rejected alien lambs dressed in jackets that were sprayed with mixtures formulated with synthetic analogues of the identified wool volatiles according to the qualitative and quantitative compositions of the experimental ewes' own lambs (1). More encouraging results were obtained with a newly developed bioassay. We have found that the severing of the bond between ewe and lamb that had been separated for longer than a certain critical period can be ascribed to the constantly changing composition of the odour of new-born lambs.

About 50% of the compounds identified in the wool of lambs were also detected in small quantities in the amniotic fluid samples. This can be construed as evidence that the identification of a lamb by its mother is probably facilitated by a maternal label transferred at birth from the amniotic fluid to the lamb. However, the odour transfer could just as well be in the opposite direction, the organic volatiles being produced by skin glands on the body of the lambs already before birth and released into the amniotic fluid, in which case the VOCs can be regarded as constituents of a recognition pheromone. Quantitative analysis and comparison of the amniotic fluid samples showed that there exists similarity in the chemical profiles of the amniotic fluid of twin lambs. Our latest results provide strong evidence in favour of the neonatal recognition cue being a pheromone. The role of residual proteins in the dissemination of the semiochemicals involved in ewe–lamb communication was investigated and six proteins were identified as amniotic fluid and wool-associated proteins.

An interesting observation is that the amniotic fluids of first- and second-born lambs of twin-bearing ewes are qualitatively and quantitatively different. It is possible that the duration of the birth process could contribute to this change in amniotic fluid composition.

Acknowledgements

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References

1. Journal of Chemical Ecology. 2011. Vol. 37: 1150–1163.

SOME TERRITORIAL MARKING SUBSTANCES FROM ANTORBITAL GLANDS OF THE MALE KLIPSPRINGER ANTELOPE *OREOTRAGUS OREOTRAGUS* (ZIMM)

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Antelopes are strongly territorial, hoofed mammals inhabiting the savannahs of the eastern and southern parts of Africa. They are richly endowed with skin glands. Males of the klipspringer antelope, *Oreotragus oreotragus* (Zimm) (Ungulata: Artiodactyla), has well developed antorbital glands that are frequently used to mark prominent features within its territory. The smell of the fresh, clear mucous secretion, which quickly solidifies to a black rubber-like matrix, has a fresh, camphoraceous, anise-like note and is distinctly different from secretions of the Thomson gazelle (*Eudorcas thomsoni*) occurring in East Africa.

The author characterized several of these volatile components and identified some major less volatile components as the hydrocarbons *n*-heptadecane and *n*-eicosane. Additionally, some hydrocarbons of higher molecular mass, in conjunction with the mucous material, probably serve as fixatives for the volatile compounds. These were characterized as aliphatic, branched ketones with 5 to 7 carbon atoms by their R_Fs 0.54, 0.65, 0.71 and 0.79 on normal phase silica TLC plates, which were developed in the solvent system benzene / hexane 3:1 after derivatization of a stream distillate of the male antorbital secretion with acidified 2,4-dinitrophenyl hydrazine solution.

In addition to the ketone fraction, the *Oreotragus* secretion contained short chain aliphatic, in the majority branched, esters, notably again in the range of five to seven carbon atoms.

In biological tests of two territorial male klipspringers kept at the zoos of Frankfurt (Germany) and Naples (Italy), both males quickly noticed artificial marks prepared from steam distillates and placed within their territories at prominent places. These artificial marks were frequently overmarked. Females took much less, and if so, only superficial notice of the marks. Juveniles disregarded them.

29 years after these investigations of Hummel (1), Burger et al. (2, 3) independently reinvestigated the *Oreotragus* secretion with greatly defined modern chromatographic-spectrometric methods. Significantly, both studies agree in their major conclusions. The progress of analytical methodology within the three intervening decades is highly noteworthy.

References

1. Hummel H. E. 1968. PhD-thesis, University Marburg. 124 pp.
2. Burger B. V. et al. Journal of Chemical Ecology. 1997. Vol. 23: 2383–2400.
3. Burger B. V. 2005. Topics in Current Chemistry. 231–278.

CHEMOSIGNALING OF ESTRUS IN DOMESTIC HORSE *EQUS FERUS CABALLUS* L. (PERISSODACTYLA, EQUIDAE)

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Like many other mammalian species, horses use olfactory communication to coordinate their reproductive and social activities (1). Behavioral observations indicate that smearing of an estrous mare's urine onto castrated males, or non-estrous females, elicits a mounting response and copulation by a stallion, which has previously shown no interest in unsmeared individuals (2). The goal of the present study is to identify volatile chemical compounds for which concentrations correlate with ovulation in mares and to determine the behavioural function of those compounds. Urine samples from 14 mares, belonging to five breeds, were collected at estrus and diestrus to search for estrous specific volatile compounds. Around 150 volatiles were trapped from urine head-space samples by solid phase micro extraction technique, and analyses were conducted by gas chromatography–mass spectrometry methods (3). Comparison of chromatographic profiles of volatile substances revealed that concentrations of *m*- and *p*-cresols were significantly greater during estrus while diethylphthalate was more abundant at diestrus. Monitoring of *m*- and *p*-cresols during the period of estrus and a few days before and after estrus revealed irregular changes in amounts of cresols until 3 to 4 days before ovulation when the concentration of the compounds began to increase with peaks 1 day before ovulation. On the day when ovulation occurred, amounts of the metabolites decreased sharply, almost to basal concentrations, and remained at these concentrations for 6 days – when sampling was finished. In four of the mares changes in the concentration of diethylphthalate were less pronounced and more temporally variable compared with those of cresols (4). Based on reproducible temporal changes in concentrations of *m*- and *p*-cresols, with respect to the time of ovulation, a noninvasive test to determine a precise insemination time could occur. Data from a two choice behavioral test demonstrate that stallions spent significantly more time sniffing *p*-cresol as compared to *o*-, and *m*-cresols. The stallions' extent of erection differed significantly in response to each sample type. The lowest level of erection was recorded for pure water, moderate level was determined for the diestrous urine of a mare, and the highest extent of reaction was scored for a sample comprised of the diestrous urine of a mare containing synthetic *p*-cresol at a level, which is equivalent to half of the amount of *p*-cresol found in the estrous urine samples. Consequently, *p*-cresol should be considered as the sex pheromone component of horses. The pheromonal activity of *p*-cresol is reported in number of insect species; thus our data provide one more linkage to the intriguing similarities between insect and mammal pheromones of which few are known.

Acknowledgements

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References

1. The Behaviour of Horses. 1987. Allen JA, London.
2. Fourth International Congress of Animal Reproduction. 1961. The Hague, Netherlands.
3. Zeitschrift für Naturforschung. 2010. Vol. 65c: 127–133.
4. Animal Reproduction Science. 2012. Vol. 130: 51–56.

PLASTICITY IN PRIMATE OLFACTORY SIGNALS: TESTING THE HORMONAL MODULATION OF SCENT VIA NATURAL AND CONTROLLED EXPERIMENTS

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Many species use olfactory signals to assess potential social and sexual partners. Although some of the information encoded in odorants may be static (e. g., individual or kinship signatures), semiochemical profiles also vary with changing circumstances (e. g., dietary or seasonal variation). The ring-tailed lemur (*Lemur catta*), a strepsirrhine primate endemic to Madagascar, provides a unique model for examining the complex interaction between changing physiological conditions and the communication of static information. Through behavioral assays, genetic profiling, and chemical analyses, we have shown that lemur scent secretions encode honest and discernible information about identity (1), sex (1), reproductive status (1–2), genetic quality (i. e., heterozygosity; 3–5), and pairwise relatedness to conspecifics (3–6). Using a combination of natural experiments and controlled endocrine manipulations, we explore the hormonal mediation of these scent signals: specifically, we link variation in female reproductive hormones to variation in female semiochemical expression. We have found that changes in the chemical composition of odorants parallel changes in endocrine function associated with seasonal breeding (1), different stages of pregnancy, and the administration of hormonal contraceptives (2). For instance, the synthetic hormone medroxyprogesterone acetate alters the expression of semiochemicals used to encode important information about genetic quality and kinship in this species (2). These studies reveal that even moderate changes to endocrine profiles can dramatically alter lemur semiochemical profiles and the information they encode.

Acknowledgements

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References

1. Chemical Senses. 2007. Vol. 32: 493–504.
2. Proceedings of the Royal Society of London B. 2011. Vol. 278: 122–130.
3. Molecular Ecology. 2008. Vol. 17: 3225–3223.
4. Journal of Evolutionary Biology. 2010. Vol. 23: 1558–1563.
5. Animal Behavior. 2010. Vol. 80: 101–108.
6. BMC Evolutionary Biology. 2009. Vol. 9: 281.

IDENTIFICATION AND CHARACTERIZATION OF FOUR BOVINE ODORANT-BINDING PROTEIN ISOFORMS

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Bovine Odorant-binding Protein (bOBP) is described as a dimer that bounds a broad range of odorant molecules without specificity for one function to another one, or for molecule conformation. Based on those properties, bOBP was defined as a non-specific carrier protein. Purification and characterization of bOBP has usually been made on its capacity to bind some compounds as 2-isobutyl-3-methopyrazine leading to a single bOBP isoform isolation.

Here, we show the existence of various bOBP isoforms in the nasal mucus of the bull, which differ by their ionic properties. A fine separation by two successive strong anion exchange chromatography HPLC steps allowed us to distinct four bOBPs isoforms. Mass spectroscopy (MALDI-TOF) analysis permitted to confirm that separated proteins were bOBPs and underlined few primary sequence differences. Western-blot using specific antibodies indicated the presence of post-translational modifications (PTM), phosphorylation and *O*-GlcNacylation, on each isoform. Experiments were performed by fluorescence spectroscopy to analyze their binding properties for the bOBP natural ligand, 1-octen-3-ol, and one structural analogue, octanoic acid. Each bOPB displayed different binding properties for the two ligands. Affinity for 1-octen-3-ol was higher for isoforms 2 and 4 than for isoforms 1 and 3. Isoform 4 bound octanoic acid with less affinity than the 3 other isoforms. The localization of PTM on each isoform primary sequence is ongoing by using high-resolution mass spectrometry (FT-ICR and ECD) in order to determine their involvment in bOBP binding properties.

The precise role of OPB in the olfactory transduction process is still speculative but the existence of various bOBP isoforms with different binding specificities suggests that bOBP is not a passive carrier of odorant ligands. These findings support the hypothesis that OBP participates in the first step of olfactory coding.

THE PIG NASAL MUCUS "SECRETOME" REVEALED BY PROTEOMIC TOOLS: CHARACTERIZATION OF NEW ODORANT-BINDING PROTEIN ISOFORMS

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One of the arguments against an active participation of odorant-binding proteins (OBP) in the first step of olfactory coding is their low diversity in each animal species. In the pig, *Sus scrofa*, three genes only encode the three different primary sequences of OBP (*stricto sensu*), VEG (Von Ebner's Gland protein), and SAL (Salivary Lipocalin). In previous work, we have shown that pig OBPs are modified by post-translational modifications (PTM) such as phosphorylation (1) and glycosylation (2). These PTM generate different isoforms with different binding properties for odorant and pheromonal molecules (3). To go further in the investigation of OBP diversity, we have used proteomic tools to analyse the fine composition of the pig nasal mucus "secretome". Thirty OBP isoforms were identified, about ten for each sequence, differing not only by their PTM, but also by their molecular weight. In particular, a new VEG isoform, of lower molecular weight (15 kDa) than the published ones (18 kDa) was characterized and its expression comes from alternative splicing of VEG messenger RNA. This is the first time that this post-transcriptional mechanism is evidenced to be involved in OBP expression.

Acknowledgements

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References

1. Journal of Chemical Ecology. 2009. Vol. 35(7): 752–60.
2. Journal of Chemical Ecology. 2009. Vol. 35(7): 741–51.
3. Journal of Chemical Ecology. 2010. Vol. 36(8): 801–13.

Symposium 7. Climate Change, Invasive /Alien Species and Chemical Ecology

Moderator: John Byers

Monitoring invasive insect species with semiochemicals and predictive models

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The *effective attraction radius* (EAR) describes the monitoring power of a semiochemical lure with regard to blend and release rate as well as the responding insect species. A specific EAR is a spherical radius that would intercept the same number of insects as that caught by a particular semiochemical source (1–5). The pink bollworm, *Pectinophora gossypiella*, has been largely eradicated from Arizona using a combination of pheromone monitoring traps, release of sterile males, and growing BT (*Bacillus thuringiensis* toxin) cotton. Eradication has continued in Arizona by means of releasing sterile male moths from airplanes over cotton fields three days a week throughout the summer of 2011 (USDA APHIS). The male PBW were grown on diets that caused their bodies to become reddish-pink and easy to identify on sticky traps. We determined the EAR of synthetic sex pheromone lure for pink bollworm in the field by counting numbers caught on baited and unbaited sticky traps. The EAR for a lure was calculated to be about 1.03 m. Six clear sticky cylinders baited with PBW lures were placed on 3-m poles to determine the mean flight height (0.82 m) and standard deviation, SD, (0.26 m) of the vertical distribution that are used to convert the EAR into a circular EARc (2.61 m) that can be used to model two-dimensional encounters of rare insects with monitoring traps over large areas. The models indicate which population densities and distributions of monitoring traps would likely detect invading moths or residual populations in non-BT cotton in Arizona.

References

1. Environmental Entomology. 2007. Vol. 36: 1328–1338.
2. Journal of Chemical Ecology. 2008. Vol. 34: 1134–1145.
3. Journal of Theoretical Biology. 2009. Vol. 256: 81–89.
4. Environmental Entomology. 2011. Vol. 40: 1210–1222.
5. Journal of Chemical Ecology. 2012. in press.

UNDERSTANDING THE CHEMICAL ECOLOGY OF THE PUSH-PULL STRATEGY: A CASE STUDY WITH THE SUGARCANE BORER *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE)

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The push-pull strategy uses indigenous plants to attract (pull) or deter (push) crop pests from the fields (1). This method was shown to significantly improve the harvest of small scale farmers in Kenya (2), while reducing the use of insecticides. The South African sugarcane industry is trying to reproduce this approach in order to control *Eldana saccharina* Walker (Lepidoptera: Pyralidae) which has been an important sugarcane pest for more than 40 years (3).

Field experiments showed that the push plant *Melinis minutiflora* P. Beauv. (Poaceae) significantly decreases the infestation of *E. saccharina* in sugarcane fields. Behavioural experiments under laboratory conditions confirmed that the odour of this plant significantly repels the moth, but does not affect its larvae. Electroantennogram recordings demonstrated that *E. saccharina* adults detect eight of 13 compounds naturally released by *M. minutiflora*. Behavioural experiments with these single compounds permitted the identification of three attractive and one repulsive compound for the adult and larval stages of *E. saccharina*.

These compounds could be used to bait traps which are needed for timely detection of pest infestation and monitoring of the pest problem. In addition, results may lead to the selection of new push- and pull-plants based on their odorant profile.

Acknowledgements

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References

1. Nature. 1997. Vol. 388: 631–632.
2. Crop Protection. 2008. Vol. 27: 1084–1097.
3. Insect Science and its Application. 1997. Vol. 17: 69–78.

PACT WITH THE ENEMY: CONSUMER-FACILITATED INVASION OF A WELL-DEFENDED RED ALGA DRIVEN BY REFUGE-MEDIATED APPARENT COMPETITION

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Biological invasions pose a risk to biodiversity and functioning of ecosystems in invaded areas (1). Why some introduced species become dominant in their new environments whereas others stay minor members of the community is still equivocal. Introduced plants are predicted to invade when they are less affected by herbivores, as this will provide the introduced species with a competitive advantage over native plants (2). Consequently, well-defended introduced species have been suggested to become most likely successful invaders (3, 4).

Having its origin in the Northwest Pacific, the filamentous red alga *Bonnemaisonia hamifera* has invaded large parts of the North Atlantic rocky shores and locally dominates the seaweed community (5, 6). In feeding choice experiments, native generalist herbivores explicitly preferred native seaweeds to the invader. Using a bioassay-guided fractionation it was shown that *B. hamifera* is chemically defended against native herbivores by producing 1,1,3,3-tetrabromo-2-heptanone as the main feeding deterrent. Further experiments showed that in the presence of herbivores the abundance of *B. hamifera* in the community increases due to both consumption of neighboring algal competitors and an enhanced performance of the invader. In return, the invasive species provides a superior refuge to herbivores from fish predation compared to native seaweeds, which may explain the rich species diversity and abundance associated with this species.

Bonnemaisonia hamifera is an excellent example of how a well-defended introduced species providing a refuge to herbivores can invade via refuge-mediated apparent competition by increasing the grazing pressure and decreasing the performance of nearby competitors. These findings highlight that plant-herbivore interactions can determine invasion success and that it is important to consider these in a tri-trophic context.

Acknowledgements

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References

1. Ecological Applications. 2000. Vol. 10: 689–710.
2. Trends in Ecology & Evolution. 2002. Vol. 17(4): 164–170.
3. Biology Letters. 2005. Vol. 1(4): 435–438.
4. Biology Letters. 2006. Vol. 2(2): 189–193.
5. Biological Invasions. 2001. Vol. 3(1): 9–21.
6. Marine Biology Research. 2007. Vol. 3(2): 61–72.

THE EFFECTS OF OZONE ON PLANT-PLANT COMMUNICATION: CHEMICAL ECOLOGY IN A CHANGING GLOBAL AND POLLUTED ENVIRONMENT

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Herbivore-induced plant volatiles (HIPV) mediate a wide array of interactions among plants and other organisms, including, for example, repellence of herbivores, attraction of natural enemies of herbivores, and inter- and intra-plant communication. Many HIPVs are highly reactive and can react rapidly with many oxidative pollutants during their transport in the atmosphere, thus potentially leading to an appreciable loss of their ecological functions mentioned above. One such pollutant is tropospheric ozone, which is phytotoxic and can degrade many HIPVs. The tropospheric ozone level has risen since pre-industrial times and is predicted to continue rising (1). Previous laboratory studies in our research group have revealed that ozone can reduce the foraging efficiency of parasitoids and predators (2) as well as the distance over which airborne plant-plant communication occurs (3). In both cases, the degradation of signaling compounds has been identified as the likely mechanism. However, the effects of ozone pollution on HIPV-mediated ecological processes such as plant-plant communication have not been explored in sufficient detail, especially under natural conditions. Here, we used a system consisting of hybrid aspen (*Populus tremula* × *tremuloides*) and a specialist leaf beetle (*Phyllodecta laticollis*) to investigate whether and how moderately-elevated tropospheric ozone concentrations impact HIPV-mediated plant-plant communication in both laboratory and natural conditions. We have recently demonstrated in the laboratory that hybrid aspen can respond to the volatile signals emitted from conspecific neighbours damaged by generalist herbivores by directly inducing the secretion of extrafloral nectar, a widely accepted indirect defence strategy, and by priming the emission of terpene volatiles (4). The study in this presentation is a follow-up, with a view to further testing this hypothesis using a specialist herbivore under both laboratory and field conditions, and more importantly the effects of ozone. We will present the experimental data and discuss the ecological implications of this study. To better understand the mechanisms responsible for the observed effects of ozone on plant-plant signalling, additional results showing the transcriptional changes will also be presented.

References

1. Sitch S., Cox P. M., Collins W. J., Huntingford C. 2007. Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature. Vol. 448: 791–794.

2. Pinto D. M., Nerg A-M., Holopainen J. K. 2007. The role of ozone-reactive compounds, terpenes, and green leaf volatiles (GLVs) in the orientation of *Cotesia plutellae*. Journal of Chemical Ecology. Vol. 33: 2218–2228.

3. Blande J. D., Holopainen J. K., Li T. 2010. Air pollution impedes plant-to-plant communication by volatiles. Ecology Letters. Vol. 13: 1172–1181.

4. Li T., Holopainen J. K., Kokko H., Tervahauta A. I., Blande J. D. 2012. Herbivore-induced aspen volatiles temporally regulate two different indirect defences in neighbouring plants. Functional Ecology. (in press).

DOES HARMONINE-BASED CHEMICAL DEFENSE MEDIATE INVASIVE SUCCESS OF THE HARLEQUIN LADYBIRD *HARMONIA AXYRIDIS*?

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The harlequin ladybird *Harmonia axyridis* has emerged as a model species in invasion biology. Its remarkable capacity to outcompete native ladybird species upon introduction into new habitats has been linked to its prominent resistance against pathogens and intraguild predation. Recently, we discovered a strong constitutive antibacterial activity in the hemolymph of *H. axyridis* beetles which has been attributed to the chemical defense compound harmonine exhibiting a broad-spectrum activity against pathogens including those causing human diseases such as the infective agents of tuberculosis and malaria (1). We elucidate that *H. axyridis* differs from other insect species, including the native ladybird *Coccinella septempunctata*, by showing decreasing antimicrobial activity in the hemolymph upon injection of bacteria which elicit innate immune responses. Using mass-spectrometry based imaging we demonstrate that harmonine disappears from the hemocoel upon bacterial challenge, implicating fitness-related resource allocation from constitutive chemical defense to inducible innate immune responses. The constitutive chemical defense against pathogens may mediate the invasive success of *H. axyridis* since harmonine, the causing agent behind, has been implicated to play an additional role in defense against intraguild predation.

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References

1. Biology Letters. 2012. Vol. 8: 308–311.

CAN CHEMICAL PROFILING OF THE SEX PHEROMONE GLAND BE USED TO DETERMINE THE ORIGIN OF INVASIVE SPECIES?

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The number of species invading new habitats is growing due to the expanding global trade network. Introduction of new species can have negative ecological and economic impacts in the new habitat. Fall webworm (FWW), *Hyphantria cunea* is native to North America, however since World War II; this insect has spread to new habitats in Europe, Asia and recently South America. Fall webworm has been discovered in New Zealand recently, if it was to establish, it would have a severe ecological and economical impact. Determination of the origin of invasive species might help in the eradication effort and reduce the risk of any future invasion. Usually molecular markers are used to determine the origin of an invasive. However this requires analysis of a large number of individuals, and sometimes the variation in the genetic material is small. In this work, we have tested whether chemical profiling of the sex pheromone gland of the FWW can be used to determine the origin of the population discovered in New Zealand. According to our chemical analysis of the New Zealand population and published data of the sex pheromone gland composition in other populations, three pheromone blends were tested in ten different locations in North America, Europe, China and Japan. In this talk we will demonstrate that chemical profiling of the sex pheromone gland combined with pheromone trapping experiments in various locations can be used as an additional tool in determining the origin of invasive species.

Symposium 8. Applied Chemical Ecology

Moderators: Miklos Toth and Christer Löfstedt

Keynote speaker

SEMIOCHEMISTRY OF CERAMBYCID BEETLES: PARSIMONY IN PHEROMONE STRUCTURES, ANTAGONISM BY PHEROMONE ANALOGS, AND SYNERGISM BY HOST PLANT VOLATILES

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In 2004, pheromones had been identified for less than 10 cerambycid species, and all were male-produced. Some 8 years later, pheromones or likely pheromones now have been identified from well over 100 species, including several examples of female-produced sex pheromones from two different cerambycid subfamilies. Within some groups, pheromone structures are highly conserved, whereas in others, pheromones appear to be relatively species-specific. It is also becoming apparent that host plant volatiles strongly synergize attraction to some but by no means all pheromones. Furthermore, as with other insect groups, attraction to pheromones may be strongly inhibited by pheromone analogs produced by congeneric species, to minimize cross attraction. Our current knowledge of these pheromones and their roles will be summarized, and the implications for development of practical uses for these pheromones, particularly for detection of invasive species, will be discussed.

Acknowledgements

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NEW SELECTIVE ATTRACTANTS FOR THE INVASIVE SOCIAL WASP, *VESPULA VULGARIS*

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A serious and increasing threat to geographically isolated natural ecosystems is invasion of Hymenopteran species. One example is social wasps, which are extremely successful invaders of new environments. As well as being agricultural and urban pests, they pose a significant ecological problem to native biodiversity in many areas around the world.

At the peak of the summer season, in the honeydew laden beech forests in Nelson region of New Zealand, the biomass of *V. vulgaris* is greater than or equal to the combined biomass of rodents, birds, and stoats. Carbohydrate consumption by these wasps from the secretions of the beech scale insects *Ultracoelostoma assimile* (Maskell) and *U. brittini* (Morales) greatly reduces both the abundance and quality of honeydew available for native wildlife and makes the resource virtually unavailable for native birds during the summer months, impacting significantly on forest biodiversity. The production of honeydew in the southern beech forests has been estimated to be around 4,000 kg dry weight/ha per year and it has been reported that wasps harvest 90% of this honeydew during five months of the year.

There is clearly a need for sophisticated means of controlling social wasps.

In a research program aimed at the development of new, more specific tools for control of invasive social wasps in threatened ecosystems we have isolated and identified attractants from natural sources in the wasp environment. The foraging strategies of these insects rely heavily on olfaction and these odor attractants may form the basis of a new generation of selective lure-and-kill solutions for invasive wasps that can be applied either aerially or manually over large areas.

The study was executed in New Zealand southern beech forests.

ONE SECRETION TO DEFEAT THEM ALL? – THE CHEMICAL DEFENSE OF THREE DERMAPTERAN SPECIES

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Earwigs, insects of the order Dermaptera, are very common in orchards and backyards in Middle Europe. On the one hand, they are recognized as beneficial because they feed on various insects, including several pest species. On the other hand, they are considered as a pest themselves, for feeding occasionally on buds, florescences and fruits. Clearly the most noted feature is that earwigs generate an unpleasant odor when irritated. However, to date only two earwig species have been investigated with respect to their chemical armature. Therefore, we analyzed the defensive secretion of three dermapteran species using gas chromatography-mass spectrometry. In total, four alkylated 1,4-benzoquinones were identified; one thereof is hitherto known exclusively from opilionid exocrine secretions. Furthermore, species- and sex-specific differences in the chemical composition were observed. Previous studies suggest that the secretion is used primarily as repellent against various predators. As earwigs live in aggregations in narrow crevices and practice maternal care, e. g. by hibernating with their brood in nests belowground, their subsocial behavior and habitat preferences increase the need for enhanced defense mechanisms against co-occuring microorganisms. 1,4-Benzoquinones in general, are renowned for their antimicrobial action and in particular, are part of the aggregation pheromone of some earwigs, and thus most likely surrounding the insects permanently. Therefore, they are suitable candidates to accomplish this task. To evaluate the antimicrobial activity of the different secretions, we performed inhibition zone assays with gram-positive and – negative bacteria and two entomopathogenic fungi. Our results indicate that the defensive secretion is not only used to repel predators but also to disinfect the microhabitat.

Acknowledgements

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DIURNAL EMISSION OF TEPHRITIDAE VOLATILES EXPLORED USING AN AUTOMATED SEQUENTIAL SPME-GCMS ANALYSIS APPROACH

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Identification of pheromones in insects is not a simple enterprise due to low concentrations and novel compounds. Identification of behavioral modifying volatiles has usually relied on difficult *in-vitro* chemical extractions of emitting glands and intensive chemical separation and analysis procedures, which are followed by extensive behavioral bioassays. Recently our laboratory successfully isolated and characterized the pheromone of the economically important lesser date moth using SPME-GCMS (Solid Phase MicroExtraction-Gas Chromatography Mass Spectrometry) coupled with an automatic sampling system that allowed continuous sequential collection of air environments enclosing single, or a few, living insects (1). As an initial step to investigate the application of this method in the characterization of unknown Tephritidae pheromones, we studied the behavior of a partially known pheromone system: the olean (1,7-dioxaspiro[5.5]undecane) pheromone of the olive fly *Bactrocera oleae* (Diptera: Tephritidae). The automatic sequential-sampling SPME-GCMS method was able to characterize the *in-vivo* diurnal emission patterns of single wild female olive fly throughout several days. We present the results of this study, and additional results related to the chemical composition (specifically chirality) of olean emissions from wild female and male olive flies of different ages. The results are discussed in relation to the possibilities that this novel method enhances the exploration of Tephritidae pheromone systems.

References

1. Tetrahedron Letters. 2011. Vol. 52: 4550–4553.

POTENTIAL FUNCTION(S) OF PBAN/PYROKININ PEPTIDES IN AN INVASIVE ANT

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Insect neuropeptide hormones represent more than 90% of all insect hormones. The PBAN/pyrokinin family is a major group of insect neuropeptides. These species-specific neuropeptides have been shown to have a variety of functions from immature to adult. PBAN is well understood in moth species relative to sex pheromone biosynthesis, but other functions have not been determined. Recently, we defined the PBAN gene and peptides in fire ants to better investigate their function(s). RNA interference (RNAi) technology is a convenient tool to investigate unknown physiological functions in insects. In this study, we selected the PBAN gene as a target for RNAi suppression for the fire ant (*Solenopsis invicta*). We report negative phenotypic impacts during developmental and adult stages of after PBAN dsRNA treatment, e. g. increased adult and larval mortality, delayed pupal development and decreased sex pheromone production in a control moth species. This is an important first step in determining the function of the PBAN gene in the fire ant and suggests the possibility for novel biologically-based fire ant control.

Keynote speaker

FIELD OBSERVATIONS OF BEHAVIORAL RESPONSES OF EUROPEAN OAK BUPRESTIDS TO VISUAL STIMULI, PLANT VOLATILES, AND CUTICULAR HYDROCARBON PHEROMONES

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We have investigated the mating and host-finding behaviours of *Agrilus* species found in European Oak forests, with a primary focus on *Agrilus biguttatus*, a known forest pest. By using pinned dead models we were able to evoke visual mating approaches by males of *Agrilus biguttatus, Agrilus sulcicollis,* and *Agrilus angustulus*. This behaviour was similar to that previously observed in males of *Agrilus planipennis* (1). In *A. planipennis* it is also known that female-produced cuticular hydrocarbons function as contact pheromones that affect the duration of male copulatory contact (2). A high degree of cross-species compatibility with respect to these visual and chemical cues were observed in the field when *Agrilus biguttatus* were allowed to approach a number of different species of pinned, dead females (3). Finally, traps were deployed in a European oak forest, adding lures such as visual decoys and different chemical formulations. Traps with 4 × 8 cm green plastic surfaces caught more *Agrilus* if visual decoys were included. Formulations of tree-produced volatiles such as manuka oil and Z3-hexen-1-ol also increased *Agrilus* captures on these traps. An additional lure consisting of Z9-tricosene, which is chemically similar to the *A. planipennis* contact sex pheromone Z9-tricosane, also significantly increased trap captures.

Acknowledgements

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References

1. Journal of Insect Behavior. 2007. Vol. 20: 537–552.
2. Journal of Chemical Ecology. 2009. Vol. 35: 104–110.
3. Entomologia Experimentalis et Applicata. 2011. Vol. 140(2): 112–121.

VARIATION IN ATTRACTION TO HOST PLANT ODORS IN AN INVASIVE MOTH HAS A GENETIC BASIS: ECOLOGICAL AND PRACTICAL IMPLICATIONS

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A number of attractant-based approaches for insect pest management rely on the assumption that adult response to distinct volatiles is a species-specific trait, neglecting the option for potential significant intra-specific variations. Thus, knowledge of such potential variation in olfactory attraction may determine the promise and the long-term success of tactics based on behavioral manipulation with plant volatiles. Here, we used the cosmopolitan oriental fruit moth *Grapholita* (=*Cydia*) *molesta*, an oligophagous lepidopteran invasive insect that attacks stone and pome fruit trees, to investigate whether and to what extent attraction of mated females to host plant odors has a genetic component. Results document a considerable genetic basis for female olfactory attraction, as well as a genetic trade-off with fecundity. These estimations were empirically corroborated when comparing two strains maintained in the laboratory for different numbers of generations. A long-term reared strain lost its olfactory discrimination ability but achieved significantly higher fecundity compared to a short-term reared strain. The ecological consequences of our findings and their implications for applied chemical ecology, in particular for insect behavioral manipulation with plant volatiles, will be discussed.

FUNCTIONAL CHARACTERIZATION OF GROOVED-PEG SENSILLA OF FEMALE *AEDES AEGYPTI*

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Different functional types of grooved-peg sensilla are distributed across the antennae of female *Aedes aegypti*. Based on the response profile to 25 odorants, including amines, ketones, alcohols, aldehydes, esters and fatty acids, we characterized five different functional types and verified these through cluster analysis. Several of the physiologically active stimuli, including ammonia and isobutylamine, elicited excitatory or inhibitory responses in the olfactory receptor neurons housed in these sensilla in a dose-dependent manner. Response profiles confirmed that compounds sharing similar functional groups are detected by a distinct set of olfactory receptor neurons housed in female grooved-pegs. This reinforces the notion that this type of sensilla houses olfactory receptor neurons expressing highly conserved olfactory receptors fundamentally dedicated to the detection of amines and fatty acids. Based on their distribution, grooved peg sensilla appear to be segregated along the female *Ae. aegypti* antennae. The conserved role of grooved-pegs is discussed in terms of their evolutionary relevance.

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THE POTENTIAL OF *AZADIRACHTA INDICA* AND *NIGELLA SATIVA* AS BIOPESTICIDE AGAINST MOSQUITO

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The insecticidal effect of *Nigella sativa* towards *Aedes albopictus* was investigated. Dried black seeds was extracted for 24 hours at 40 g : 200 ml. The extract at 5,000 ppm, 10,000 ppm, 25,000 ppm, 50,000 ppm and 75,000 ppm was poured in 9 cm petri dish. Ten second instar larvae of *Aedes albopictus* were placed in each petri dish. Ten replicates of each treatment were prepared. Mortality was counted at each hour for 24 hours. Using probit analysis LD_{50} was achieved at 1 hour with 126,084 ppm, at 2 hr, LD_{50} was achieved at 56, 065 ppm. The minimum ppm for *N. sativa* was at 2,935 ppm at 15th hour after application. As a comparison, similar experiment was conducted using neem extract, *Azadirachta inidica* against *Aedes aegypti*, at 1 hour after application, 178,521 ppm was needed for LD_{50} to be achieved. LD_{50} at 2 hours was achieved at 64,630. While LD_{50} was achieved at 2,652 after 15 hours application. Both black seed and neem has potential as a biopesticide. Mortality was achieved faster at lower dosage using black seed.

Acknowledgements

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References

1. Dua V. K., Gupta N. C., Pandey A. C., Sharma V. P. 1996. Repellency of Lantana camara (Verbenaceae) flowers against Aedes house flyes. Am. Mosq. Cont. Assoc. Vol. 12: 406–408.

2. Jacobson M. 1975. Insecticides from plants: a review of the literature, agricultural handbook. Willhington, U. S. Dep. Agriculture. Vol. 138: 1954–1971.

3. Sivagnaname N., Kalayanasundaram M. 2004. Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of house flyes and non-target organisms. Mem. Inst. Oswaldo Cruz. Vol. 99: 115–118.

4. Perich M., Wells C., Bertsch W., Tredway K. E. 1995. Isolation of the insecticidal components of Tagetes minuta (Compositae) against house fly larvae and adults. J. Am. Mosq. Cont. Assoc. Vol. 11: 307–310.

STUDY OF ATTRACTIVE AND POSSIBLY REPELLENT STIMULI ON ADULT GREEN LACEWINGS (CHRYSOPIDAE)

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Green lacewings are an important group of natural enemies, because larvae of all species and adults of *Chrysopa* genus are predatory. Previously, attraction of common green lacewings (*Chrysoperla carnea* complex) to floral volatiles and attraction of *Chrysopa* spp. to aphid sex pheromone compounds were reported. However, when using these stimuli in combination, attraction of common green lacewings decreased significantly.

Chrysopa lacewings are known to emit defensive compounds when disturbed or attacked. Therefore, it seemed reasonable that instead of the aphid sex pheromone compounds themselves being repellent for the *C. carnea* complex, it was possibly the odour of the trapped *Chrysopa formosa* adults which had been attracted to aphid sex pheromone compounds. To study this phenomenon, we set up field experiments. When dead *Chrysopa* lacewings (which bear the specific '*Chrysopa* smell'), were placed in the traps, no decrease of *C. carnea* complex catches was found. Furthermore, when skatole, a characteristic compound of *Chrysopa* defensive secretions, was added to the traps, no effect was observed on either *C. carnea* complex or *C. formosa* adults, thus suggesting that this compound serves only for defense and does not have an alarm function.

An early season experiment was also conducted during the short period when *C. carnea* complex adults are already on the wing, but *C. formosa* adults are not yet present, to test whether the aphid sex pheromone compounds themselves had an effect on the catches. This experiment showed some effect, but not as marked an effect as previously observed.

In summary, our results suggest an antagonistic effect between attractants of *C. formosa* and *C. carnea* complex. Although no unambiguous explanation has yet been found for this phenomenon, this issue is well worthy of further research and may reveal important interspecific relationships between these species, which were previously unknown.

Acknowledgements

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Keynote speaker

PHEROMONES FOR MONITORING AND CONTROL OF INSECTS IN SPRUCE SEED ORCHARDS

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Seed orchards are important for the production of high-quality seeds for reforestation, but infestation by cone and seed insects can severely reduce the seed harvest in such orchards. The aim of this project is to identify sex pheromones for monitoring and control of three major moth pests in European spruce seed orchards: *Cydia strobilella* (Tortricidae), *Dioryctria abietella* (Pyralidae), and *Eupithecia abietaria* (Geometridae). For *C. strobilella*, we have identified the sex pheromone as a blend of (8*E*,10*E*)-dodecadienyl acetate and (8*E*,10*Z*)-dodecadienyl acetate (1). Females produce the isomers in extremely small amounts (<1 pg per compound), and the antennae of males are remarkably sensitive to these compounds. Mating disruption experiments on this pest have produced mixed results. Although trap catches of males in pheromone-treated areas dropped to almost zero, we found no reduction in number of larvae per cone in treated areas versus control areas. For *D. abietella*, we have identified a second pheromone component, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene, which has a strong synergistic effect when added to the previously identified (9*Z*,11*E*)-tetradecadienyl acetate (2). Monitoring of both *C. strobiella* and *D. abietella* in seed orchards has been conducted for several years to establish relationships between trap catches and cone damage. Finally, the identification of the sex pheromone for *Eupithecia abietaria* is ongoing, and preliminary electrophysiological data indicate that the species uses two components as pheromone, although behavioural data are so far lacking.

Acknowledgements

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References

1. Journal of Chemical Ecology. 2010. Vol. 36: 305–313.
2. Journal of Applied Entomology. 2012. Vol. 136: 70–78.

IDENTIFICATION AND FORMULATION OF PHEROMONES OF THE AMBROSIA BEETLE *MEGAPLATYPUS MUTATUS* AND FIELD MANAGEMENT USING RESERVOIR AND MONOLITHIC DELIVERY SYSTEMS

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Megaplatypus mutatus (Chapuis) (Coleoptera: Curculionidae: Platypodinae) is an ambrosia beetle native to South America, but it 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 devices in half sphere shape were made with different mixtures of waxes and polymers with inert components. They followed first-order kinetics according to the curve $y = y_0 + ae^{-bx}$ and released significant amounts of pheromone during a period of until 33 days in the 4–400 mg/day range.

We performed field trials of mating disruption of *M. mutatus* and trapping in baited traps 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.

FIRST STEPS TO THE DEVELOPMENT OF A "PUSH-PULL" STRATEGY TO PROTECT BROCCOLI PLANTS AGAINST THE CABBAGE ROOT FLY, *DELIA RADICUM*

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Volatile organic compounds (VOCs) emitted by plants are used by phytophagous, predatory and parasitoid insects to adjust behavioural decisions in complex environments. The modification of these plant signals could represent a promising approach to manage pest insects but documented applications remain scarce. *Delia radicum*, the cabbage root fly, is a major pest of brassicaceous crops for which classical control strategies are currently very limited. Associating lab and field experiments, we selected a set of synthetic VOCs that have contrasted effects on *Delia radicum* host plant selection behaviour and on the activity of its natural enemies. We conducted additional field experiments where broccolis plants were surrounded with a belt of another brassicaceous plant species selected on the basis of its high attractiveness for *D. radicum* and that could act as trap plants. Ultimately we plan to apply repulsive synthetic VOCs in the broccoli parcel and attractive ones in the trap belt in an original push-pull design aiming at protecting broccoli plants against the cabbage root fly, *D. radicum*.

FACTORS AFFECT THE TRAP EFFICIENCY OF DOGWOOD BORER: SEX PHEROMONE DISPENSER AND TRAP DESIGN

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The trap efficiency of dogwood borer (DWB), *Synanthedon scitula* Harris (Lepidoptera: Sesiidae), was evaluated in field trapping studies using wing-style sticky traps baited with rubber septum or polyethylene vial dispensers containing the most effective ternary blend [86:6:6 v:v:v (*Z,Z*)-3,13-octadecadienyl acetate : (*E,Z*)-2,13-octadecadienyl acetate : (*Z,E*)-3,13-octadecadienyl acetate] in an apple orchard in West Virginia. Traps baited with a polyethylene vial dispenser captured significantly more male DWB than those baited with a rubber septum dispenser in first two months. Although the release rate from vials did not change, captures in vial-baited traps decreased considerably after two months, possibly due to the presence of *3,5-di-tert-butyl-4-hydroxy acetophenone from the polyethylene vials and its antagonistic effect on the response of males.* Traps baited with rubber septum dispensers effectively captured DWB males for at least 6 months and significantly more than traps with vials during the last four months of the flight season. A release rate study using lab and field-aged dispensers demonstrated that the DWB sex pheromone was desorbed from polyethylene vials and rubber septum dispensers following first order kinetics, with half-life ($t_{1/2}$) values of 1.6 and 10.7 months, respectively. Several trap designs including wing and delta style sticky traps and white and green "bucket" style traps baited with rubber septum dispensers were also compared for their effectiveness at capturing DWB in commercial apple orchards in WV and VA. Bucket traps clearly demonstrated greater trapping efficiency of male DWB during a trial when moth populations were high and traps with sticky surfaces such as 1C and delta-style were rapidly saturated by male DWB moths.

CHEMICAL ECOLOGY OF GENETICALLY MODIFIED POTATO PLANTS

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Metabolomic techniques are a promising tool for studying the impact of genetically modified (GM) plants, because unintended pleiotropic effects of genetic modification on plant physiology can be detected in a non-targeted and unbiased way. Metabolomics may therefore also contribute to the assessment of ecological risks of GM plants on insect communities via changes in primary or secondary plant metabolism. While this approach has been mentioned as an interesting perspective by the European Food Safety Agency, few studies have tested its applicability. We tested the metabolomics approach in a field study using a GM potato variety, its non-modified counterpart and four non-modified commercial varieties. Insects were counted on whole plants four times in a growing season and leaf samples of the same plants were analyzed by nuclear magnetic resonance (NMR). While important sources of environmental and genotype-dependent variation were found in both insect and metabolomics data, the two data sets were only weakly correlated, suggesting that factors other than those measured by NMR (e. g. volatile compounds, morphological characters or non-plant environmental factors) were more important in shaping insect communities. While the predictive value of NMR metabolomics in terms of ecological risk assessment is limited, it remains a powerful tool for the reverse approach of elucidating possible chemical mechanisms of observed changes in plant-insect interactions.

Keynote speaker

NOVEL MATING DISRUPTION TECHNOLOGIES AND STRATEGIES FOR MANAGING CODLING MOTH

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Recent efforts to enhance or develop new mating disruption formulations have been guided by large-cage, field and flight tunnel studies examining the mechanisms by which mating disruption is achieved (1, 2, 3). Dosage-response profiles for reservoir dispensers targeting *Cydia pomonella* L., codling moth (CM), revealed that competition between pheromone dispensers and females initiates communicational disruption in the field. Additional disruption mechanisms come into play subsequent to initial attraction. Disruption profiles indicated that one exposure to a reservoir dispenser rendered CM males incapable of normal sexual response for the remainder of a diel cycle, but males recovered and oriented to pheromone sources the following evening. Superior disruption was achieved when the initial attraction to a dispenser resulted in the complete elimination of subsequent male orientations, i. e., males were killed following attraction. Disruption profiles generated for an attract-and-remove dispenser revealed that this approach provided disruption substantially greater than that achieved using reservoir dispensers. Studies using open orchard 0.2-ha plots comparing a high density-trapping scenario using a patent-pending microtrap to mating disruption using reservoir dispensers confirm those results (4). The attract-and-remove treatment with microtraps reduced CM capture in central monitoring traps by 92%; while disruption using Isomate CM Flex reduced catch by 71%. The high cost of mating disruption is often cited as a major impediment to broader adoption of the tactic. The economics of reservoir dispensers could be improved through more efficient use of the precious active ingredient. Very similar disruption profiles and impacts were generated using dispensers that released pheromone at much lower rates than the standard dispenser. ShinEtsu (Tokyo, Japan) has produced Isomate Flex CM dispensers that have substantially lower release rates than the standard Isomate dispenser. Equivalent levels of CM disruption have been achieved using low-releasing Flex dispensers and standard dispensers in both small-plot and on-farm experiments. The greatest efficacy of reservoir dispensers should occur when numerous point sources are distributed uniformly within the orchard. Reservoir dispensers that are amenable to mechanical application should facilitate achieving this. A new pheromone delivery system, called the Tangler®, consists of a two-piece pheromone module connected by string that is launched from a compressed gas applicator. As a result of the bola design, the propelled modules readily become tangled in the tree branches. Large plot field experiments revealed that a single application of Tangler® modules provided CM control equal to commercially available hand-applied dispensers. Moreover, automated deployment of the modules was 5x faster than hand application of dispensers. Sprayable microencapsulated formulations appear to operate by camouflage. Their major limitations are that capsules only hold enough pheromone to last a few weeks and capsules are dislodged by heavy rainfall. A solid-set system that delivered a small dose of pheromone-filled sub microcapsules each evening was tested in 0.25-ac apple plots. The 'pherogation' system provided orientation disruption superior to that achieved through airblast sprayer application. Collectively, this research demonstrates several ways costs may be reduced while maintaining or improving efficacy when using sex pheromones for CM management.

References

1. J. Chem. Ecol. 2006. Vol. 32: 2089–2114.
2. J. Chem. Ecol. 2006. Vol. 32: 2115–2143.
3. Proc. Nat. Acad. Sci. 2010. Vol. 107: 22–27.
4. Physiol. Entomol. 2012. Vol. 37: 53–59.

MATING DISRUPTION OF THE SWEDE MIDGE (DIPTERA:CECIDOMYIIDAE)

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The potential for pheromone-based mating disruption of the Brassica pest *Contarinia nasturtii*, was tested in both in small-scale plots and commercial-scale fields with either Brussels sprouts, broccoli or cauliflower. The experiments in the small-scale plots were made with laboratory-reared insects released into a previously non-infested area, whereas the large-scale experiments were made in an area with a high natural population of *C. nasturtii*. Dental cotton rolls (small-scale experiment) and polyethylene caps (large-scale experiment) containing 50 µg (2*S*, 9S)-diacetoxyundecane, 100 µg (2*S*,10*S*)-diacetoxyundecane, and 1 µg (2*S*)-acetoxyundecane, served as dispensers and were spaced in 2 × 2 m squares in the test plots. In both experiments, mean catches of *C. nasturtii* males in pheromone traps were reduced to almost zero in the treated plots. Crop damage in the treated plots compared to the control plots was reduced by 60% in the small-scale and 90% in the large-scale experiments, respectively. This study shows that pheromone-based mating disruption has a high potential for management of *C. nasturtii* populations.

TRACKING DOWN THE SEMIOCHEMICALS IN VINEYARDS – A NEW APPROACH TO ENHANCE MATING DISRUPTION

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Mating disruption is widely utilised in ecological pest control. In viticulture it is used against *Lobesia botrana* and *Eupoecilia ambiguella*, which has led to a huge decline in the use of insecticides. As this method is increasingly applied in regions worldwide, wine growers and researchers also face problems in vineyards where the confusion via pheromone dispensers fails. In order to understand the circumstances that lead to these failures, analytical devices are needed that feature a high sensitivity for volatile organic compounds, short measurement time and portability. Therefore, mobile electro-antennogram (EAG) devices have been invented, but they still produce non-comparable relative units and provide distorted results in the presence of non-target volatiles.

To avoid all these disadvantages, we established an automated portable device that comprises a needle-trap device (NTD), gas chromatograph (GC), mass spectrometer (MS), and electro-antennographic detection (EAD). Testing this setup with the *Lobesia botrana* main pheromone component 7,9–12Ac, we discovered a surprisingly low detection limit and a high dynamic range. Our first results indicate a new application strategy for the pheromone dispensers. A first series of measurements will be realized in vineyards and the data will be validated by cage-release and capture tests.

Once adjusted to the morphological conditions of the insect antennae and provided that the pheromone is chemically known and synthetically available, the portable NTD-GC-MS-EAD system can be used to monitor pheromones for any pest insect species in question.

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GAS SENSORS FOR AIRBORNE PHEROMONES MONITORING IN VITICULTURE

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Pest insects have an economic impact in viticulture. Since the use of insecticides is critical in respect to the whole ecosystem, the mating disruption method is widely and increasingly used against the grapevine moth (*Lobesia botrana*). To disrupt the male moth in his approximation flight that is based on the airborne pheromone concentration, the release of synthetic pheromone components have to reach a relevant concentration threshold. To guaranty the effectiveness of the method, a monitoring system for the pheromone concentration in the vineyard is needed. With the antennae of *Lobesia botrana* mounted in an electro-antennograpic detector it is possible to detect concentration of pheromones, but they possess a short lifetime in the measurement system and are difficult to calibrate. Chemical sensors could detect insect volatiles, but so far are not as sensitive and selective as insect antennae.

In our investigation, we used a portable gas chromatograph – mass spectrometer (GC/MS) with an electro-antennograpic detector (EAD) as reference system in combination with metal-oxide semiconductor gas sensors in a SOMMSA (selective odorant measurement of a multi-sensor array) setup. To increase the sensitivity of the sensors, a preconcentration step through a needle-trap enrichment device was applied. Since gas sensors react to plenty volatiles, the selectivity had to be improved by a temperature controlled desorption of the enrichment material. Through these improvements, the aim of a handheld monitoring system for the winegrower has come a step closer.

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SOLVING THE PHEROMONE MATING DISRUPTION PUZZLE: WHERE IS THE CONFUSION?

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There is a lot of confusion in the insect world that surrounds our vegetables, fruit trees and forests. However, sexual confusion by mating disruption still cannot be relied on as a single control measure. Control tends to break down at high population densities due to the existence of many natural pheromone sources. The efficacy of pheromone mating disruption also depends on the migration of mated females into the area under management, the longevity of the disruptant formulation, environmental factors and implementation of a successful integrated pest management (IPM) programme. Maintaining a sustainable confusion through a constant pheromone release rate over time is not an easy task. Sometimes, the confusion created may only be temporary. It can vary from a few days, until the charged wax powder of female pheromones electrostatically loaded onto male insects is rubbed away, to a few weeks before the pheromone microcapsules are degraded or displaced (1). In response to mating disruption pressure, some insect species such as almond moth, *Ephestia cautella*, may display evasive behavior by increasing pheromone production while some other species may even modify their pheromone composition leading to chemo-speciation (2). Our contradictory field results suggest a difference in olfactory responses between the two generations of peach twig borer, *Anarsia lineatella,* populations. Nevertheless, there are species like Oriental fruit moth, *Grapholita molesta*, which is highly sensitive to pheromones and more susceptible to mating disruption compared with other tortricid species. To summarize, the factors affecting the practical implementation of mating disruption will be reviewed.

References

1. Journal of Economic Entomology. 2005. Vol. 98: 1248–1258.
2. Journal of Stored Products Research. 2001. Vol. 37: 237–252.

Poster Session

Symposium 1. Plant–Animal–Animal Interactions

CHEMICAL CONSTRAINT OF SOLITARY BEES IN HOST–PLANT SPECIALIZATION

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Different pollen foraging strategies have been described among bees. Some taxa display floral specificity, restricting their flower visits to closely related plant taxa (pollen specialists, i.e. oligolecty) while other bee species are more opportunistic, exploiting a wide range of different flowers (pollen generalists, i. e. polylecty). Oligolecty can be linked to various adaptations as host recognition, phenology, morphology and / or behaviour displayed by females to enhance the uptake of pollen. As bees, and especially oligoletic bees, show high requirements of pollen and high efficiency in pollen removal, host plants should balance their attraction for pollinators by some restrictions in pollen availability or quality. Various morphological traits of flower have been identified as limitation of excessive pollen losses to bees. The hypothesis of pollen chemical protection remains unexplored. Preliminary studies have shown that variability in essential nutrients of pollen is high among plants. Pollen does not represent an universally exploitable resource. Physiological adaptations to plant chemistry could be the ultimate adaptation of oligolectic bees to their host plant. We study the oligolectic bee *Andrena vaga* (Andrenidae) and its host-plant, the genus *Salix* (Salicaceae). Our results show that in addition to common plant sterols, this bee species requires erythrodiol (a natural triterpenoid), specific to *Salix* pollen; as well as an optimum level of protein, limiting its range of suitable host plants. Pollen chemistry clearly influences bees ecology.

CHEMOTAXONOMICAL RELATIONSHIPS WITHIN THE *CERATITIS* FAR COMPLEX (*C. FASCIVENTRIS, C. ANONAE* AND *C. ROSA*) BASED ON PHEROMONE ANALYSIS

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Recent investigations show that taxonomic relationships in African *Ceratitis* species *Ceratitis rosa,* C. *fasciventris and C. anonae* (African *Ceratitis* FAR complex) are quite complex. Though these species have not yet been reported outside the African continent (except La Réunion and Mauritius), they are potentially invasive and are considered as species of quarantine significance (EPPO/CABI 1997). Therefore it is important to clarify the taxonomical relationships and to develop species-specific identification markers. Chemical communication during mating is highly sex specific and it represents one of the most important species-specific reproduction barriers. Involved are chemical signals that mediate long-range chemical communication (operating via sense of smell) – pheromones, as well as short-range chemical signals (operating via sense of taste) – cuticular hydrocarbons (CHC). Long-range pheromones bring mating partners together, contact hydrocarbons function as another species and gender specific identification cues. Due to their species-specificity pheromones and cuticular hydrocarbons can provide relevant cues for taxonomical classification. We present the comparative data of sex pheromones in *Ceratitis* FAR complex obtained using GCxGC-TOFMS, GC-EAD techniques.

FACULTATIVE SOCIAL PARASITES IN PAPER WASPS OVERCOME HOST DETECTION BY OVERMARKING

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Social insect colonies contain attractive resources. Colony members defend their colony integrity and exclude parasites by discriminating between the cuticular chemical profiles of nestmates and non-nestmates. Obligate social parasites trick their hosts about their chemical identity via chemical insignificance and / or mimicry of cuticular profiles (Bagnères & Lorenzi 2010). With few exceptions (e. g., Lorenzi et al., 2007, 2011), what facultative social parasites do to trick their hosts is largely unexplored. We analyzed the chemical strategies of integration in *Polistes nimphus* facultative social-parasites of paper wasps by simulating nest usurpation in the lab. Before host-brood emergence, we cut each paper-nest into two parts and assigned a half-nest to the original foundress and the other half to a facultative social parasite. After four days in the half nests, we removed foundresses and social parasites from their half-nests. When host workers emerged from half-nests, we tested their ability to discriminate between their foundress and their usurper and then we analyzed the chemical profiles of foundresses, facultative social parasites and their half-nests. Behavioral tests and chemical analyses on the two half-nests document that facultative social parasites trick their hosts successfully about their chemical identity and particularly by overmarking. Facultative social parasites deposit their own odors on nest paper, where there are already the odors of original foundresses. Emerging workers use the odors deposited on nest paper as a reference to recognize colony members. Therefore host workers in parasitized half-nests learn multiple odors and accept their foundress and their facultative social parasite as colony members. Our results highlight that facultative social parasites get tolerance and integration in host colonies by means of overmarking, a chemical strategy common in species other than social insects but rarely used by obligate social parasites.

References

1. Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology. 2010. Cambridge Univ. Press. Eds GJ Blomquist & A-G Bagnères. Chapter. Vol. 14: 282–324.
2. Biological Journal of the Linnean Society. 2007. Vol. 91: 505–512.
3. Animal Behaviour. 2011. Vol. 82: 1149–1157.

IN THE SEARCH OF A SEXUAL CONTACT PHEROMONE IN THE HAEMATOPHAGOUS BUG *RHODNIUS PROLIXUS* (HETEROPTERA, REDUVIIDAE)

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Insect epicuticular lipids (EL) are usually involved in intraspecific chemical communication, as contact pheromones for mate recognition. Reports on the lipid composition of the cuticle of several triatomine species have focused on the characterisation of cuticular hydrocarbons. Changes in hydrocarbon profiles of the cuticle during development were reported for *Triatoma infestans* and *Rhodnius prolixus* (1, 2). Differences in lipid cuticle composition were observed between sexes for *T. infestans.* However, at present no sexual dimorphism was reported for *R. prolixus.* Our work focused on the role of EL in the mating behaviour of *R. prolixus*. Our hypothesis is that the mating behaviour is mediated by EL through contact chemoreception. EL dichloromethane (DCM) extracts of the hydrocarbon and non-hydrocarbon profiles of immature and adult bugs were analysed by GC-MS. Also, several EL fractions of increasing polarity were then obtained. Behavioural assays were conducted to analyze the sexual behaviour of males when confronted to females under different treatment cond225itions. Frequencies of copula were recorded for all treatments. We tested whether males distinguish between intact females (dead or alive) and EL-washed females (with DCM) that were painted with 1 female equivalent of EL from both, adult or 5th instar larvae, females or males. The behavioural analyses showed that copula occurred in presence of alive or dead females, the latter either intact or female-painted. The chemical analysis revealed that dichloromethane EL extracts showed differences in the hydrocarbon profile between immature and adult insects, but not between males and females. However, we found that non-hydrocarbon EL were different between males and females (adult and immature), which may explain the gender-related differences found on the male behaviour. Besides, fraction 1 corresponds to male and female hydrocarbon fraction and, as expected, no differences in mating behaviour were found when females were painted with these fractions. However, the chemical analyses of fraction 5, which corresponds to non-hydrocarbon compounds, showed a sexual dimorphism. In this fraction a greater proportion of one compound was found for *R. prolixus* males. In behavioural assays, females painted with this fraction prevent copula. The hypothesis that emerges from these results is that the male of *R. prolixus* could recognize the female by a differential proportion of a non-hydrocarbon epicuticular lipid compound (present in fraction 5) during the copulatory attempt. The knowledge of the mechanisms underlying communication, involved in sexual behaviour, might help in the development of new tools useful to control vectorial transmission.

Acknowledgements

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References

1. Comp. Biochem. Physiol. 1985. Vol. 8211(4): 793–803.
2. Comparative Biochemistry and Physiology Part B. 2001. Vol. 129: 733–746.

CHEMICAL SIGNATURE OVERTIME AND AGGRESSIVE BEHAVIOR OF TWO TERMITE SPECIES IN CONTACT WITH ALIEN HYDROCARBONS

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The role played by cuticular hydrocarbons (CHCs) in insect chemical communication is well known. From prey-predator or plant-insect interactions to sexual courtship through kin and specific recognitions, CHCs are major components. In social insects, they are particularly involved in recognition and social organization. Presence or absence of CHCs in near environment can be used to assess potential presence of predators or competitors which can influence future colonization strategies of insect societies by modifying caste ratios (1).

We studied the impact of the presence of CHCs in near environment on social organization of two *Reticulitermes* termites (*R. grassei* and *R. flavipes*). To do so, we extracted CHCs of: (i) a predator ant (*Lasius niger*), (ii) workers and (iii) soldiers of the two termite species. Solvent only was used as control. CHCs were transferred on a filter paper and added into termite nests with nutritive filter paper disks. 350 workers per nest were reared during 2 months in those conditions and 20 individuals were extracted every 2 weeks to survey chemical signature overtime. After 2 months, 50 individuals were used in several behavior tests to assess their aggressiveness. Preliminary results suggest that the presence of CHCs in near environment did influence their aggressiveness.

References

1. Nature. 1996. Vol. 379: 630–631.

FILLING DYNAMICS OF THE BRINDLEY'S GLANDS OF THE HAEMATOPHAGOUS BUG *TRIATOMA INFESTANS* (HEMIPTERA, REDUVIIDAE) AND ASSOCIATED BEHAVIOURAL RESPONSES

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When adults of *Triatoma infestans* are mechanically disturbed, the Brindley's glands emit volatile compounds that are postulated to function as alarm pheromones, and trigger escaping behaviour in the larvae. In this study we report the filling dynamics of the Brindley's glands, and determined whether the capacity to release the secretion is associated with feeding. In addition, we studied the larval behavioural response in relation to the degree of filling of the glands. Adults were disturbed weekly and either fed or left unfed, and their glands were dissected at different time intervals to analyze their main secretion component (isobutyric acid) by gas chromatography. For the behavioural response of larvae, a double choice olfactometer was used to asses their orientation with respect to volatiles released by disturbed adults with varying degrees of fasting. Unfed disturbed adults were not able to refill their glands, while fed adults recharged them (gland weight: empty 1.7 mg; full 11.3 mg). When the larvae were exposed to odours from adults that had been disturbed weekly for three weeks, they did not show escaping behaviour when volatiles from unfed adults were tested, whereas significant escaping response against odours from fed adults was observed. Taking as an indicator the isobutyric acid content, the filling dynamics of the glands depended on feeding. The recharge of the glands after disturbance occurred gradually and decreased after successive disturbance events. The fasting degree of adults would limit the recharge capacity of Brindley's glands for an eventual release of the secretion to a new disturbance event that would be reflected in the escape behaviour of larvae.

Acknowledgements

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PHENOTYPIC PLASTICITY IN *PHRATORA VULGATISSIMA*: THE WILLOW SPECIES AFFECTS ODOUR OF BEETLES AND BEETLE ORIENTATION TO HOST PLANT CUES

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Knowledge on the phenotypic plasticity in infochemical use by herbivorous insects is essential for understanding plant - insect interactions. *Phratora vulgatissima,* a main pest insect in willow plantations, is thought to use a combination of volatile cues from conspecifics and from host plants for aggregation. Here we studied whether previous host plant experience affects (A) the volatiles emitted by the beetles as well as the electrophysiological antennal response to these conspecific cues and (B) the behavioural (olfactory, feeding, and oviposition) response of the beetles to host plants.

Methods. Beetles that spent their larval development on *Salix viminalis* were fed during the adult stage either with *S. viminalis* (V-beetles) or with *S. dasyclados* (D-beetles). (A) Volatiles released by V-beetles or D-beetles were sampled and analysed by solid phase microextraction (SPME) and gas chromatography coupled with mass spectrometry (GC-MS). Antennal sensitivity of *P. vulgatissima* towards beetle volatiles was determined *via* electroantennography (EAG). (B) The beetles' olfactory preference for host plant odours was tested in a dual choice test with *S. viminalis* and *S. dasyclados* leaves as odour sources or with green leaf volatiles (GLVs) in the natural ratios of these plants. Feeding and oviposition preferences were investigated in a dual choice test with *S. viminalis* and *S. dasyclados* twigs.

Results. (A) The odour of beetles was composed of saturated, short chain carboxylic acids (C6-C9), their aldehydes (C7+C9), benzaldehyde and salicylaldehyde. Salicylaldehyde was released only by D-beetles. The beetles' antennae responded to carboxylic acids and aldehydes. Females were more sensitive for nonanoic acid and nonanal than males. (B) V-females did not differentiate between the odour of *S. viminalis* and *S. dasyclados* leaves or between the odours representing the different GLV-ratios of these plants. D-females preferred the odour of *S. dasyclados* leaves and the bouquet representing the natural GLV-ratio of this species over the odour of *S. viminalis* and its GLV-ratio. Both D- and V-females showed a feeding (and tentatively an oviposition) preference for *S. viminalis* over *S. dasyclados*.

In conclusion, both the odour emitted by *P. vulgatissima* beetles as well as the beetles' behavioural response to host plant volatiles showed phenotypic plasticity and were dependent on the willow species experienced during the adult stage. In contrast, the beetles preferred the host plant used as larval diet in feeding and oviposition assays, irrespective of adult host plant experience.

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RESPONSE OF FEMALE MELON FLY, *BACTROCERA CUCURBITAE* (DIPTERA, TEPHRITIDAE) TO VOLATILES OF DIFFERENT CUCURBIT HOST PLANTS

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Bactrocera cucurbitae (Diptera, Tephritidae) is one of the major pest for cucurbits (cucumber, zucchini, melon, etc.) and can cause nearly 90% production losses on Cucurbitaceae crops in La Réunion.The objective of the present study was to improve our knowledge of olfactory stimuli used by females of *B. cucurbitae* to locate cucurbit host plants. We firstly compared different laboratory devices (a wind tunnel, olfactometers with one or four arms and small test cages) for assessing fly responses to different host plants. In the wind tunnel, females showed no orientated response in the presence of host odours. By contrast, positive response in small test cages and olfactometers was recorded. This allowed us to measure the relative attractiveness of the odours of different wild and cultivated cucurbitaceae for *B. cucurbitae* females. In parallel, the volatile composition of the odours of fruit and flowers of these different cucurbits was characterised using headspace-solid phase microextraction (SPME)/gas chromatography-mass spectrometry (GC-MS). This study should allow us to assess the host preferences of the Melon fly and eventually identify some powerful kairomonal attractants for this pest.

GROWTH RESPONSES IN THE LEPIDOPTERAN HERBIVORE *PIERIS BRASSICAE* FED WITH BLACK MUSTARD PLANT (*BRASSICA NIGRA*) FUMIGATED WITH OZONE OF VARYING CONCENTRATION

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Introduction. Concentrations of gaseous ozone present in the lower atmosphere are increasing mainly due to anthropogenic activities. Ozone, due to its strong oxidizing potential is capable of reacting with virtually any bio-macromolecules (Kelly et al., 1995). It degrades in the apoplast to form reactive oxygen species (ROS) which causes oxidative stress and activate several signal transduction pathways resulting in lesion, cell deaths, decreased photosynthetic activity and impaired fecundity (Guidi et al., 2009). Exposure to abiotic stressors like ozone are known to affect the emission of BVOCs from plants (Holopainen and Gershenzon, 2010) and this modification of BVOC emission may affect animal behavior, plant fitness and more importantly changes in the biological community associated with plants (Whitham et al., 2006). This is of particular concern because climate change may exacerbate ozone formation (Fuhrer, 2009, The Royal Society, 2008). In this experiment we studied the effects of ozone exposure on *Pieris brassicae* larvae growth and development. Results showed a negative effect of elevated ozone exposure on growth and survival of the larvae. We further investigated whether the negative effects of ozone on larval development were due to the direct effects of ozone on the larvae or due to ozone indirectly affecting larval performance through oxidative stress to the plant. To test the indirect effect we conducted a second experiment.

Methods. Four weeks old *Brassica nigra* plants grown in greenhouse conditions, were fumigated in plant growth chambers (Weiss Bio 1300) – that have been adapted so that each can have an independently regulated ozone concentration – for five consecutive days. In the first experiment, in order to check the direct effect of ozone exposure, a total of 290 *Pieris brassicae* larvae were divided among three treatments and reared on *B. nigra* plants under three different ozone concentrations (Ambient, 70 ppb and 120 ppb). The larvae were weighed and mortality noted. In the second experiment, 450 *Pieris brassicae* larvae were divided among treatments and again reared on *B. nigra* plants fumigated at the same three ozone concentrations. However, instead of rearing the larvae under the different ozone concentrations, the plants were removed from chambers after five days of ozone exposure and rearing was done in a controlled conditions insect rearing room. The larvae were weighed every second and third day. The number of resulting pupae, and the time to pupation were noted.

Result. The mean weight and survival of the larvae reared on *B. nigra* plants fumigated with elevated ozone concentrations were lower in both the experiments. In the second experiment, the development of pupae from larvae, that had been fed *B. nigra* plants fumigated with elevated ozone concentrations were slower and reduced.

Conclusion. In the first experiment the effect in terms of mortality and growth was clearly visible in larvae exposed to the highest ozone concentration (120 ppb). In the second experiment, the mortality and growth of the larvae was affected by both elevated ozone concentrations (70 ppb and 120 ppb). In general, the rearing of the *P. brassicae* larvae with ozone treated *B. nigra* plants resulted in reduced mean weight of the larvae and slower pupae development. In nature, this delay in pupation may have profound negative effects on larval populations.

Acknowledgements

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FOLIAR MONOTERPENE EMISSION PROFILES IN CORK OAK INFESTED BY *CERAMBYX WELENSII* KÜSTER (COLEOPTERA: CERAMBYCIDAE)

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Cerambyx welensii (Coleoptera: Cerambycidae) can be considered the most important xylophagous species on *Quercus ilex* and *Quercus suber* in western Andalusia (Spain). The feeding activity of larvae weakens tree branches and trunks, and facilitates their falling during storm days; furthermore, adults of this species can act as vectors of both pathogenous and wood-rotting fungi. The pattern of host-plant selection by this cerambycid is unknown, but recent studies using EAG have shown the ability of *C. welensii* to detect a wide spectrum of VOC, especially the main constitutive foliar monoterpenes characteristic of *Q. suber* and *Q. ilex* emissions. *Q. suber* is actually considered a strong monoterpene emitter (mainly α-pinene, β-pinene, limonene, sabinene and myrcene). Its monoterpene emission pattern shows intraspecific variation at the population level. Two emission profiles (or "emission signatures") have been proposed which are characterized by the major monoterpenes emitted from individual *Q. suber*: (1) pinene-type (α-, β-pinene plus sabinene emitted mainly) and (2) limonene-type (limonene emitted mainly). This work is part of a larger project in progress aiming to determine *Q. suber* volatiles that are used by *C. welensii* for host selection. We present the results of a study that compared monoterpene emissions of highly infested trees and uninfested trees. A dynamic headspace procedure was used, followed by GC-FID analysis. The main monoterpenes detected were α-pinene, β-pinene, limonene and sabinene; α-phellandrene, ocimene, cineol, ρ-cymene and γ-terpinene were detected in lower quantities. Neither absolute emission levels nor emission percentages of the main monoterpenes were significantly different between infested and non-infested trees. The major emission signature detected was the limonene-type (25 of 40 trees); the number of trees showing this signature in the infested group was significantly higher than in the group of non-infested trees. Future studies need to elucidate how *C. welensii* responds behaviourally and physiologically to the odour of the limonene-type signature of *Q. suber*.

INTERSPECIFIC HYBRIDIZATION OF *EUCALYPTUS* SPP. AS A SOURCE OF BEHAVIORALLY ACTIVE COMPOUNDS AND THEIR ENANTIOSELECTIVITY AGAINST *AEDES ALBOPICTUS*

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Dengue and yellow fever are human viral diseases transmitted by mosquitoes, principally by *Aedes aegypti* (L.) and *A. albopictus* (Skuse) which are endemic in many tropical regions, including Latin America. Natural products such as volatiles from *Eucalyptus* essential oil may act as strong mosquito repellents. Previous studies of our research group have demonstrated that some terpene compounds in *Eucalyptus* essential oil elicit an enantioselective electrophysiological response by *Aedes aegypti* antennae (1). The interspecific hybridization in *Eucalyptus* species is a useful tool that allows improving the chemical composition of the essential oil and its enrichment with more active compounds (2). Essential oils from *Eucalyptus* hybrids were obtained by steam hydro distillation and analyzed by GC-MS using a chiral stationary phase. The essential oil was also fractionated by RP-HPLC with a photodiode array detector. The aqueous fractions obtained were partitioned with hexane, concentrated and analyzed by GC-MS. All essential oils and extracts were analyzed for their electrophysiological activity. Gas chromatography coupled to electroantennography detection (GC-EAD) was performed by using the same chiral stationary phase as used for the GC-MS analyses. Potential bioactive compounds which elicited an enantioselective electrophysiological response by *A. albopictus* antennae are reported here.

Acknowledgements

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References

1. First Latin American Meeting of Chemical Ecology P-29: 122.
2. Entomologia experimentalis et applicata. Vol. 129(1): 107–114.

EVALUATION OF PLANT VOLATILE COMPOUNDS AS FEEDING ATTRACTANTS FOR *AEDES AEGYPTI*

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Aedes aegypti is a well-known vector of dengue fever, yellow fever and other diseases. The role of the hematophagous mosquito females in pathogen transmission has been widely acknowledged. Most current attractants, used in lures for monitoring and controlling the mosquito population are based on human host odour with carbon dioxide. However, this odour blend fails to attract male and non-blood seeking female mosquitoes.

Nectar feeding has been observed in males and females throughout the gonotrophic cycle. Although it was reported that plants and volatile emitting sugar sources attract mosquitoes into field traps, little is known about the attractiveness of single plant volatiles or volatile blends. The aim of this study is to gain a better understanding of the role of major plant volatile compounds involved in the mosquitoes' attraction.

The behavioural response of mosquitoes to plant volatiles was tested in a two-choice behavioural assay. The bait of volatile compounds from a whole plant and 10% sucrose solution versus the plain sucrose were offered for feeding. The plants which volatiles were preferred by *A. aegypti* over the plain sucrose were *Lobularia maritima* and *Plectranthus neochilus*. Volatile compounds of those plants were identified by SPME-GC-MS.

The identification of volatile compounds, which are potentially involved in the nectar seeking behaviour of *Aedes aegypti*, may be valuable in increasing the effectiveness of mosquito population monitoring and control.

FALL AND BEET ARMYWORM DIFFERENTIALLY AFFECT EMISSIONS OF MAIZE VOLATILES

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Plants employ a broad spectrum of inducible defence mechanisms to combat their enemies. Direct defences, such as the production of toxic compounds, have immediate negative effects on insect herbivores. Plants may also protect themselves indirectly by means of herbivore-induced volatile organic compounds (VOCs) that attract natural enemies of these herbivores. Yet, not all herbivores are totally at the mercy of plant defence mechanisms. Some have adapted to resist plant defences and may even manipulate the physiology of their host plant to their own benefit.

This study aims to examine to which extent indirect defences in maize (*Zea mays* ssp. *mays*), domesticated in Mexico, are affected by two leaf-chewing herbivores that co-occur in Mexico, fall armyworm (*Spodoptera frugiperda*) and beet armyworm (*S. exigua*). Both are frequently reported to feed on maize plants, but while fall armyworm is well-known as a major pest, beet armyworm is not. In order to obtain information about the possible importance of inducible VOCs for this difference in pest status, we profiled caterpillar-induced VOCs by GC-FID/MS analysis and studied their impacts on the attraction of parasitoid wasps, using a six-arm olfactometer.

Our results show that fall armyworm triggered the emission of considerably less VOCs than beet armyworm. Furthermore, we found that fall armyworm-infested maize plants were less attractive to the parasitoid wasp *Cotesia marginiventris*, an important natural enemy of both caterpillar species, suggesting that fall armyworm benefits from its capacity to repress host VOC induction. We are currently trying to identify the mechanism behind this phenomenon, with the aim to unravel the complexity of the interactions between plants and insects in the context of an evolutionary arms-race between plants and their pests.

TERPENE ACCUMULATION IN *PICEA ABIES* IS NEGATIVELY CORRELATED TO COLONIZATION OF *IPS TYPOGRAPHUS*

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Tree-killing bark beetles (Coleoptera, Scolytinae) are among the most economically and ecologically important forest pests in the northern hemisphere. Induction of terpenoid-based oleoresin has long been considered important in conifer defense against bark beetles, but it has been difficult to demonstrate a direct correlation between terpene levels and resistance to bark beetle colonization. Improved knowledge about tree defenses is needed to understand how ecological disturbances influence interactions between insects and their host trees. To test for inhibitory effects of induced terpenes on colonization by the spruce bark beetle *Ips typographus* (L.) we inoculated 20 mature Norway spruce *Picea abies* (L.) Karsten trees with a virulent fungus associated with the beetle, *Ceratocystis polonica* (Siem.) C. Moreau, and investigated induced terpene levels and beetle colonization in the bark. Fungal inoculation induced very strong and highly variable terpene accumulation 35 days after inoculation, and induced terpene strongly inhibited the colonization of *I. typographus*. The seven trees with the highest induced terpene levels had only 4.9% as many beetle attacks and 2.6% as much gallery length as the six trees with the lowest terpene levels. The relationship between induced terpene levels and beetle colonization was not linear but thresholded: above a low threshold concentration of ~100 mg terpene g^{-1} dry phloem trees suffered only moderate beetle colonization, and above a high threshold of ~200 mg terpene g^{-1} dry phloem trees were virtually unattacked. This is the first study demonstrating a dose-dependent relationship between induced terpenes and tree resistance to bark beetle colonization under field conditions, indicating that terpene induction may be instrumental in tree resistance.

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FERTILIZATION AND DEFOLIATION EFFECTS ON TOLERANCE, INDUCED RESISTANCE TO *ORMISCODES* SP. (LEP. SATURNIIDAE), AND FOLIAR CHEMISTRY IN TWO SOUTHERN BEECH SPECIES (*NOTHOFAGUS ALPINA* AND *N. OBLIQUA*)

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Plant phenotypic plasticity in the expression of defoliation induced responses could be modulated by resource availability and allocation trade-offs. How soil fertility level may affect the expression of defoliation induced responses to herbivore insects in trees of southern temperate forests and their native insects remains largely unexplored. In this study, it was determined that *Nothofagus obliqua* expressed defoliation induced resistance to its native outbreak defoliator *Ormiscodes* sp. in low nutrient treatments, it expressed no resistance at medium fertility level, and it expressed induced susceptibility in high fertility treatments. The defoliation treatment induced an increase in foliar concentration of alpha-agarofuran terpenoid, across fertility levels. However, defoliation induced a decrease in foliar phenylpropanoids only in tress with high fertility treatments. These effects were species specific, as *N. alpina* did not express induced resistance to this insect. Conversely, both *N. obliqua* and *N. alpina* expressed growth compensatory responses after the defoliation treatment, an effect that was independent of the fertilization treatment. The variation in the expression of induced resistance to a defoliator insect but not in the expression of tolerance could represent adaptive responses to different environmental challenges.

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THE EFFECT OF THE *ORYZOPHAGOUS ORYZAE* (COSTA LIMA) (COLEOPTERA; CURCULIONIDAE) HERBIVORY ON THE CHEMICAL PROFILE RELEASED BY RICE PLANTS

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Oryzophagous oryzae known as rice water weevil (RWW) is the major insect pest of flooded rice in Brazil. Adults feed on rice leaves. However, the damage to rice plants is caused primarily by root system larval pruning provoking yield looses of 10 to 18%. Aiming to minimize the pesticides application and meet a more ecological method of control, the chemical communication between RWW and rice plants is been studied to evaluate if semiochemicals from rice plants play on the attraction of insect. For this, volatiles compounds of the following treatments were collected using air-entrainment: plantlet of rice plants + 5 mL of water, as control, 40 males of *O. oryzae* + 5 mL of water, 40 females of the RWW + 5 mL of water, plantlet of rice with 40 males + 5 mL of water or with females of the RWW + 5 mL of water. These treatments were placed in glass chambers of 500 mL and the volatiles were collected using Super Q, and eluted at each 24 hour, during five consecutive days. The principal component analysis of the chemical profile of the treatments separate the volatiles released from undamaged plants from the volatiles released by herbivory damaged plantsThe chemical profile of damaged rice plants was composed of the following compounds: α-pinene, camphene, β-phellandrene, β-terpinene, 6-methyl-5-hepten-2-one, limonene, β-ocimene, 2-nonanone, methyl benzoate, methyl salicylate, benzothiazole, 2-undecanone, octanal, linalool, α-satalene, α-bergamotene, β-sesquiphellandrene, curcumene, α-zingiberene, β-farnesene, β-bisabolene, and five sesquiterpenoids were not identify. The compounds α-pinene, camphene, limonene, ethyl ester decanoic acid and benzothiazole were the main compounds identified on volatiles collections from males and females without the presence of rice plantlet. In addition, all monoterpenes and sesquiterpenes were released in higher amounts by herbivory damaged plants when compared to undamaged plants. Bioassays are being conducted to evaluate if RWW use these volatiles as cues to find their host.

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INFLUENCE OF NATURALLY OCCURRING PLANT ACTIVATOR IN THE SEARCHING BEHAVIOUR OF THE EGG PARASITOID *TELENOMUS PODISI*

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It is known that salicylic acid (SA) and jasmonic acid (JA) signalling pathways interact antagonistically and that SA can suppress JA-dependent defense responses (1). The aim of this study was to evaluate whether naturally occurring plant activator changes the volatile organic compounds (VOC) profile of soybean (cv Dowling) and modify the searching behavior of egg parasitoid *Telenomus podisi*. Soybean plants in the V3 stage were subjected to spray treatments, manually applied over all aerial parts of the plant. The activator treated plants were used in bioassays with 72, 96, 120 or 144 h after spraying and the volatile emitted by these plants were used as odour source in a double choice olfactometer (Y type), to evaluate their influence on foraging behavior of *T. podisi*. The following spray treatments were used: (1) distilled water (control), (2) Tween 20 (0.1% in distilled water), (3) methyl salicylate, (4) methyl jasmonate, (5) *cis*-jasmone, (6) methyl salicylate+methyl jasmonate at concentration of 1.4 mmol l^{-1}, solubilized in distilled water by Tween-20 (0.1% vol/vol in distilled water, added prior to the compounds), and (7) plants induced by *Euschistus heros* herbivory for 96 h. The first choice of egg parasitoid was preferentially to plants sprayed by: methyl salicylate 72 and 96 h after treatment; methyl jasmonate 96 h; *cis*-jasmone 96 h; methyl salicylate + methyl jasmonate 72 h and herbivory 96 h. The parasitoid remained more time in the arm with these treatments, however spent less time in the combination methyl salicylate + methyl jasmonate, showing that the odor attracted the *T. podisi*, but it was not sufficient to retain it on the treatment arm. Thus, the tested compounds appear to induce defense pathways in soybean cv Dowling similar to those induced by stink bug feeding damage. The results suggest that the methyl salicylate + methyl jasmonate treated plants produce a different chemical profile of volatiles from the plants treated with individual compounds, since plants sprayed with the methyl jasmonate attracted the parasitoid 96 h after treatment and when volatiles from methyl salicylate + methyl jasmonate treated plants was tested, *T. podisi* responded only to 72 h after treatment and it was not arrested. Chemical analysis of the volatiles collections are being conducted to evaluate if methyl salicylate and methyl jasmonate plays on the metabolic pathways of soybean plants.

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References

1. Plant Physiology. 2008. Vol. 147: 1358–1368.

HERBIVORY INDUCED DEFENSES MEDIATE SPECIFIC INTERACTION IN THE TRI-TROPHIC SYSTEM SOYBEAN – STINK BUGS (PENTATOMIDAE) – EGG PARASITOIDS (SCELIONIDAE)

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In this work the tri-trophic interactions mediated by semiochemicals in the system soybean-stink bug-egg parasitoids were studied. The study focused on the evaluation of: (1) the qualitative and quantitative composition of volatiles released by plants after herbivory by the stink bugs *Euschistus heros* (Fabricius) and *Nezara viridula* (L.); (2) the response of the parasitoids *Telenomus podisi* (Ashmead) and *Trissolcus basalis* (Wollaston) to the volatiles of soybean plants at different times after injury by *E. heros* and *N. viridula* and (3) the relation between the parasitoid response to the volatiles induced by the injury by the stink bugs *E. heros* and *N. viridula* with the host preference of the parasitoids. The chemical composition of the volatiles mixtures released by soybean plants after the injury by the stink bugs *E. heros*, *N. viridula* or both showed quantitative and qualitative differences and were also different when compared to plants without injury (control). The compound (Z) 3-hexen-1-ol was most related to the treatment with both stink bugs, *E. heros* + *N. viridula*; limonene and α-farnesene to the treatments with *E. heros* and *N. viridula* and 6-methyl-5-hepten-2-one was associated to the control plants. Olfactometer bioassays with plant volatiles from the different treatments showed that the parasitoid *Te. podisi* had a selective response to the plants injured by their preferential host, *E. heros*. The parasitoid *Tr. basalis* did not discriminate between volatiles induced by the injury of the two stink bugs. The results suggest that the composition of the guild of herbivorous stink bugs that attack soybeans could influence the host searching behavior of the egg parasitoids by inducing differential defenses.

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SEMIOCHEMICALS FROM HERBIVORY INDUCED COTTON PLANTS INFLUENCING THE FORAGING BEHAVIOUR OF THE COTTON BOLL WEEVIL

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Anthonomus grandis Boheman (Coleoptera: Curculionidae) is the main pest on cotton crops being responsible for millions of tons of pesticides used for their control every season around the world including Brazil. In an attempt to minimize the abusive use of insecticide in cotton crops the MIP is encouraging the use of the aggregation pheromone for monitoring the populations. However, when the plants start to produce the reproductive structures, trap capture of males and females, *A. grandis*, diminished substantially because the pest prefers to go directly to the plants. Several studies have proposed that volatile compounds emitted by reproductive cotton plants are responsible for this attraction. However, a blend of volatiles that could be the responsible for this attraction is not known. Therefore, the aim of this work was to identify the volatiles released from cotton plants when damaged by different pest species and to elucidate the effect of these volatiles on the *A. grandis* behaviour. Cotton plants damaged by herbivory by *A. grandis*, *Euschistus heros* and *Spodoptera frugiperda* were considered for this study. The chemical analysis of air-entrainment extracts from cotton plants subjected to different treatments (undamaged plants, mechanically damaged plants, and *E. heros*, *A. grandi*s and *S. frugiperda* herbivory damaged plants) were carried out. The results showed that the differently treated cotton plants released different chemical profiles. In addition, bioassays conducted by using a "Y" tube olfactometer showed that *A. grandis* did not respond to volatiles emitted by cotton plants damaged by *E. heros* or *S. frugiperda* when contrasted to hexane, but responded to volatiles from herbivory damaged cotton plants when damaged by conspecifics. The main volatiles responsible to separate the attractive cotton plants from the other treatments were: α-pinene, β-pinene, β-myrcene, (*Z*)-3-hexenyl acetate, β-ocimene, DMNT and TMTT. These compounds were released in significantly higher amounts by the attractive plants and thus, might be involved in the attraction of *A. grandis*.

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ENHANCED PHENOLIC ACID CONTENTS IN PEST INFESTED CASTOR BEAN, *RICINUS COMMUNIS* PLANTS

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Phenolic compounds have been known to occur in all plants investigated so far either constitutively, or formed in response to pest or pathogen stress and associated as part of an active defense response in the host. Qualitative and quantitative estimation of phenolic compounds was done through high-performance liquid chromatography (HPLC) in leaves of castor plant, *Ricinus communis* L. after feeding by a few major insect pests, such as the borer pest, *Dichocrosis punctiferalis* (Guenee), the leaf feeders, *Spodoptera litura* L. and *Achaea janata* L., Major phenolic compounds detected by HPLC were vanillic acid, syringic acid, cinnamic acid and p-coumaric acids. Synthesis of phenolic compounds was enhanced in all the pest infested plants compared with non-infested plants. Insect antifeedant assays were performed using the above phenolic acids to determine their effects on insect pests. The enhanced levels of phenolic compounds of the plant altered the activity of the detoxifying enzymes like esterase, β-glucosidase, and glutathione-S-transferase in the larval midgut. Insects showed enhanced activity of these enzymes to cope with the toxic nature of plant phenolics. This study supports the role of phenolic compounds in plant defense through their impact on the feeding herbivore.

SELENIUM CONTAMINATION AFFECTS HONEY BEE FEEDING, BEHAVIOR, AND SURVIVAL

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Selenium (Se) has contaminated soils and plants in areas where pollination can be critical to the functioning of both agricultural and natural ecosystems, yet we know very little about how pollutants can impact insect pollinators. Se can be biotransferred and biomagnified throughout the food web, and has negative impacts on plants and the herbivores that feed on them. The overall goal of our studies was to investigate both the sublethal and lethal effects of a plant-accumulated pollutant on a common insect pollinator. Using a plant species known to accumulate Se in the pollen and nectar, we conducted a two-year field study examining the pollination ecology of a common, weedy plant (radish, *Raphanus sativus* L.) and its pollinator, the honey bee (*Apis mellifera* L.), by quantifying floral traits, pollen limitation, as well as honey bee foraging visits. Se-treated plants remained attractive to honey bees and were not pollen limited. In laboratory studies, honey bee foragers and larvae were fed Se in artificial diet and survival was quantified. Inorganic forms of Se were most toxic to foragers, whereas selenomethionine was only toxic at the highest concentrations. Given that pollinators such as the honey bee do not avoid Se compounds in the plant tissues they are foraging on, they will suffer similar adverse effects as seen in other insect guilds. Our study sheds light on how plant-accumulated pollutants such as Se can impact pollinators through a lack of recognition and / or modification in foraging behaviors that result in reduced survival.

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References

1. Environmental and Experimental Botany. 2011. Vol. 74: 90–97.
2. PLoS One. 2012. e34137.

LOCALLY DISTINCT VOLATILE EMISSION FROM HERBIVORE-DAMAGED POPLAR AND ITS IMPLICATION FOR INDIRECT TREE DEFENSE

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After local herbivore damage, plants often emit volatiles systematically from nearby undamaged organs. Such systemic emission is usually reported as being qualitatively similar but lower in intensity to that from locally damaged, but systemic vs. local emission patterns have not been well described in most species. We investigated local and systemic volatile emission in *Populus nigra* (black poplar) after damage by *Lymantria dispar* (gypsy moth) and *Phyllodecta vulgatissima* (blue willow beetle) under field and laboratory conditions and tested the effect of the odour blends on the behaviour of the braconid parasitic wasp *Glyptapanteles liparidis*. While terpenes are abundant in both local and systemic blends, nitrogen containing compounds (oximes and nitriles) and green leaf volatiles are only found as locally released volatiles. Moreover, the parasitoid clearly orients towards the herbivore-induced local poplar odour, rather than the systemic odour in an olfactometer experiment, and thus should be readily able to locate its lepidopteran hosts. Locally distinct expression patterns were supported by qRT-PCR data of three terpene synthases. The distinct volatile signatures of local vs. systemic *P. nigra* foliage may have an important role in the attraction of herbivore enemies.

HOST-PLANT VOLATILES ATTRACT ADULT FEMALES OF THE BLACK-BANDED OAK BORER *COROEBUS FLORENTINUS* (COLEOPTERA: BUPRESTIDAE): ELECTROPHYSIOLOGICAL AND BEHAVIORAL RESPONSES

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The black-banded oak borer (BBOB) *Coroebus florentinus* attacks the cork oak *Quercus suber* by laying eggs in groups or separately inside the bark of young and healthy branches. After eclosion, the endophyte larvae start feeding, constructing large galleries (up to 1 m). Presence of the insect is not lethal to standing trees but its damage provokes dryness and yellow leaves, and finally wounds and death of branches and shoots.

Previous studies on the BBOB had demonstrated that adult females, but not males, are attracted to volatiles and leaf extracts of *Q. suber* in behavioral bioassays (1). To identify active compounds from the host plant we performed GC-MS analyses, and electrophysiological and Y-tube olfactometer tests. Headspace volatile collections of freshly cut cork oak branches showed the presence of 32 compounds. Basically, two groups of compounds were identified, green leaf volatiles (GLVs) and isoprenoids / terpenoids (mono-, homo-, and sesquiterpenes). Antennae of both sexes displayed GC-EAD responses towards (*E*)-2-hexenal, (*E*)-2-hexenol, 1-hexanol, (*Z*)-3-hexenyl acetate, and *n*-hexyl acetate but in dual-choice experiments, only females were attracted to synthetic GLVs, particularly to (*E*)-2-hexenol, 1-hexanol, and (*Z*)-3-hexenyl acetate. The electroantennographic and behavioral activity elicited by the GLVs suggest that these compounds may play an important role as attractants in the foraging / oviposition behavior of BBOB females.

References

1. Journal of Chemical Ecology. 2012. Vol. 38: 378–388.

CHEMOECOLOGICAL INTERACTIONS BETWEEN TWO VARIETIES OF THE PURPLE WILLOW *SALIX PURPUREA* L. AND ITS PEST *PONTANIA VESICATOR* (HYMENOPTERA, TENTHREDINIDAE)

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The Purple willow *Salix purpurea* L. is a valuable species, suitable for the production of high technological quality willow withies. For the manufacture of wickerware, *Salix purpurea* L. is cultivated in short-rotation willow stands, special plantations. Requirements that willow plantations have to meet are high enough, productivity and quality being the most important. *S. purpurea* and *Salix vinimalis* L. as well as their intraspecies taxa and hybrids are the most suitable for cultivation. However, the mentioned species are very susceptible to pests and pathogen-induced diseases. Willows often suffer from insects causing galls on the leaves. One of such pest species is *Pontania vesicator* Bremi. Larvae of this pest damage *S. purpurea* leaves by forming galls, which are 2 cm in length.

Only two *S. purpurea* varieties, i. e. *S. purpurea* var. *rubra* and *S. purpurea* var. *lutea*, picked by clone selection are usually cultivated in Lithuania. These two varieties are grown together in the same plantations. However, galls caused by pest *Pontania vesicator* occur only on 'Rubra' leaves. In order to determine what influences such pest preference, we compared chromatographic profiles of *S. purpurea* var. *rubra* and *S. purpurea* var. *lutea* leaf extracts prepared by hydrodistillation. We also investigated which compounds present in leaf extracts of different willow varieties elicited responses in antennae of *P. vesicator*. Electrophysiological tests showed that the purple willow pest responds to six compounds emitted by *S. purpurea* leaves: n-hexanol, n-nonanal, β-linalool, β-citronellol, E – geraniol and eugenol. All these chemicals were present in leaf extracts of both *S. purpurea* var. *rubra* and *S. purpurea* var. *lutea*, but in different amounts. Behavioural tests examining the attractiveness of EAG-active compounds toward *P. vesicator* are in progress.

SCOTS PINE VOLATILES EMITTED BY NEEDLES FOLLOWING *DIPRION PINI* L. LARVAL OR MECHANICAL DAMAGE

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Diprion pini L. is among the major folivorous insects causing defoliation of pine, *Pinus* spp., in Europe. Pines are known to react to damage (even to potential damage) by either quantitative or qualitative modification of their of secondary metabolites' emission (Boland et al., 1999). *Pinus sylvestris* L. headspace emissions were collected using SPME and monoterpene composition was analyzed both after pine sawfly larval and mechanical damage of needles. Quantitative difference was established only. Relatively higher amount of (*E*)-beta-ocimene in the Scots pine headspace was induced by damage caused by *Diprion pini* L. larvae, compared to that recorded after mechanical damage of needles. This compound is involved in plant's indirect defense mechanisms as attractant for parasitoid wasps (Hymenoptera: Braconidae) (Sasso et al., 2009). Further studies on perception of (*E*)-beta-ocimene by and behavioral effect on *D. pini* predators are in progress.

References

1. Boland W., Koch T., Krumm T., Piel J., Jux A. 1999. Induced biosynthesis of insect semiochemicals in plants. D. J. Chadwick and J. A. Goode (eds.) Insect–Plant Interactions and Induced Plant Defence. P. 110–126.

2. Sasso R., Iodice L., Woodcock C. M., Pickett J. A., Guerrieri E. 2009. Electrophysiological and behavioural responses of *Aphidius ervi* (Hymenoptera: Braconidae) to tomato plant volatiles. Chemoecology. Vol. 19: 195–201.

Symposium 2. Chemical Ecology Meets Evolutionary and Molecular Biology

EVOLUTIONARY PATTERNS OF MALE SECONDARY SEXUAL CHARACTERS IN MYCALESINA BUTTERFLIES

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The Mycalesina (Bush brown) butterflies comprise a subtribe within the Nymphalidae family, with around 300 recognised species. Many of the species are morphologically very similar, but the males show to be involved in sexual selection (even though their function is not fully understood) we aim a wide range of secondary sexual characters of which one in particular, the androconial structures, has formed the basis of the taxonomy of the six genera within the subtribe. The speciose genus *Bicyclus* is found all over sub-Saharan Africa together with the smaller genus *Hallalesis*. *Heteropsis* is the most widespread genus – some species co-occur with the two former genera on the African mainland while most of the Afro-tropical species are part of a radiation endemic to Madagascar, and there are also a few Asian representatives. *Mycalesis* has radiated mainly in South and South-East Asia whereas *Mysodama* has radiated in South-East Asia and the Australasian region. *Lohora* is endemic to the Sulawesi and co-occurs with *Mycalesis* on the island.

The males of *Bicyclus* butterflies produce large quantities of sex pheromones used at close range during courtship (1, unpublished). Recently similar pheromone systems have been documented in *Hallalesis*, *Mycalesis* and *Mysodama* (unpublished) and the remaining genera are under investigation. Comparing *Bicyclus*, *Heteropsis*, *Mycalesis* and *Mysodama* it appears like *Bicyclus* have the most complex androconial system (structures that to some degree are linked with the chemical communication), while the other three genera have a much more complex patterning on the dorsal wing surface, a trait shown to be involved in mate choice (2). The male genitalia structure among species I also variable but here the variation appears to be more complex with no clear pattern to date. For many species-groups variation at species level is minimal while separate species group can be very different.

Since all these traits are likely to measure these traits across the range of Mycalesina butterflies and map them onto a multi-gene molecular phylogeny. By comparing correlative patterns and relative rate of evolution of these traits between genera and species-groups we hope to get a better understanding on the functional roles of these traits in the mating systems of butterflies.

Acknowledgements

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References

1. PLoS ONE. 2008. 3:e2751: 1–12.
2. Proceedings of the Royal Society B-Biological Sciences. 2009. Vol. 276: 2369–2375.

TRACKING DOWN THE DIVERGENCE OF FATTY ACID DERIVED COMPONENTS OF BUMBLEBEE MARKING PHEROMONES

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Bumblebee males use marking pheromones (MPs) produced in cephalic labial gland to attract conspecific females. The major components of MP vary dramatically among closely related *Bombus* sp.: *Bombus terrestris*, an established greenhouse pollinator, uses terpenoid compounds, whereas *B. lucorum* (both *Bombus s.s.*) uses C14-fatty acid derived ethyl esters (ethyl tetradec-9-enoate). *B. lapidarius*, representative of further related subgenus *Melanobombus*, uses exlusively C16-fatty acid derived alcohols (hexadecanol and hexadec-9-enol).

To gain more information on molecular mechanisms leading to the divergence of fatty-acid derived MPs, we have identified multiple homologs of fatty acid desaturases (FADs) present in the male cephalic labial gland and fat body of studied bumblebees. The mRNA levels of desaturases in both tissues were quantified and compared using qPCR and the FADs activities were assayed upon heterologous expression in yeast. Surprisingly, we found that substrate preferences and the expression patterns of the most abundant cephalic labial gland FADs are conserved among studied bumblebees, suggesting that the composition of fatty acid derived MPs diverged at the level of other biosynthetic steps, e. g. fatty acid reduction or esterification. The role of other FADs homologs identified using RNA-seq of cephalic labial gland cDNA library is also discussed.

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CUCKOO VERSUS PREDATORY *MACULINEA* BUTTERFLY SPECIES: DISTINCT CHEMICAL INTEGRATION STRATEGIES INSIDE THE HOST ANT COLONIES

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The insects' communication system is mainly based on chemical cues. Cuticular hydrocarbons, in particular, have been assumed to play a fundamental role in the nest-mate recognition of social insects, since individuals living in the same society share a bouquet of chemicals, which serves as a "colony odour" and enables them to discriminate between nest mates and strangers. Therefore it is clear that each organism that enters the colony to exploit its resources has to bypass this chemical recognition barrier (1).

Among the numerous insect *taxa* that live in association with social insects, lycaenid butterflies of the genus *Maculinea* van Eecke, 1915 are obligate parasites of *Myrmica* ants. The caterpillars start their life feeding on the developing buds of specific food plants, but once the fourth larval instar is reached, larvae drop to the ground where they are adopted by *Myrmica* ants. *Maculinea* butterfly complete their development during the last larval instar in an ant nest (2). The level of the parasites' integration within the host colony results from two distinct strategies. In the so-called "cuckoo" species, *Maculinea* larvae become perfectly integrated members of the colony and are actively fed by worker ants by food regurgitation (trophallaxis). In contrast, larvae of other species, known as "predator" species, actively feed on the ants' brood. In all cases, however, the larvae are attended by ants until pupation. To achieve social integration within the ant nests, the caterpillars mimic the acoustic (3) as well as the chemical signals of their host ants (4).

We present results on pre-adoption and post-adoption hydrocarbon expression obtained on larvae of a cuckoo and a predatory species of *Maculinea, M. alcon* and *M. teleius* respectively, living sympatrically and exploiting the same *Myrmica* species (i. e. *M. scabrinodis*). The study system allow us to explore the changes in chemical profiles occurring in the two parasite species and in their ant host colonies during the post-adoption phase, as well as to verify if there are any differences in the chemical adaptation to the host colony, between the "cuckoo" and the "predatory" *Maculinea* species.

Acknowledgements

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References

1. Annual Review of Entomology. 2001. Vol. 46: 573–599.
2. Oecologia. 1989. Vol. 79: 452–457.
3. Science. 2009. Vol. 323: 782–785.
4. Journal of Chemical Ecology. 2004. Vol. 30: 91–107.

CHEMOTAXONOMICAL RELATIONSHIPS WITHIN THE CERATITIS FAR COMPLEX (*C. FASCIVENTRIS, C. ANONAE* AND *C. ROSA*) BASED ON CUTICULAR HYDROCARBONS ANALYSIS

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Recent investigations show that taxonomic relationships in African *Ceratitis* species *Ceratitis rosa, C. fasciventris and C. anonae* (African *Ceratitis* FAR complex) are quite complex. Though these species have not yet been reported outside the African continent (except La Réunion and Mauritius), they are potentially invasive and are considered as species of quarantine significance (EPPO/CABI 1997). Therefore it is important to clarify the taxonomical relationships and to develop species-specific identification markers. Chemical communication during mating is highly sex specific and it represents one of the most important species-specific reproduction barriers. Involved are chemical signals that mediate long-range chemical communication (operating via sense of smell) – pheromones, as well as short-range chemical signals (operating via sense of taste) – cuticular hydrocarbons (CHC). Long-range pheromones bring mating partners together, contact hydrocarbons function as another species and gender specific identification cues. Due to their species-specificity CHCs can provide relevant cues for taxonomical classification. We present the comparative data of CHC in *Ceratitis* FAR complex obtained using GCxGC-TOFMS.

IDENTIFICATION AND SYNTHESIS OF POLAR CUTICULAR COMPOUNDS FROM THE ANT *LEPTOGENYS DISTINGUENDA* IMPLICATED IN MYRMECOPHILY

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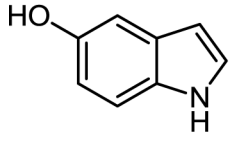
The Malaysian ponerine army ant *Leptogenys distinguenda* is a model species to investigate the interactions between myrmecophilic species like spiders and silverfish. The latter live undetected in the nest of the army ant. The symbionts mimic the chemical profile of the ant cuticle, which are covered by a lipid layer composed preferentially of hydrocarbons, to remain undetected (1).

Nevertheless, GC-MS analysis of polar extracts of the exocuticle have shown that they contain several unknown polar compounds, in addition to the already known hydrocarbons.

Preliminary results indicate that these compounds might also play a role in the interactions of these species. We were able to identify these compounds as indole derivatives like 5-hydroxyindole (**1**) and indole-3-acetic acid (**2**). The identification of the compounds will be described.



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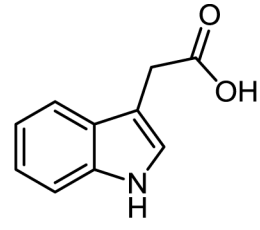

2

References

1. Witte V., Foitzik S., Hashim R., Maschwitz U., Schulz S. 2009. Journal of Chemical Ecology. Vol. 35: 355–367.

CAN "CRY WOLF" STABILIZE SYMBIOTIC RELATIONS?

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We consider a simple model of the evolution of Hervibore Induced Plant Volatiles (HIPV), which mediate tritrophic (plants-herbivores-carnivores) interactions. In an environment with plants sending alarm only when induced by herbivory, a mutant plant may gain by sending the same alarm signal even when there are no or only few herbivores on that plant, thereby receiving early protection against herbivory. Indeed, such a 'cry wolf' strategy has been identified in a Japanese variety of cabbage (1). 'Cry wolf' strategies are likely to have a tremendous impact on the communication between plants and the enemies of its herbivores then chemical alarm 'languages' of plants change over generations and become complex; once plants sending the honest signal dominate, new opportunities arise for 'cry wolf' plants mimicking this signal, yet harboring no or only few herbivores (2, 3, 4). We have modeled the tritrophic system and have been confirmed that HIPV stabilizes symbiotic relations (3) and we also have proposed a model of evolution of HIPV and shown that "Cry wolf" strategy evokes various types of HIPV (3, 4) and in this contribution we indicate that there is a possibility that "Cry wolf" strategy stabilizes the symbiotic relation.

Acknowledgements

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References

1. Shiojiri K., Ozawa R., Kugimiya S, et. al. 2010. PLoS ONE. Vol. 5(8).
2. Sabelis M. W., Janssen A., Takabayashi J. 2011. J. of Plant Interactions. Vol. 6: 2–3, 71–75.
3. Sakai M., Suzuki Y. 2011. ISCE 2011 Annual meeting.
4. Suzuki Y., Sakai M., Adachi K. 2012. Five Approaches to Lang. Evolution, 95–103.

FERTILITY SIGNALS IN THE SPECIES COMPLEX *PACHYCONDYLA APICALIS* (HYMENOPTERA, FORMICIDAE): PROXIMATE AND EVOLUTIONARY IMPLICATIONS

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Reproductive biases are a general rule in social groups. This phenomenon is deepened in insect societies, where only one or few individuals monopolize the reproduction of the entire colony. Despite this reproductive division of labour, workers in most species have retained the capacity to lay unfertilized male-destined eggs, which creates an opportunity for reproductive conflicts. Several mechanisms have then evolved to counter the potential high-cost these conflicts may entail on the colony productivity. In orphaned colonies, workers reproductive competition over male parentage is typically regulated by means of dominance hierarchies. Minimizing the costs of dominance interactions therefore requires the individuals to recognize the reproductive status of their nestmates. In this context, honest–evolutionarily stable–fertility signals are supposed to have a crucial importance in informing about the reproductive individuals' fertility state. However, we know virtually nothing about the evolution of fertility signals, in particular if they are a prerequisite for or a consequence of these dominance hierarchies. Here we investigated the convergence of putative fertility signals in *Pachycondyla apicalis* ants, a complex of closely-related species. We first determined the hierarchical rank of all workers in orphaned colonies of seven different species, before dissecting them to assess their ovarian activity. Using gas chromatography / mass spectrometry, we then studied the differences in the cuticular hydrocarbon profiles between high-ranking and low-ranking individuals. Highlighting interdependency between hierarchical rank, ovarian activity and chemical profile allow inferring the existence of putative fertility signals in these species, and then analysing their nature in a comparative study. We discuss the implication of these olfactory signals in the speciation processes among this species complex.

FLORAL SCENT PROFILES AND POLLINATOR HOST SPECIFICITY IN A NARROW HYBRID ZONE OF TWO JOSHUA TREE VARIETIES

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The obligate mutualism between yuccas and yucca moths is a major model system for the study of coevolution. The Joshua tree *Yucca brevifolia* is divided into two varieties based on morphology: the western *Y. b. brevifolia* and the eastern *Y. b. jaegeriana*. Two *Tegeticula* moths are the exclusive pollinators of these yuccas: *T. synthetica* on western plants, and *T. antithetica* on eastern plants. The two moth-plant pairs have a parapatric distribution, and only coexist in a narrow (≈4 km) contact zone in Tikaboo Valley, Nevada. Here, the two plant varieties grow side by side, and the area thus represents a unique opportunity to study the importance of olfactory signals for host discrimination in an obligate pollination mutualism. Previous genetic data have shown a clear asymmetry in gene flow between hosts, with significant pollinator-mediated gene flow from eastern to western populations (1). These data indicate that *T. antithetica* exhibits low host fidelity, visiting and successfully pollinating western plants much more frequently than *T. synthetica* visits and pollinates eastern plants. In addition, moth larvae from fruits of the two varieties have been assigned to species by genetic markers, and whereas no larvae of *T. synthetica* was found on the foreign host, clutch size for the two moths on fruits of western plants was equal, indicating weak prezygotic barriers against gene flow from eastern to western plants (2). In this study, headspace collections and GC-MS were used to analyse the blends of volatiles emitted by the two varieties. In addition to the homoterpenes and aliphatic hydrocarbons previously found in other studied yucca species (3), both varieties of Joshua tree produce several octane-derived compounds, e. g. 1-octen-3-ol. Small but significant differences were detected in the odour profiles between the two varieties, a pattern which could facilitate host discrimination in the pollinators. Passive sticky traps were placed close to the inflorescence on trees for each host type, as well as on ambiguous trees, to monitor moth visits. Contrary to our prediction, both pollinators were captured on all tree types, indicating that these moths do a poor job of distinguishing native and foreign hosts based on olfactory cues, which correlates well with the pattern of substantial ongoing gene flow observed between the two varieties in the contact zone.

Acknowledgements

The research was supported by the Crafoord Foundation.

References

1. Evolution. 2008. Vol. 62: 2676–2687.
2. Molecular Ecology. 2009. Vol. 18: 5218–5229.
3. Oikos. 2011. Vol. 120: 1577–1583.

Symposium 3. Biosynthesis and Chemistry of Natural Products

DECARBOXYLATION IN THE POLYENE PRODUCTION AND PBAN-REGULATED TRANSPORTATION IN THE WINTER MOTH, *OPEROPHTERA BRUMATA*

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In addition to the previously identified single component sex pheromone (*Z,Z,Z*)-1,3,6,9-nonadecatetraene (1, 2), the trienoic and tetraenoic polyenes, (Z,Z,Z)-3,6,9-nonadecatriene, (*Z,Z,Z*)-3,6,9-heneicosatriene and (*Z,Z,Z*)-1,3,6,9-heneicosatetraene were found in both abdomen cuticle and pheromone gland of the Winter Moth, *Operophtera brumata* L. (Lepidoptera: Geometridae). Deuterium-labelled fatty acid precursors, (*Z,Z,Z*)-2582[17,17,18,18,18-$^{2}H_{5}$]-9,12,15-octadecatrienoic acid and (*Z,Z,Z*)-[3,3,4,4,5,5-$^{2}H_{6}$]-11,14,17-icosatrienoic acid were applied *in vivo* to investigate the polyene biosynthesis. The results showed that linolenic acid ((*Z,Z,Z*)-9,12,15-octadecatrienoic acid) was used as precursor by the female adult to produce the triene (*Z,Z,Z*)-3,6,9-nonadecatriene, after a 2C-unit elongation to (*Z,Z,Z*)-11,14,17-eicosatrienoic acid followed by decarboxylation. The role of the pheromone biosynthesis activating neuropeptides (PBAN) in wintermoth pheromone biosynthesis was also evaluated in this study. After decapitation, the titre of glandular polyenes significantly decreased, and partly recovered when *O. brumata* head extract or synthetic Bom-PBAN were supplementary injected. However, among the groups of intact, decapitated, and decapitated plus supplementary-PBAN females, the total amount of each polyene from abdomen and gland did not change significantly, indicating that PBAN may affect the polyenes transportation from abdomen to gland, but not influence the polyene production in female abdomen.

Acknowledgements

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References

1. Tetrahedron Lett. 1982. Vol. 23: 4007–4010.
2. Science. 1982. Vol. 217: 657–659.

A NEW MULTI-DIMENSIONAL GC-MS SYSTEM (MD-GC-MS) AIMED TO FACILITATE THE IDENTIFICATION OF COMPLEX MIXTURES INCLUDING CHIRAL VOLATILES IN PLANT-INSECT-MICROBE INTERACTIONS

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The instrument is an Agilent-Gerstel hybrid comprised of two Agilent 7890A GC systems each combined with one 5975C inert MSD with triple-axis detector. A transfer line with liquid nitrogen cryo cooling combines the two GC-MS units to focus the analytes before entering them into the second GC. The capillary column in the first GC is a DB-5 (30 m × 0.25 mm × 0.25 μm, Agilent J & W Scientific), and in the second GC a Cyclodex-B (30 m × 0.25 mm × 0.25 μm, Agilent J & W Scientific) suitable for separation of most monoterpene enantiomers (1). The elution from column in GC 1 is split 1:1 to MS 1 and to GC 2 for subsequent separation and detection on MS 2 or to vent.

Comparisons of different techniques of injections using conifer seedlings as a model in order to increase the number of potential bioactive compounds are made. The sample introduction methods include liquid injection, high volume injection, manual or automated solid phase micro extraction, and thermal desorption from living organisms or volatiles adsorbed on a organic polymer e. g. Tenax TA or Porapak Q.

References

1. Borg-Karlson A.-K., Lindström M., Norin T., Persson M., Valterová I. 1993. Enantiomeric composition of monoterpene hydrocarbons in different tissues of Norway spruce, Picea abies (L.) Karst. A multi-dimensional gas chromatography study. Acta Chem. Scand. Vol. 47: 138–144.

CHEMICAL REACTIONS IN NORWAY SPRUCE (*PICEA ABIES*) CAUSED BY NATURAL INFECTION OF *HETEROBASIDION ANNOSUM*

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Serious forest damage is caused by root and butt rot fungus, *Heterobasidion* ssp., on conifers such as larch, spruce, pine and fir. In Sweden, 15 to 20 per cent of planted Norway spruce (*Picea abies* L.) of different ages is infected by this root-rot fungus. To be able to identify high resistant trees has since long been strongly emphasized in forest management and production (1). Conifers produce inheritable and / or acquired metabolites for resistance against e. g. herbivores, insects, and pathogenic fungi.

Basic research aimed to evaluate the relationships between metabolites and anti-fungal activitities is still needed. There has been research focused on the constitutive trunk constituents of Norway spruce such as terpenes, stilbenes and lignans comparing chemical composition between conifer species. However, very few studies have been reported about the chemical response of the trees during natural infection of *H. annosum*.

The aim of this research is to investigate the chemical responses in the trunk induced by the natural root infection of *H. annosum*, comparing the quantitative and qualitative differences of components from the horizontal and vertical borders of fungi on growing xylem parts.

Samples were provided from about 50 years old *P. abies* clones, both healthy and naturally infested by *H. annosum*. Wood disks were taken from three different height i. e. low, middle and high part due to the decay degree. The lower part was seriously decayed, the middle part was taken just at the border of fungal growth from the pith of heart wood and the highest part was healthy. Yields and water contents were calculated after extraction of the inner and outer parts of each sample disk first by *n*-hexane and then by methanol.

The yield of decayed parts (*n*-hexane: 1.0–1.3%/dw, methanol: 1.0–10.0%/dw) were much higher than that of healthy parts (*n*-hexane: 0.7–0.9%/dw, methanol: 0.8–1.2%/dw). There were quantitative differences both low polar and polar extracts were higher in decayed part. Qualitative differences in the extracts were not observed by GC-MS at horizontal border; however, considerable compound (m/z 188) were observed in low polar extract from vertical border. Thus, it was presumed that the spruces react and produce compounds during the progress of growing *H. annosum*.

Acknowledgements

The research was supported by Grant-in-Aid for Japan Society for the Promotion of Science (JSPS) Fellows (225181).

References

1. Silvae Genetica. 1997. Vol. 46: 369–374.
2. Journal of Chemical Ecology. 2010. Vol. 36: 1381–1386.

SUSTAINABLE GREEN STYRENE FROM FOREST WASTES

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Everyday life is highly dependent on plastic products. Styrene being the principal monomer of ubiquitous polystyrene plastics and resins is synthesized from petroleum (1). In the search of repellent and antifeedants for the pine weevil produced by microbes associated to pine weevil (*Hylobius abietis*), we have isolated a strain of *Penicillium expansum* producing styrene in huge quantity by culturing it on weevil frass medium. The fungal strain was grown on various forest related waste materials supplemented with yeast extract and the volatiles were collected using Tenax TA tubes (2). The collected volatiles were desorbed in hexane and the amounts produced by the fungus were quantified by comparing injected amounts with a standard curve of pure styrene sample using GC-MS. Pine needle and grated pine bark media with yeast extract were found to be best in styrene production as compare to wood yeast extract, glucose yeast extract and potato dextrose broth. The maximum production rate was 38 ug/h form pine bark broth and 50 ug/h from pine needle broth after 5 and 15 days of incubation respectively, when cultured on 100 ml broth in 1 Liter flasks. These promising results suggest that the isolated fungal strain can be used to produced green styrene plastics from a renewable and waste material of forest industry.

Acknowledgements

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References

1. Denis H. James William M. Castor. 2005. “Styrene” in Ullmann’s Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim.

2. Fäldt J., Jonsell M., Nordlander G., Borg-Karlson A. K. 1999. Volatiles of bracket fungi Fomitopsis pinicola and Fomes fomentarius and their functions as insect attractants. J. Chem. Ecol. Vol. 25: 567–590.

BIOACTIVITIES OF BRANCH HEARTWOOD EXTRACTS OF *CHAMAECYPARIS OBTUSA*

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Chamaecyparis obtusa Endl. (hinoki) is one of the important Japanese conifer trees. Hinoki heartwood has been known to show high resistance to termites and wood decay fungi and its bioactivity is related to extractive compounds in the heartwood. At present, branches of Hinoki are regarded as waste material in Japanese wood industry. Therefore, It would be useful,to develop a method to use this material. In this study, we determined the bioactivities of extracts from branch heartwood of Hinoki against ten fungi and termite (*Reticulitermes speratus*) and compared them with the bioactivities of extracts from trunk heartwood.

Branch and trunk heartwood were extracted successively with hexane, ethyl acetate, and methanol. Bioactivities of each extract was tested against ten fungi (*Trametes versicolor, Lenzites betulinus, Fomitopsis palustris, Gloeophyllum trabeum, Trichoderma virens, Chaetomium globosum, Aspergillus niger, Penicillium citrinum, Rhizopus oryzae,* and *Cladosporium cladosporioides*) and subterranean termite (*Reticulitermes speratus*).

The hexane and ethyl acetate extracts of branch and trunk heartwood showed antifungal activities. There were greater yields of the active extracts from branch heartwood (28.1%/dw) than from trunk heartwood (4.7%/dw). The active extracts (hexane and ethyl acetate extracts of branch heartwood) were fractionated by silica gel column chromatography. We obtained sesquiterpene hydrocarbon fraction (H-1), germacra-1-(10),5-dien-4β-ol, t-cadinol, t-muurolol, hinokiresinol, and hinokinin. According to the results of antifungal tests using above fraction and compounds, t-muurolol and hinokiresinol showed strong activities. The germacra-1-(10),5-dien-4β-ol, t-muurolol and t-cadinol, showed strong antitermite activities. As determined by GC-MS analyses, germacra-1-(10),5-dien-4β-ol and hinokiresinol were the minor components in trunk heartwood, however, major components in branch heartwood. Hinokiresinol showed bioactivities against *F. palustris, T. virens, and R. oryzae*. Germacra-1-(10),5-dien-4β-ol was lethal to termite. t-Muurolol and t-cadinol showed strong activity against wood rot fungi and termite. These compounds were commonly found in both branch and trunk extracts. These active sesquiterpenes are more abundant in branch heartwood than trunk. In addition, the active compounds hinokiresinol and germacra-1-(10),5-dien-4β-ol were characteristic components of branch heartwood.

Branches get damaged easier than trunk. Thus, branch heartwood has abundant bioactive components against several ecological influences.

STEREOSELECTIVE SYNTHESIS OF LILAC ALCOHOLS AND ALDEHYDES

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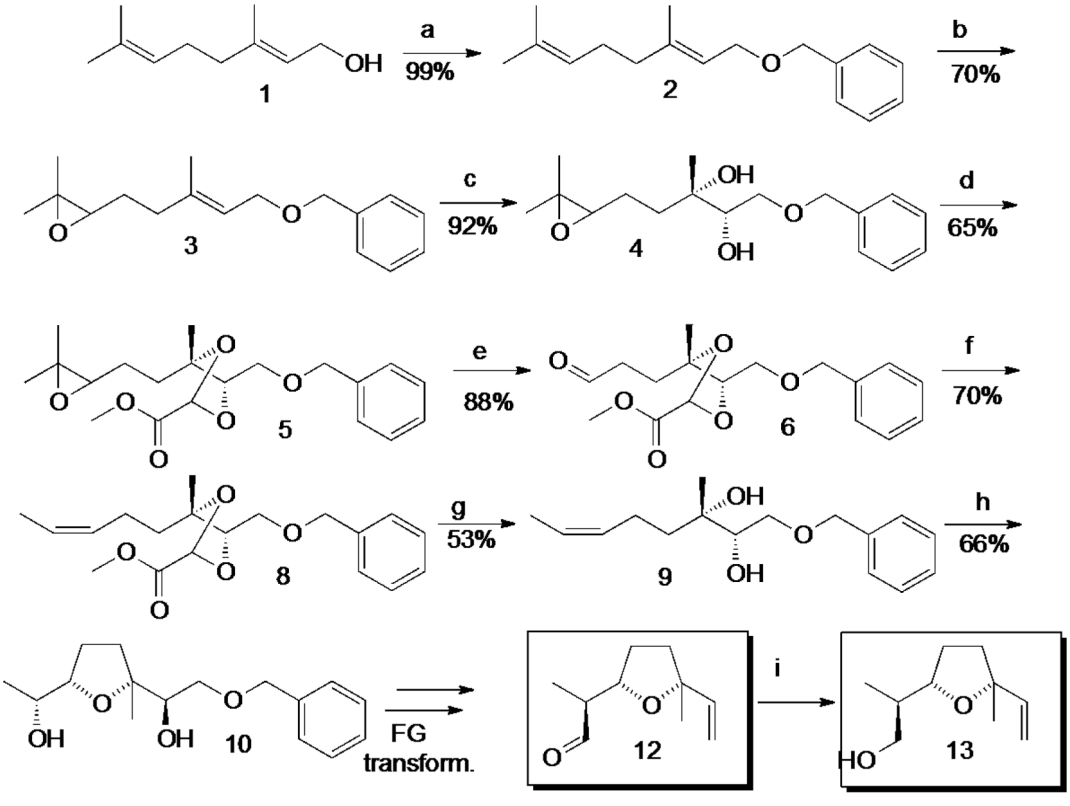
Lilac alcohols and aldehydes are natural flower scent compounds having important role in pollination aspect. They have complex structure with three chiral centers, therefore there are eight stereoisomers both of alcohols and aldhydes. The stereoselective synthesis of lilac compounds is not published and pure stereoisomers are not available for biological tests.

Here we present the scheme of stereoselective synthesis of lilac compounds. Our route involves Sharpless asymmetric dihydroxylation, Wittig reaction and Ru-catalyzed oxidative cyclization of dihydroxy alkenes[1].



Scheme. *Reagents and conditions:* (a) NaH, Benzyl bromide, THF; (b) m-CPBA, CH_2Cl_2; (c) K_2CO_3, $K_3[Fe(CN)_6]$, $(DHQD)_2PHal$, $K_2OsO_2(OH)_4$, $CH_3SO_2NO_2$, H_2O : *t*-BuOH – 3 : 1; (d) Me-propynoate, DMAP, AcN; (e) H_5IO_6, THF : H_2O – 1 : 1; (f) $EtP^+Ph_3Br^-$, $[(CH_3)_3Si]_2NNa$, THF; (g) Pyrrolidine, *n*-BuLi, THF; (h) TPAP, NMO, *t*-BuOH, CH_2Cl_2; (i) $NaBH_4$, EtOH

Acknowledgements

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References

1. Angewandte Chemie Int. Ed. 2010. Vol. 49: 1587–1590.

STEREOSELECTIVE SYNTHESIS OF LILAC ALCOHOLS

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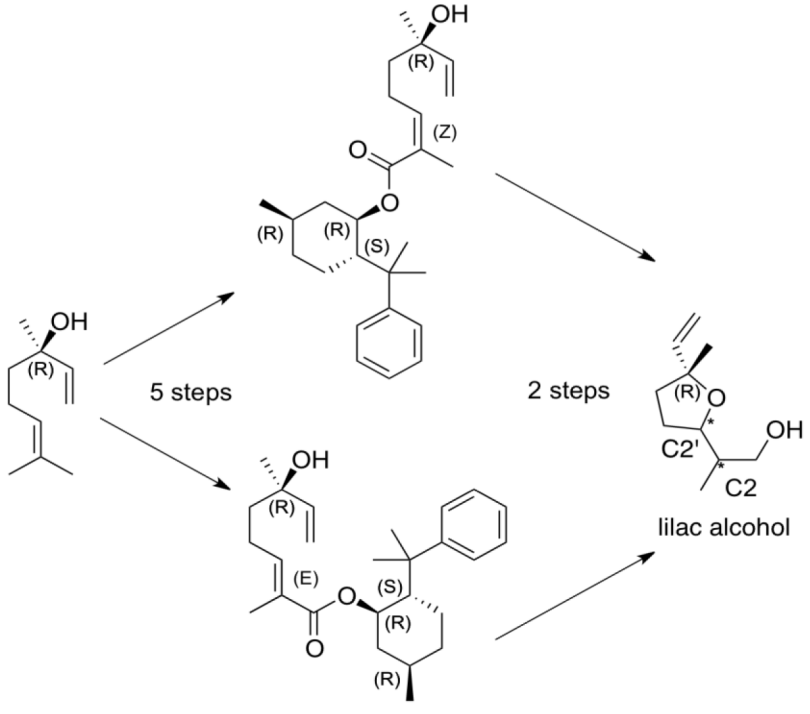
Lilac alcohols and lilac aldehydes are naturally occurring flower scent compounds with a complex chemical structure. The compounds possess three stereogenic centers which give rise to eight different stereoisomers. Lilac compounds represent important chemical signals between pollinators and flowering plants. Although studies indicate that insects are affected by each of the isomers (1), biological studies with pure isomers need to be carried out to confirm the type of interaction.

The aim of this research has been to work out a synthetic route to obtain isomers of lilac alcohols in high purity. The key step of the chosen synthetic route was the intramolecular Michael conjugate addition, which introduced two new chiral centers into the molecule. In short, chiral ligand (*1R,2S,5R*)-(*-*)-8-phenylmenthyl was applied in the key step to try out its effect to the formation of new stereocenters.



The results showed slight tendency toward *S*-configuration in C2-center when *Z*-alkene was cyclisized. In the case of *E*-alkene, the tendency was toward an isomer with *S*-configuration at C2-center and *R*-configuration at C2'-center. Further experiments are needed for achieving stereoisomeric purity high enough for application in the subsequent research involving insect–plant communication.

Acknowledgements

The research was supported by grants Mobilitas Top Researcher MMT2 "Chemical Ecology" and GLOTI 75 17 from the Estonian Science Foundation.

References

1. Journal of Chromatography A. 2006. Vol. 1113: 231–238.

THUJOPSENE AND ITS AUTOXIDATION PRODUCTS AS PLANT SELF-DEFENSIVE COMPOUND

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Sesquiterpene hydrocarbons are found widely in plant extracts. In several woody plant, the sesquiterpene hydrocarbons are contained as main components in the extract of heart wood, leaf, bark, and resin oil. However, most of these hydrocarbons have low bioactivities against plant harmful insects and fungi. And it is known that some sesquiterpenes are easily autoxidized in natural condition.

We have studied the autoxidation of sesquiterpenes and the bioactivity of autoxidation products to consider the ecological meanings of sesquiterpene production in the plant body. In our former reports (1), autoxidation of sesquiterpene hydrocarbons, caryophyllene and longifolene, were investigated and bioactivities of these autoxidation products were tested against termite and wood decay fungi to comfirm anti-termite and antifungal activities.

Thujopsene, a sesquiterpene hydrocarbon, is known as a main component in low polar extract of Hiba (*Thujopsis dolabrata* Sibe. et Zucc. Ver Hondai) heartwood which has high durability, and easily autoxidized in a similar manner as caryophyllene and longifolene. Nagahama et al. reported the natural autoxidation mechanism of the thujopsene (2). But the bio-activities of the autoxidation products have not been reported yet. In this study, anti-termite activities of thujopsene and its autoxidation products were investigated against subterranean termite (*Reticulitermes speratus* Kolbe) which is known as a popular harmful insect to Japanese wood.

Thujopsene was autoxidized naturally for 2 years in the room temperature. From the results of GC analysis, the autoxidation product was the mixture which contained mayurone as a main component. The anti-termitic activities of autoxdation product and original thujopsene were tested by no choice and dual choice methods. Thujopsene had weak antifeedant activity, and did not show significant termiticidal activities. However, the autoxidation product had high termiticidal and antifeedant activities.

Thus, it was shown that thujopsene can easily turns into strong plant defensive compounds by natural autoxidation. Therefore, autoxidation of sesquiterpene hydrocarbons might be related to the plant self-defensive system.

References

1. Ashitani T. et al. Abstract 25th Annual meeting of ISCE 223.
2. Nagahama S., Tazaki M. Bull. Chem Soc. Jpn. Vol. 60: 4453–4454.

BIOSYNTHESIS OF LEUCINE-DERIVED NITRILE IN THE EVENING PRIMROSE, *OENOTHERA* SP. (ONAGRACEAE) BY INSECT-FEEDING

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Plants emit a series of characteristic volatile blends, such as terpenes and green leaf volatiles when they are damaged by insect feeding. These herbivore-induced plant 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.

Previously, we found phenylacetonitrile, (*E*)-β-ocimene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*,*E*)-α-farnesene from the leaves of the giant knotweed, *Fallopia sachalinensis* (Polygonaceae) that was infested by the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae) or treated with a cellular signalling molecule, methyl jasmonate (MeJA) (1). These terpenes are well known volatiles induced by insect feeding, while nitrile emission is not usual as herbivore-induced plant response. Nitriles were thought to be synthesized from their corresponding amino acids in plants (2). We determined that phenylacetonitrile was synthesized from phenylalanine (Phe) in the leaves of *F. sachalinensis* using deuterium-labeled Phe and an inhibitor of Phe biosynthesis, glyphosate. We also found that Phe, the precursor of phenylacetonitrile, was synthesized *de novo* in the leaves of *F. sachalinensis* by insect-feeding or MeJA.

Here, we reported the identification of another nitrile, isovaleronitrile, 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 MeJA. We hypothesized that isovaleronitrile was synthesized from amino acid, leucine (Leu), as *F. sachalinensis*, and we investigated the biosynthetic pathway of the nitrile. Exogenous deuterium-labeled Leu was incorporated into the nitrile and four other volatiles in the leaves of *Oenothera* sp. Two of them were tentatively identified as *syn*- and *anti*-3-methylbutylaldoxime by GC/MS. These results suggest that isovaleronitrile was synthesized from Leu via the corresponding aldoxime in the plant.

Acknowledgements

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References

1. Molecules. 2011. Vol. 16: 6481–6488.
2. Medicinal Natural Products. 1997. 418–420.

PURIFICATION AND CDNA CLONING OF A PHLOEM LECTIN-LIKE ANTI-INSECT DEFENSE PROTEIN BPLP FROM THE PHLOEM EXUDATE OF WAX GOURD, *BENINCASA HISPIDA*

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Latex and other exudates in plants contain various proteins that are thought to play important defensive roles against herbivores and pathogens (1, 2). We investigated the defensive effects of phloem exudates against herbivorous insects (Lepidopteran larvae) in several Cucurbitaceous plants. We found that phloem exudates are responsible for the defensive activities of Cucurbitaceous plants such as the wax gourd *Benincasa hispida* and *Cucumis melo*, especially in *B. hispida*, whose leaves showed the strongest growth-inhibitory activity of all the Cucurbitaceous plants tested (3). Therefore, we purified and isolated a 35 kDa proteinaceous growth-inhibitory factor against insects designated BPLP (*Benincasa hispida* Phloem Lectin-like Protein) from *B. hispida* exudate using anion exchange and gel filtration chromatographies (3). A very low concentration (70 μg/g) of BPLP significantly inhibited the growth of lepidopteran larvae (3). The full-length cDNA (1076 bp) encoding BPLP was cloned and its nucleotide sequence was determined based on information on the internal amino acid sequence, degenerate RT-PCR, 3'RACE, and 5'RACE (3). The deduced amino acid sequence of BPLP exhibited 51% homology with Cucurbitaceous phloem lectin (phloem protein 2, PP2) and showed binding specificity to oligomers of N-acetylglucosamine while some features of BPLP indicated that it does not have a cysteine residue and it is composed of two repeats of similar sequences, suggesting that BPLP is distinct from PP2 (3). The present study provides decisive experimental evidence that phloem exudates of Cucurbitaceae plants, analogous to plant latex, play defensive roles against insect herbivores, especially against chewing insects, and contain defensive substances toxic to them.

References

1. Konno K. 2011. Phytochemistry. Vol. 72: 1510–1530.
2. Agrawal A. A., Konno K. 2010. Annu. Rev. Ecol. Evol. Syst. Vol. 40: 311–331.
3. Ota E., Nakamura M., Hirayama C., Konno K. (submitted)

THE BIOSYNTHETIC PATHWAY OF CERBINAL IN *GARDENIA JASMINOIDES*

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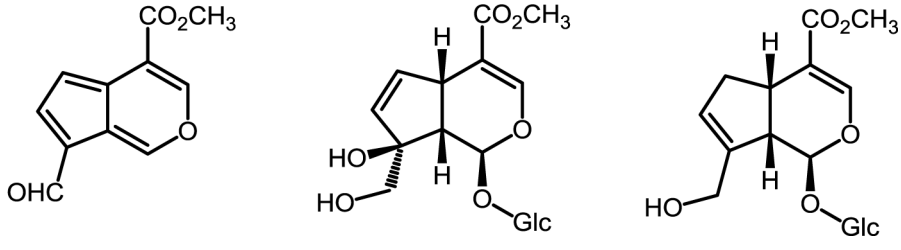
Cerbinal, a pseudoazulene iridoid, has been isolated from *Gardenia jasminoides* Ellis and recognized as a potent antifungal compound (1). The chemical structure of cerbinal shows its characteristic Δ-3,5,7,9-tetraene aromatic 10π-system. Although the efficient chemical synthesis of cerbinal from (+)-genipin was reported (2), the biosynthetic pathway has been completely unknown. In this study, we investigated the biosynthetic pathway of cerbinal in leaves of *G. jasminoides*.

Cerbinal was detected in methanol extracts of damaged leaves of *G. jasminoides* by LCMS. However, this compound was not found in the undamaged leaves, nor in the preheated leaves. This strongly suggests that cerbinal is not constitutively present in *G. jasminoides* leaves, but is formed enzymatically after an induction by mechanical damage. Furthermore, two iridoid glycosides, gardenoside and geniposide, were identified in young leaves, but a tiny amount of geniposide in mature leaves. Since cerbinal was induced in mature leaves, we assumed that gardenoside could be the precursor of cerbinal. To test the hypothesis, quantitative analyses of cerbinal and gardenoside were conducted in preheated leaves and homogenized leaves. As a result, 15 times higher amount of gardenoside was detected in preheated leaves than in the homogenized leaves, while cerbinal corresponded in reverse order. More importantly, the homogenized leaf tissues with additional gardenoside produced a larger amount of cerbinal than the control. Consequently, it is considered that gardenoside is to be the precursor of cerbinal.



Cerbinal Gardenoside Geniposide

References

1. Agricultural and Biological Chemistry. 1986. Vol. 50: 2655–2657.
2. Chemistry Letters. 1992. 139–140.

N-LINOLENOYL-L-GLUTAMIC ACID KNOWN AS A PLANT VOLATILE ELICITOR, FOUND IN FIVE TRUE CRICKETS (ORTHOPTERA: GRYLLIDAE)

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Since the discovery of volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine], an insect-produced plant volatile elicitor in the regurgitant of larval *Spodoptera exigua* (1), glutamine- and glutamic acid-based fatty acid-amino acid conjugates (FACs) have been identified from gut contents of lepidopteran caterpillars (2). Interestingly, glutamic acid-based FACs were also identified from gut contents of adults of two closely related cricket species, *Teleogryllus taiwanemma* and *T. emma* (Orthoptera: Gryllidae), and from larvae of the fruit fly, *Drosophila melanogaster* (Diptera: Drosophilidae) (3).

In this study, we reported that glutamic acid-based FACs were also identified from 5 Gryllidae species: *Teleogryllus ezoemma*, *Gryllus bimaculatus*, *G. firmus*, *G. ovisopis*, and *Platygryllus maurus*, by comparing the retention times and fragmentation patterns obtained from MSMS experiments with those of authentic samples. When these crickets were fed on different types of diets, interestingly, the difference in the composition of glutamic acid-based FACs was observed. Thus, the composition of FAC mixture was strongly influenced by the diets crickets feed, as in the case observed in caterpillars.

References

1. Alborn et al. 1997. Science. Vol. 276: 945–949.
2. Yoshinaga et al. 2010. Journal of Chemical Ecology. Vol. 36: 319–325.
3. Yoshinaga et al. 2007. Journal of Chemical Ecology. Vol. 33: 1376–1381.

BIOGENESIS OF (*E*)-1-NITROPENTADEC-1-ENE IN SOLDIERS OF THE TERMITE GENUS *PRORHINOTERMES*

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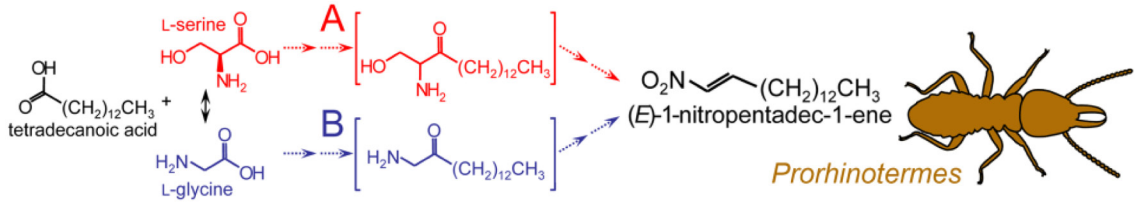
Though structurally diverse and frequent in bacteria, fungi and plants, naturally occurring nitro compounds were only very rarely documented in arthropods. (*E*)-1-nitropentadec-1-ene (NPD) was the first described insect-produced nitro compound, identified in the defensive secretion of soldiers of the termite *Prorhinotermes simplex* (Rhinotermitidae) (1). NDP has since been confirmed to be the dominant defensive chemical in three other *Prorhinotermes* species, together with a few minor nitroalkanes, nitroalkenes and nitrodienes (2, 3) NPD is a potent lipophilic contact poison; its toxicity is assigned to the strong electrophilic nitroalkene group, which reacts by Michael reactions with nucleophilic moieties such as sulfhydryl, hydroxyl, or amino groups. Although the functional aspects of this unusual nitrocompound have received considerable attention in the past, its biogenesis was not yet well understood. Therefore, in an attempt to clarify the biosynthetic origin of (*E*)-1-nitropentadec-1-ene, we performed a series of experiments to test the hypothesis about the biogenesis of the compound through initial condensation of amino acids with a fatty acid and subsequent series of transformations (4, 5). Using radiolabelled precursors, L-serine, L-glycine, and tetradecanoic acid, injected into living soldiers, we confirmed this hypothesis and proposed two alternative biosynthetic scenarios for the origin of (*E*)-1-nitropentadec-1-ene, summarized in the schema below.



Acknowledgements

Financial support provided by the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic (RVO: 61388963).

References

1. Tetrahedron Letters. 1974. Vol. 15: 1463–1464.
2. Journal of Chemical Ecology. 1990. Vol. 16: 685–692.
3. Journal of Chemical Ecology. 2007. Vol. 33: 1787–1794.
4. Science. 1981. Vol. 214: 1363–1365.
5. Tetrahedron. 1982. Vol. 38: 1921–1930.

SEX PHEROMONE OF STREPSIPTERA

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Strepsiptera are rare endoparasites of various insects including bees. We studied the mating system of *Stylops* nr. *melittae*, parasite of the solitary bee *Andrena vaga*. *Stylops* female is not leaving the host, and protrudes from it only by the fore body part, the cephalothorax. Cephalothorax is filled with the secretory cells of Nassonow's gland producing the sex pheromone (so called stylopsal) serving for male attraction. Adult males leave the host bee right after their emergence, and, according to their life span of few hours only, they have just few chances to mate. Stylopsal is highly unusual compound ensuring a quick mate location at *Andrena vaga* nesting sites.

The production of the stylopsal starts during overwintering, and the final compound originates from the fatty acid transported to the Nassonow's gland from the adipocytes of the fat body.

We report on the quantity and also time dependence of the stylopsal release before, during and after mating.

Acknowledgements

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DETERMINATION OF LIPOLYTIC ENZYMES IN LABIAL GLAND OF MALES *BOMBUS TERRESTRIS*

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The study of fat metabolism in insects has received considerable attention over the last years. Although the lipases from insects, their properties and importance are poorly investigated area. Fatty acids stored as triglycerides in the fat body serve as precursor in multiple processes including energy production and synthesis of cellular components. In bumblebees, lipases can participate in addition to the hydrolysis of storage lipids, in the biosynthesis of different fatty acids, which serve as pheromones for very specific sexual communication of this species. We detected lipase activity in labial gland of *Bombus terrestris* and performed characterization of these novel lipases.

The lipolytic enzymes were extracted from labial glands of the bumblebee males *Bombus terrestris*. The enzymes were isolated by a combination of precipitation procedures with column chromatography. Enzymes were separated by SDS-PAGE and molecular mass was determined. These bands also showed a lipolytic activity in zymography assay.

The identity of the peptides was confirmed by using mass spectrometry. Comparison with cDNA library of *Bombus terrestris* shoved that the main lipolytic enzymes in labial gland are phospholipases (C, B, D), secretory phospholipase, carboxylesterase and phosphodiesterase.

Acknowledgements

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BIOSYNTHESIS OF TERPENIC COMPOUNDS IN MARKING PHEROMONE BLEND OF *BOMBUS TERRESTRIS*

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Bombus terrestris, a common European bumblebee, is an important agricultural pollinator as well as a model bumblebee species. Males exhibite a patrolling behaviour as a mating strategy. They mark prominent objects on their flight routes with a marking pheromone to attract conspecific females. The marking pheromone is a complex mixture released from the cephalic part of labial gland and its composition is highly species-specific. Labial gland secretion of *B. terrestris* males contains mixture of terpenic and aliphatic compounds and 2,3-dihydrofarnesol, geranylcitronellol and ethyl dodecanoate are the most abundant components.

In our study we focused on detailed analysis of biosynthesis of terpenic compounds in pheromone gland of *B. terrestris*. *In vitro* incubations of labial glands with radioactive [1,2-14C]acetate and deuterium labelled acetate proved that pheromonal components are synthesized *de novo*. We measured the relative mRNA abundance of genes coding enzymes from mevalonate pathway in different aged labial glands. We compared the results with analytical measurements of age dependent concentration changes of selected compounds in the labial glands of *B. terrestris* and identified the enzymes regulating a terpenic compounds biosynthesis.

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DEVELOPMENT OF SEMIOCHEMICALS SLOW-RELEASE FORMULATIONS AS BIOLOGICAL CONTROL DEVICES

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Semiochemicals have been widely considered within various integrated pest management (IPM) strategies. In the present work, two sesquiterpenoids, *E*-β-farnesene and *E*-β-caryophyllene, were formulated for their related properties as aphid enemy attractants. *E*-β-farnesene, the alarm pheromone of many aphid species, was also identified as a kairomone of aphid predators (*Episyrphus balteatus* De Geer (Diptera: Syrphidae)) and parasitoids (*Aphidius ervi* Haliday (Hymenoptera: Braconidae)). *E*-β-caryophyllene was identified as a potential component of the aggregation pheromone of the Asian ladybird, *Harmonia axyridis* Pallas, another aphid predator. The two products were purified from essential oils of *Matricaria chamomilla* L. (*Asteraceae*) and *Nepeta cataria* L. (*Lamiaceae*) for *E*-β-farnesene and *E*-β-caryophyllene, respectively. Natural and biodegradable slow-release formulations were then investigated in order to deliver these molecules on crop fields for a long period of time as biological control devices. Due to their sensitivity to oxidation, both sesquiterpenes needed to be protected from degradation. For this purpose, alginate – hydrophilic matrix with low oxygen permeability – was used as polymer for the formulations: the main objective was to deliver semiochemical substances in the air in a controlled way. Consequently, a careful selection of alginates was realised. Formulated beads showed different structural and encapsulation properties depending on various formulation factors. Alginate formulations were characterized by texturometry and by confocal microscopy in order to observe the distribution of semiochemicals in alginate network. The last step of alginate bead characterisation consisted in studying release rate of semiochemicals in laboratory-controlled conditions by optimised trapping and validated Fast-GC procedures. Finally, the efficiency of formulations as aphid predator (*Syrphidae*) and parasitoid (*A. ervi*) attractants was demonstrated by field trapping and olfactometry experiments.

TIMING OF MALE SEX PHEROMONE BIOSYNTHESIS IN A BUTTERFLY – DIFFERENT DYNAMICS UNDER DIRECT OR DIAPAUSE DEVELOPMENT

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Pieris napi has two generations per year, different selection pressures affect the different generations, depending on the season, and behavioural and physiological seasonal polyphenisms have earlier been shown.

Here we explore the dynamics in sex pheromone production in males. Butterflies were fed ^{13}C labelled glucose either in the larval or adult stage and incorporation into the sex pheromone geranial and neral and other volatiles produced was studied.

Labelled glucose ingested in the larval stage showed up in geranial and neral produced by adult butterflies. This demonstrates that the pheromone synthesized by newly eclosed adult males is based on materials ingested in the larval stage. In addition adult butterflies were also able to synthesise geranial and neral and the related alcohols from adult intake of glucose. This was shown using flying males with access to ^{13}C labelled glucose during 48 hours.

The two generations have significantly different scent composition early in life, as the direct developers – who have shorter time for pupal development – need the first 24 hours of adult life to synthesise geranial and neral after eclosion, whereas the diapausing individuals who have spent several months in the pupal stage eclose with almost complete adult scent composition.

Our study shows that time-stress changes the timing in biosynthesis of the complete pheromone between generations, and increases the understanding of the physiological basis of life history traits.

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IDENTIFICATION AND SYNTHESIS OF POSSIBLE CONTACT PHEROMONES OF *ARHOPALUS FERUS*, THE BURNT PINE LONGHORN BEETLE

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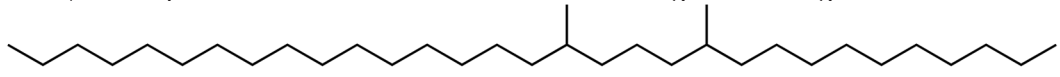
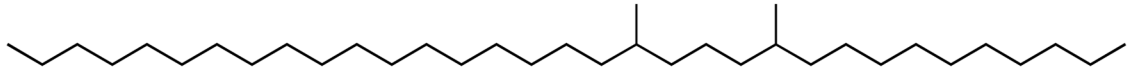
Cerambycid beetles are among the most important pests of woody plants worldwide. One example is *Arhopalus ferus*, the burnt pine longhorn beetle; a northern hemisphere native that has unintentionally been introduced to New Zealand (NZ) (1). Apart for the damage it can cause to felled timber, it is also a major quarantine pest in NZ. To meet regulations, timber shipments must be fumigated with methyl bromide to remove potential pests. With methyl bromide use being phased out, new control tactics for these pests are of upmost importance. A deeper knowledge of the chemical signaling of the beetles could aid in the development of alternative methods. It is known that cerambycid beetles are dependent on very specific contact pheromones in mating (2). If these can be identified and synthesized it might be possible to construct new types of lures to selectively catch male longhorn beetles. Two alkanes (11,15-dimethylhentriacontane and 11,15-dimethyltritriacontane) have been identified as potential contact pheromones in this project, based on the analysis of whole body extracts of male and female beetles. The long-chain alkanes were synthesized in 7 steps, including two Grignard reactions, and confirmed to be identical to the compounds found in the extracts. The activity of the produced alkanes will be evaluated during the coming field season in NZ



11,15-dimethylhentriacontane



11,15-dimethyltritriacontane

References

1. Kimberley M. O., Suckling D. M., Donaldson T. 2006. Nationwide survey for invasive wood-boring and bark beetles (Coleoptera) using traps baited with pheromones and kairomones. EG Brockerhoff, DC Jones, Forest Ecology and Management. Vol. 228(1): 234–240.

2. Ginzel M. D., Hanks L. M. 2003. Contact pheromones as mate recognition cues of four species of longhorned beetles (Coleoptera: Cerambycidae). Journal of Insect Behavior. Vol. 16(2): 181–187.

Symposium 4. Chemical Ecology of Microorganisms

NEMATODE DISPERSAL IS REGULATED BY AN EVOLUTIONARY CONSERVED NEMATODE COMMUNICATION SYSTEM

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Despite recent discoveries of signaling compounds, called ascarosides, regulating mating and dauer formation in the model nematode *Caenorhabditis elegans,* very little has been known about the dispersal behavior of the dauer larvae. Furthermore, the dauer dispersal stage is analogous to the infective second stage juveniles (J2) of root knot nematodes (*Meloidogyne* spp.) and infective juveniles (IJ)s of insect parasitic nematodes (EPN), e. g. *Steinernema feltiae*. We discovered that a blend of four ascarosides (ascr#2, ascr#3, ascr#8, icas#9) found in dauer forming growth media dispersed *C. elegans* dauer even in the presence of food and also caused dispersion of IJs of *S. feltiae* as well as J2s of plant parasitic *Meloidogyne* spp. Assay guided fractionation revealed structural analogs as major active components of the *S. feltiae* (ascr#9) and *C. elegans* (ascr#2) dispersal blends. Further analysis revealed ascr#9 in insect host cadavers infected by several species of *Steinernema* spp. and *Heterorhabditis* spp. Ascaroside blends represent evolutionarily conserved, fundamentally important communication systems for nematodes from diverse habitats, and thus may provide sustainable means for control of beneficial as well as parasitic nematodes.

AMMONIA PRODUCTION BY INTESTINAL MICROBES IN THE LARGE PINE WEEVIL (*HYLOBIUS ABIETIS*)

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The pine weevil, *Hylobius abietis* (L.), is a harmful insect that feeds on conifer seedlings in reforestation areas in Northern Europe. The weevil have no sex pheromone which can be used for control of the pine weevil, instead, we screen for effective pine weevil repellents and antifeedants. As *H. abietis* survive in nutritionally poor environment, the insect microbial communities may contribute to its development and survival through synthesis of essential nutrients. For the control of the pine weevil, these communities also might be an effective target.

Fifty-four microbes have been isolated from the gut and feces of *H. abietis*. They were classified into six groups depending on their volatile profile measured by SPME-GC-MS. A few microbes isolated from the gut produced ammonia on nutrient broth but they did not have the ability to reduce acetylene to ethylene. Investigations of characterization of these microbes are underway.

METHYL SALICYLATE PRODUCING MICROBES FROM *HYLOBIUS ABIETIS* FRASS

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Methyl salicylate is a ubiquitous molecule carefully examined in many biological systems (1). In many plants, it is produced as a systemic defense response to herbivore attack (2). The aim of this study is to identify the origin of the pine weevil (Hylobius abietis L.) antifeedants and repellents present in the weevil frass. We isolated a number of microbial strains from aseptically collected pine weevil frass producing methyl salicylate as a major volatile metabolite when cultured on weevil frass broth. Those microbes were identified by morphological comparison and DNA sequencing methods as *Ophiostoma piceae*, *O. pluriannulatum, Mucor racemosus, Eucasphaeria capensis* and a yeast species. Pure microbial strains were cultured on weevil frass liquid medium without shaking and the head space volatiles were collected by SPME and analyzed on GCMS. The attraction / repellency of methyl salicylate for pine weevil was tested by using a big circular arena with multi choice traps, fewer weevils entered the traps having host plant plus methyl salicylate as compare to traps with only host plant odor. Methyl salicylate showed a medium antifeedant activity towards pine weevils. These results show that methyl salicylate has an effect on the pine weevil orientation and feeding so can be a candidate for protecting small seedlings of pine and spruce in managed forests.

Acknowledgements

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References

1. E. H. Koschier, Hoffmann, D., Riefler J. 2007. Influence of salicylaldehyde and methyl salicylate on post-landing behaviour of Frankliniella occidentalis Pergande. J. App. Entom. Vol. 131: 362–367.

2. Snoeren T. A. L., Mumm R., Poelman E. H., Yang Y., Pichersky E., Dicke M. 2010. The Herbivore-Induced Plant Volatile Methyl Salicylate Negatively Affects Attraction of the Parasitoid Diadegma semiclausum. J. Chem. Ecol. Vol. 36: 479–489.

IDENTIFICATION OF VOCS RELEASED BY *BURKHOLDERIA AMBIFARIA* AND THEIR EFFECTS ON PLANTS, FUNGI AND BACTERIA

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Bacteria are known to produce a wide variety of volatile organic compounds (VOCs). Some strains emit volatile mixtures with up to 80 different compounds like fatty acid derivatives, aromatic compounds, nitrogen-containing compounds, terpenoids, or sulphur-containing compounds (1). However, the biological functions of VOCs and their effect on target organisms are still poorly understood. They might be infochemicals for inter- and intra-specific communication, cell-to-cell communication signals, carbon release valve or growth promoting or inhibiting agents (2). While many rhizobacteria are known to influence plant growth with volatiles (3), very few bioactive compounds have so far shown to be responsible for these effects.

We observed that bacterial volatiles released by *Burkholderia ambifaria* strains originating from different environments (root tissues, rhizosphere or clinical environment) showed strong effects on the model plant *Arabidopsis thaliana* (higher biomass and shorter root length), as well as inhibitory effects on the phytopathogenic fungus *Alternaria alternata*. Moreover, cells of *Escherichia coli* exposed to the VOCs produced by the three *B. ambifaria* strains showed higher antibiotic tolerance than the non-exposed control cells.

To find out which substances are responsible for the observed effects, we collected the headspace extracts of the different strains via closed-loop stripping analysis (CLSA) and analysed the compounds using GC-MS. Overall, more than 40 different compounds were identified, e. g. ketones, thioesters, aromatic compounds, terpenoids, and alcohols. We are presently testing the biological activity of the most promising compounds on plant, fungi and bacteria.

References

1. Schulz S., Dickschat J. S. 2007. Natural Products Report. Vol. 24: 814–42.

2. Kai M., Haustein M., Molina F., Petri A., Scholz B., Piechulla B. 2009. Applied Microbiology and Biotechnology. Vol. 81: 1001–1012.

3. Blom D., Fabbri C., Connor E. C., Schiestl F. P., Klauser D. R., Boller T., Eberl L., Weisskopf L. 2011. Environmental Microbiology. Vol. 13: 3047–3058.

MYRCENE-RESISTANT BACTERIA ISOLATED FROM THE GUT OF THE PHYTOPHAGOUS INSECT *IPS TYPOGRAPHUS* L.

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Bark beetles *Ips typographus* L. (Coleoptera, Scolytidae) feed on conifers, which produce myrcene among many other defensive compounds. It has been suggested that microorganisms present in beetle guts could be involved in detoxication of this defensive compound.

The beetles were collected from the bark of Norway spruce (*Picea abies* L.) in Lithuania. Gram-negative-staining bacteria that were the most resistant to monoterpene myrcene (7-methyl-3-methylene-1.6-octadiene, $C_{10}H_{16}$, at concentrations of up to 10 µl ml^{-1} in TSB) were isolated from gut contents of adult bark beetles and characterized by conducting phenotypic assays as well as fatty acid analysis, 16S rRNA gene sequencing, multilocus sequence analyses (MLSA) based on the *rpoB*, *atpD* and *infB* genes and DNA–DNA hybridization. The biochemical characterization indicated that the bacteria belonged to the family Enterobacteriaceae. The phylogenetic analyses of 16S rRNA gene sequences and MLSA of novel strains revealed that they belonged to the genus Erwinia and represented a novel species. The dominant cellular fatty acids were $C_{16:0}$ and $C_{17:0}$ cyclo. The DNA G+C content was 49.1 mol%. The bacteria from the bark beetle gut represented a novel species, to which the name *Erwinia typographi* sp. nov. is proposed, with the type strain DSM 22678^T (=Y1^T=LMG 25347^T).

Symposium 6. Aquatic Chemical Ecology

"DIAGNOSING" DISEASE BY SMELL: THE SYMBIOTIC HARLEQUIN CRAB IS ABLE TO CHEMICALLY DETECT IF ITS SEA CUCUMBER HOST IS INFECTED BY SKIN ULCERATION DISEASE

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Skin Ulceration Disease (SUD) is a bacterial infection that induces serious lesions on the body wall of sea cucumbers. It is highly contagious and can cause the death of 95% of sea cucumbers when they are reared in aquaculture ponds. The harlequin crab *Lissocarcinus orbicularis* is a common ectocommensal of the sea cucumber *Bohadschia vitiensis*. Using host choices experiments in a Davenport olfactometer, we recently demonstrated that these crabs are attracted by kairomones that enable them to specifically recognize their hosts by means of chemical sensing. In this study, we observed that individuals of *B. vitiensis* presenting skin ulcerations are no longer attractive to the crabs. Moreover, when given the choice between two sea cucumbers, harlequin crabs are able to distinguish healthy individuals from diseased ones, with a significant preference for sea cucumbers that are not infected by skin ulceration disease. Three hypotheses were tested in order to discover the reason of this avoidance behavior: the effect of the bacteria responsible of SUD, a possible change in the quantity of saponins produced by reared sea cucumbers, and the release of other secondary metabolites that could be emitted in surrounding water of skin ulcerated holothurians. This study is the first to highlight that a symbiont would be able to discriminate if its host is ill or not by sniffing its surrounding water. This ability certainly confers a selective advantage to these crustaceans which are obligatory symbionts of sea cucumbers.

WHEN A REPELLENT BECOMES AN ATTRACTANT: ANTIFEEDANT QUINONES ARE THE POTENTIAL KAIROMONES THAT MAINTAIN THE SYMBIOSIS BETWEEN THE SNAPPING SHRIMP *SYNALPHEUS STIMPSONI* AND THEIR CRINOID HOSTS

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Quinones are one of the major pigments that provide such bright colors to feather stars (Echinodermata, Crinoidea). Furthermore, these secondary metabolites act as a chemical defense mechanism to crinoids, rendering them unpalatable to predatory fish. However, feather stars are usually infested by a lot of symbiotic organisms, amongst which the ectocommensal snapping shrimp *Synalpheus stimpsoni* (De Man) which occurs under the calyx of several tropical crinoid species. We recently highlighted that host recognition is mainly mediated by chemical communication. Using a Y-tube olfactometer, we showed that quinones could be the attractive kairomones allowing the shrimps to recognize their host. Multiple chemical extracts were isolated from three different crinoid host species and directly tested in the olfactometer in order to test their behavioral effects on the symbionts. The chemical extracts which potentially contain the quinones were attractive while those that potentially contain carotenoids were not. This hypothesis is also supported by the fact that shrimps are chemically attracted to synthetic anthraquinones. In addition to their traditional defensive role (allomones), quinones, therefore would also function as kairomones, maintaining the symbiosis between *S. stimpsoni* and its sea cucumber host.

OVIPOSITION HABITAT SELECTION BY *ANOPHELES GAMBIAE* IN RESPONSE TO CHEMICAL CUES BY *NOTONECTA MACULATA*

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A number of mosquito species avoid predator-inhabited oviposition sites by detecting predator-released kairomones. In the laboratory, we found that when offered de-ionized water and de-ionized water conditioned with *Notonecta maculata*, gravid *Anopheles gambiae* females preferentially oviposited into the former. We then conducted further experiments using two chemical components found in *Notonecta*-conditioned water, chemically pure *n*-tricosane and / or *n*-heneicosane, which were previously shown to repel oviposition by *Culiseta longiareolata*. These hydrocarbons failed to deter oviposition by *An. gambiae* females. Thus, different mosquito species may rely on distinct chemical cues to avoid predators. Identification and chemical characterization of such kairomones could facilitate innovative, environmentally sound mosquito control.

ANTIFUNGAL ACTIVITIES OF CONIFEROUS BARK COMPONENTS ON THE GENUS *SAPROLEGNIA*

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Saprolegniasis, one of the serious fish diseases, caused by the fungal species of the genus *Saprolegnia*. Nowadays, developments of new control agents using ecologically safe chemicals are strongly required on the aquaculture. On the other hand, Japanese cedar (*Cryptomeria japonica* D. Don), most major conifer tree in Japanese forest, have been planted and accumulated in large amounts for housing materials. From our previous researches, the needle and bark components of *C. japonica* showed antifungal activities against the genus *Saprolegnia (*1) and growth inhibition activities against the red tide planktons(2, 3), respectively. In this study, the antifungal activities of the *C. japonica* bark components were examined against saprolegniasis related fungi, *Saprolegnia parasitica* and *S. diclina*.

The inner bark and outer bark of *C. japonica* were successively extracted with *n*-hexane, ethyl acetate, and methanol. Antifungal tests for these two fungi were performed on GYA (glucose yeast agar) medium. The low polar extracts obtained from *C. japonica* bark showed mycelium growth inhibition against *S. parasitica* and *S. diclina*. Ferruginol was detected as a one of main components in the active extracts by GC-MS analysis. The components in the active extracts were isolated by SiO_2 column chromatography, and antifungal activities of the isolated compounds were examined. Strong growth inhibitions against *S. parasitica* and *S. diclina* were observed in ferruginol.

As the results, it was suggested that the *C. Japonica* bark extracts might be valuable as the natural pest control agents against the *S. parasitica* and *S. diclina.*

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References

1. Kurihara Y. et al. 2009. Asia-Pacific Conference on Chemical Ecology 2009, Hawaii, United States of America.

2. Saijo H. et al. 2010. International Society of Chemical Ecology Annual Meeting 2010, Tours, France.

3. Tsuruta K. et al. 2011. Inhibition activity of essential oils obtained from Japanese trees against *Skeletonema costatum.* Journal of Wood Science. 57: 520–525.

MOLECULAR ANALYSIS OF THE BACTERIA-DIATOM ASSOCIATION IN PHAEODACTYLUM TRICORNUTUM

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Microbes are ubiquitous interaction partners in various ecosystems. Here, they may act as symbiotic partners or as pathogenic threats. Therefore, it is important for many life forms to actively recognize the presence of microbes. In higher organisms, the first line of recognition of invading microbes is by the perception of microbe-associated molecular patterns (MAMPs). These are perceived by highly specific pattern recognition receptors (PRRs) at the plasma membrane, such as the mammalian toll-like receptors or the flagellin sensing receptor FLS2 in plants. When comparing plant and animal PRRs, surprising similarities can be observed: Many PRRs comprise of a single transmembrane domain and an extracellular leucine-rich repeat domain, which is reported to be essential for binding of the MAMP. Homo- or heterodimerization of the receptor molecule leads to activation of intracellular kinases, which are responsible for further signal transduction (1).

Also unicellular algae, such as marine and fresh water diatoms live in tight interaction with bacterial microbes. These bacteria may either positively or negatively influence the diatoms' growth rates (2). Furthermore, under laboratory conditions, some diatoms only exhibit production of extracellular polysaccharides (EPS) and develop biofilms when co-cultivated with bacteria (3). Moreover, the sterile culture supernatant of bacteria is sufficient to trigger EPS and biofilm production in diatoms, suggesting the existence of soluble bacterial signals. Thus, also unicellular algae seem to possess the ability to recognize microorganisms and adapt to their presence.

We have screened the genome of the diatom *Phaeodactylum tricornutum* for potential proteins which exhibit typical structures of PRRs. Several proteins carrying LRR domains were identified and classified according to their domain architecture. Although *P. tricornutum* encodes several single transmembrane LRR-proteins, none of these carry additional domains typical for mammalian or plant like PRRs. Thus, the signal recognition pathway seems to be substantially different in this alga. To further evaluate the role of specific LRR proteins, these will be cloned and further analyzed by producing over expression and knock-down lines in *P. tricornutum*. Bioassays on EPS and biofilm production should characterize their role in bacteria-diatom interactions.

References

1. Journal of Biological Chemistry. 2010. Vol. 285: 9444–9451.
2. Environmental Microbiology. 2011. Vol. 13: 1052–1063.
3. Environmental Microbiology Reports. 2012. Vol. 4: 133–140.

EFFECTS OF ALLELOPATHICALLY ACTIVE MACROPHYTE *MYRIOPHYLLUM SPICATUM* ON NATURAL PHYTOPLANKTONIC COMMUNITY – A MESOCOSM APPROACH

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One of the most troublesome symptoms of nutrient over enrichment is the proliferation of toxic, food-web altering and hypoxia generating harmful algae blooms which are expanding geographically and now threaten the ecological integrity and sustainability of the most resourceful water bodies world wide (1). The treat is mainly caused by cyanobacteria which outcompete other algae species due to the possession of various functional features. In order to evaluate how allelopathically active macrophyte *Myriophyllum spicatum* affects harmful algae blooms, a coexistence experiment lasting for 13 days was designed in 4 macrophyte-full and 4 macrophyte-free mesocosms filled with cyanobacteria dominated blooming water from the Curonian lagoon. Fluorimetric chlorophyll a concentration, algae primary productivity measured by oxygen evolution, cyanobacteria nitrogenase activity measured by acetylene reduction analyses showed that *M. spicatum* lowers cyanobacterial and total chlorophyll a concentrations by 48% and 30%, respectively, has negative effects on algae primary productivity and has different effects on cyanobacterial nitrogenase activity. Our results prove that growth promotion of allelopathically active macrophyte *M. spicatum* can help to control harmful algae blooms.

References

1. Science of the Total Environment. 2010. Vol. 409: 1739–1745.

Symposium 7. Climate Change, Invasive /Alien Species and Chemical Ecology

SEED GERMINATION INHIBITION BY HOGWEED *HERACLEUM SOSNOWSKYI*

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Assessment of the total phenols content (TPC) and biochemical impact of *H. sosnowskyi* on perennial ryegrass (monocots) and winter rapeseed (dicots) seed germination was carried out *ex situ*. Aqueous extracts of 2-yr. *H. sosnowskyi* exhibited higher phytotoxicity if compared with 1-yr. plant extracts. According to the results obtained, all parts of *H. sosnowskyi* produce phenolics, and thus inhibit acceptor-species seed germination. The character of phytotoxic impact of *H. sosnowskyi* aqueous extracts on the germination data significantly depended on plant age (1-year, 2-year), plant parts extracted (shoot; stem, leaf, blossom, seed; root), growth stage (rosette-ripening) and extract concentration (0.02–0.2%) (1). The most pronounced phytotoxicity of *H. sosnowskyi* was recorded at flowering stage due to highest TPC (30.42 mg ml^{-1}). The results suggested that the invasive plant species may acquire spreading advantage in new territories by using allelochemicals to inhibit germination. Nonetheless, species evidence for allelopathic effects should not be restricted to analysis of the plant extracts in the lab, but also based on research in natural environment.

References

1. Allelopathy Journal. 2009. Vol. 23: 229–236.

ISOLATION AND IDENTIFICATION OF AN ALLELOPATHIC COMPOUND IN THE INVASIVE PLANT SOSNOVSKYI HOGWEED, *HERACLEUM SOSNOWSKYI*

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Heracleum sosnowskyi Manden. originates in the central and eastern Caucasus, western, central, eastern and southwestern Transcaucasia and northeaster Turkey (1). The plant was introduced into Lithuania in 1950 (2). The spread of invasive plant species has threatening effects on native communities, biological diversity, and is one of the most serious problems today. We evaluated allelopathic properties of *H. sosnowskyi* in laboratory conditions. Statistically significant inhibition of seed germination by water extracts of Sosnowskyi hogweed was established in all the test-plant seeds: *Triticum aestivum* L., *Sinapsis alba* L., *Avena sativa* L. and *Lactuca sativa* L. *S. alba* seed germination was completely inhibited when exposed to acidic fraction, less when exposed to neutral and the least when exposed to alkaline fraction compounds. *L. sativa* seed germination was completely inhibited when exposed to neutral fraction compounds, whereas *T. aestivum* and *A. sativa* seed germination was inhibited nearly to the same level by the compounds of all fractions. GC/MS analysis (after extraction with diethyl ether and fractionation on silica gel column) allowed us to identify isopsoralene as the main compound in the alkaline fraction of *H. sosnowskyi*. This compound (like many other furanocoumarins (3), possesses allelophatic activity. Synthetic isopsoralene was active at concentration of 40 μg/mL and suppressed *S. alba* seed germination to appr. 94%, while at concentration of 1 μg/mL it suppressed seed germination appr. to 6%. The identification of other allelopathic compounds is still in progress.

References

1. Diversity and Distributions. 2007. Vol. 13: 99–114.
2. The Giant Hogweed Best Practice Manual, 2005.
3. Int. J. Biol. Chem. 2011. Vol. 5: 86–90.

FLEXIBLE AGGREGATIVE BEHAVIOR OF *HARMONIA AXYRIDIS* ACCORDING TO THE FRESHNESS OF AREA MARKING IN OVERWINTERING SITES

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The multicolored Asian ladybeetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) aggregates inside dwellings and buildings during winter to survive cold. This adaptive behavior causes annoyances to the occupants from their number and the induction of allergic reactions. Although this species has aroused a great interest these last years, the factors involved in the selection of its overwintering site remain misunderstood. The work presented herein was oriented to the study of the non-volatile chemical compounds involved in this behavior. Chemical analyses revealed the presence, in aggregation sites, of an area marking made up of saturated and unsaturated hydrocarbons. Afterwards, behavioral investigations showed that *H. axyridis* preferentially aggregates in sites previously marked by congeners, indicating the retention potential of this blend on overwintering individuals of this invasive species. In the second instance, the same analyses were performed on an area marking aged of one year. The chemical investigations showed that only saturated hydrocarbons can still be detected after that period of time but the remaining blend does not induce anymore an aggregation of *H. axyridis* at the place where it was deposited. This difference of *H. axyridis* response according to the freshness of the area marking suggests that this species would not be prisoner of the marking previously deposited on the substrate if the surrounding has changed and the site is not suitable anymore.

VOLATILE CHEMICALS OF ADULTS AND NYMPHS OF THE *EUCALYPTUS* PEST, *THAUMASTOCORIS PEREGRINUS* (HETEROPTERA: THAUMASTOCORIDAE)

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Thaumastocoris peregrinus is an introduced "true bug" that is now a severe pest in *Eucalyptus* plantations of various Southern Hemisphere countries. The semiochemicals of thaumastocorids are completely unknown. Therefore, volatile chemicals from *T. peregrinus* nymphs and adults were identified as possible leads for pheromones potentially useful for control. The contents of nymphal exocrine glands, which are shed at molting, were identified from extracts of exuviae. Adults lack functional metathoracic scent glands that are characteristic of most heteropterans; however, both males and females possess a glandular-appearing hold-fast organ that they quickly extrude posteriorly when disturbed. Whole body hexane extracts from males and females were prepared by freezing the insects in a flask so that they extruded the hold-fast organ, and then they were extracted with hexane. Volatiles from nymphal exuviae included benzaldehyde, octanol, (*E*)-2-octenol, octanoic acid, decanal, and hexanoic acid. Adult volatiles included 3-methyl-but-2-enyl butyrate, 3-methyl-but-3-enyl butyrate.

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DIFFERENCES IN CUTICULAR COMPONENT COMPOSITION AS AN IDENTIFICATION KEY FOR TWO ECONOMICALLY IMPORTANT *LIRIOMYZA* SPECIES

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The comparative analysis of cuticular compound profiles of *Liriomyza bryoniae* Kaltenbach and *Liriomyza huidobrensis* Blanchard (Diptera, Agromyzidae) showed that these morphologically very similar species could be easily distinguished one from another by their cuticular components. The results obtained revealed that for comparative analysis, extracts from insect cuticle could be prepared from either dried or frozen individuals of each species. The identification of two cuticular compounds characteristic only of *L. bryoniae* cuticle was carried out. These components were identified as (*Z*,Z)-9,12-octadecadienoic acid and (*Z,Z,*Z)-9,12,15-octadecatrienoic acid methyl ester. These epicuticular fatty acids were not detected in the cuticular compound profile of *L. huidobrensis* and can be used as identification keys to distinguish *L. bryoniae* species from *L. huidobrensis.*

IMPACT OF *MYZUS PERSICAE* INFESTATION ON THE VOLATILE EMISSION OF *ARABIDOPSIS THALIANA* COL-0

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Being members of complex communities, plants often emit a wide range of volatile organic compounds to defend themselves against insect invasions. Although many studies exist on insect-induced plant volatile emission, most of them either compare the influences of various herbivore species on one plant species or the impact of a given herbivore on several host plant species. Moreover, informations related to the influence of insect density as well as the infestation duration are still needed. Here, we showed that a sucking insect – *Myzus persicae* (green peach aphid) induced the volatile emission from *Arabidopsis thaliana* Columbia wild-type (*A. thaliana* Col-0) under laboratory conditions based on results obtained by solid-phase micro-extraction coupled with gas chromatography – mass spectrometry (SPME-GC-MS). The released volatile blend was discussed in relation to related biosynthesis pathways and functions. These included terpenoids, green leaf volatiles, alcohols and isothiocyanate. The qualitative and overall proportion of volatile components differed depended on the number and residence duration of aphids on leaves. By studying the effects of sucking insect stresses to plant, we not only aim to contribute to the fundamental understanding of the emission of volatile components in the interaction between plants and pests, but also to provide standardised and easy to use assays to assess *A. thaliana* volatile changes according to cross stresses, including both biotic and abiotic ones in ongoing experiments.

References

1. Pareja M., Qvarfordt E., Webster B., Mayon P., Pickett J., Birkett M., Glinwood R. 2012. Herbivory by a Phloem-Feeding insect inhibits floral volatile production. Plos one. Vol. 7(2): 1–11.

2. Snoeren A. L. T., Kappers F. I., Broekgaarden C., Mumm R., Dicke M., Bouwmeester J. H. 2010. Natural variation in herbivore-induced volatiles in *Arabidopsis thaliana*. Journal of Experimental Botany. Vol. 61(11): 3041–3056.

3. van Poecke M. P. R., Posthumus A. M., Dicke M. 2001. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. Journal of Chemical Ecology. Vol. 27(10): 1911–1028.

Symposium 8. Applied Chemical Ecology

ARE THERE OPTIMAL STRATEGIES FOR FINDING AN ODOR PLUME? LESSONS FROM MOTHS

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To maximize the probability of rapid contact with a female's pheromone plume, the trajectories of male foraging flights might be expected to be directed with respect to wind flow and also to be energetically efficient. Flights directed either upwind, downwind, or crosswind have been proposed as optimal strategies for rapid and / or energetically efficient plume contact. Other possible strategies are random and Lévy walks, which have trajectories and turn frequencies that are not dictated by the direction of wind flow. The planar flight paths of males of the day-active bog moth *Virbia lamae* were recorded during the customary time of its sexual activity. We found no directional preference in these foraging flights with respect to the direction of contemporaneous wind flow, but, because crosswind encompasses twice the possible orientations of either upwind or downwind, a random orientation is in effect a *de facto* crosswind strategy. A crosswind preference should be favored when the plume extends farther downwind than crosswind and this strategy is realized by *V. lamae* males by a random orientation of their trajectories with respect to current wind direction.

References

1. Journal of Animal Ecology. 2012. Vol. 81: 268–276.

ERGOGRAPHY: A NOVEL METHOD FOR ANALYZING AND UNDERSTANDING TEMPORAL WORKING PATTERNS OF PROLIFIC CHEMICAL ECOLOGISTS

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Ergography is defined as a method with temporal resolution power for plotting, viewing, documenting, analyzing and understanding working patterns of prolific entomologists. To this end, the frequency and number of papers, theses, and books are plotted against time to yield an "ergogram". R. L. Metcalf (1916–1998), H. Schmutterer (born 1926), E. O. Wilson (born 1929) and T. Eisner (1929–2011) were selected as examples of entomologists from the New and Old world whose work was subjected to this novel approach. Unlike static bibliographies, ergograms reveal dynamic, interconnected and integrated working patterns and make them transparent. Thus, ergographic analysis is able to "demystify" to some extent the hidden creative processes underlying research in chemical ecology. In the case of R. L. Metcalf to whose work this quantitative approach was first applied by Hummel and Lampman (1), ergography immediately revealed at least half a dozen research fields (agricultural entomology, integrated pest management, chemical ecology of plant-insect interactions, pheromones and kairomones, ecotoxicology and pesticide toxicology, delayed neurotoxicology, medical entomology, application technology, and 40+ contributions to the *Diabrotica* pest complex). These fields become immediately evident and understood in a clarity hidden before even to Metcalf's closer associates.

Similarly, H. Schmutterer's contributions to the at least six different research fields become transparent by subjecting his life's work to this kind of quantitative analysis.

T. Eisner leaves behind an immensely rich oevre of 11 books, 490+ original publications in several languages in which he was fluent, films, numerous recorded public appearances and testimonials before the US Congress on professional and societal issues, and of course his regular academic "fireworks" for students of Cornell University.

Up to the year 2006, E. O. Wilsons bibliography lists 414 original papers and 22 books.

Ergography nicely depicts both similarities, but also individual characteristic differences between researchers. Metcalf's ergogram reveals a peak of publication productivity around 1974 in his 58th year. His 450+ publications, 7 books and dozens of doctoral theses cover some 55 years of restless activity, whereas Schmutterer's ergogram lists 225 publications, 90 doctoral theses and 9 books. His output peaked around 1990 at age 64 with 12 publications. Notably, in the field of the insect antifeedant and natural product azadirachtin alone, he published 70 papers and 5 books in the course of 2 decades, all with a major worldwide impact on agricultural entomology. More details on the life's work of these authors based on careful numerical analysis will be elaborated in the future. I

In conclusion, ergographic analysis evidently is a useful, retrospective, powerful tool whose potential will be more fully appreciated after it has been applied to a larger circle of chemical ecologists.

References

1. Eisner T. American Scientist. 1988. Vol. 76: 451.
2. Hummel H. E., Lampman R. L. 2003. Comm. Appl. Biol. Sci Gent. Vol. 68(4a): 29–44.
3. Hummel H. E., Sanguanpong U. 2008. Comm. Appl. Biol. Sci Gent. Vol. 73(3): 471–480.

FIFTY YEARS OF PHEROMONE SCIENCE

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Chemical ecology is the science of species interaction via secondary chemical substances. Depending on their function as sex attractants, trail and territorial determinators, defensive secretions, group cohesion and caste determinating substances, lures for food plant and oviposition site recognition, different terms have been invented. While contemporary biologists now take the term as well as the concept of "pheromones" for granted, it was a sensational event when the January 1959 issue of the British Nature magazine published a two page communication by Karlson and Lüscher on "Pheromones, a new term for a class of biologically active substances". This seminal paper appeared simultaneously with the first identification of the chemical structure of any pheromone, bombykol. In this case, it was the main pheromone component (*E,Z*)-10,12-hexadecadien-1-ol isolated and identified from domesticated female silk moths, *Bombyx mori* (Lepidoptera: Bombycidae), and inducing very specifically and in most minute concentrations the males of this species to a mating dance which could serve as a behavioural indicator for both the presence and also the concentration of this sex pheromone. Two years later, the total synthesis of this compound via several independent routes by Butenandt, Hecker and co-workers in 1961 concluded more than twenty years of pioneering work into uncharted territory. Immediately, the impact of this discovery for chemical communication, sensory physiology and practical plant protection was recognized and paved the way for establishing chemical ecology as an academic but also as a practical endeavour. Thus, bombykol served as a prototype for an entirely new class of exogenously active natural signal compounds and paved the way for subsequent developments.

The structural motive of aliphatic, unsaturated hydrocarbon chains with or without functional groups was subsequently repeated time and again, although later on also some aromatic, cyclic, and many branched structures were discovered. Therefore it did not come as a surprise when the enterprise of analytically inclined chemical ecologists boomed. It produced, within a few decades, thousands of new pheromones described within a body of twenty thousand original research articles and at least 4 dozen monographs. Today, pheromones are known from pest insects in all major food and fiber crops and from stored products. Most prominent are still insect sex attractants because of their spectacular function, a phenomenon that already had fascinated the great French naturalist J. H. Fabre during the last decades of the 19th century, although he then was at a loss for a good explanation.

Meanwhile, crop protection via pheromones is a worldwide endeavour. In integrated pest management, pheromones and the related kairomones play a key role as lures in traps for monitoring and mass trapping, and without traps for mating disruption. In favourable cases like in cotton, in fruit, and in produce pest management or in glass house cultures, pheromones can compete with the non sustainable chemical pesticides which provoke ecotoxicity and resistance. Today, pheromones not only of insects but of arthropods like spiders and of some invertebrates are chemically known.

Progress in vertebrate pheromone research is noteworthy, but somewhat slower to develop. If exploited, its long term impact on human society, however, may be enormous.

FROM JUSTUS LIEBIG STYLE COMBUSTION ANALYTICS TO BIOFET BIOSENSORS: A QUANTUM LEAP OF TECHNICAL REFINEMENT, WITH CONSEQUENCES FOR CHEMICAL ECOLOGY

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Justus Liebig (1803-1873) introduced his famous 5-bulb-absorption-apparatus, trade mark of his lab, prior to 1831 (1). It complemented the combustion tubes known to Gay-Lussac (Paris) and Berzelius (Sweden) who needed, for one organic analysis, samples in the decagram range. Liebig greatly reduced the mass requirement by a factor of one hundred and more and could at least characterize if not identify organic substances by establishing molar composition. Combustion tubes, absorption of generated volatiles (H_2O, CO_2, N_2), sensitive analytical balances, arithmetic, and a lot of patience were his tools.

Simultaneously, R. Bunsen (1811–1899) and G. Kirchhoff (1824–1887) invented spectral analysis which during the 20th century developed into modern spectrometric techniques such as UV-Vis, IR, NMR, MS, ORD, CD. Later on, chromatographic techniques first used by M. Tsvet (1903) joined the arsenal of analytical tools by providing rapid and efficient separation of complex mixtures followed by (usually) their non-destructive physical analysis by electromagnetic radiation and revealing the chemical identity of the separated components. Hyphenated tandem chromatography-spectrometry (e. g. GLC-MS, HPLC-MS, HPLC-NMR, HPLC-UV) created the refined tools we today take for granted in rapid characterization and on-line identification of minute substances available in chemical ecology and pheromone science.

Another independent information channel emerged from using insect antennae as bioelectro-analytical tools. During the second half of the 20th century, EAG and EAD were developed from the school of D. Schneider in 1957 and his associates Kaissling, Priesner, and Boeckh. Hyphenation of these tools with gas chromatography created GLC-EAD (2) and single cell recordings (3) and facilitated the short lived but immensely specific isolated insect BioFET biosensors known today (4, 5).

The power of this approach and the ingenuity of nature in creating and optimizing these biotools are demonstrated by our present ability to use various insect antennae, including that of the Colorado potato beetle, for testing and quantifying mixtures for bioactivity.

In retrospect, a refinement in sensitivity and specificity by a factor of one million and more was achieved within the span of 150 years. This quantum leap was a blessing to analytical chemistry and chemical ecology alike. Without these tools, chemical ecology in the modern sense would be unthinkable.

References

1. Liebig J. 1831. Poggendorffs Annalen der Physik und Chemie. Vol. 21: 1–43.
2. Arn H. et al. 1975. Z. Naturforsch. Vol. 30c: 722–725.
3. Wadhams L. J. 1984. Techniques in Pheromone Research. 179–189.
4. Schütz S. et al. 1995. Naturwissenschaften. Vol. 84: 86–88.
5. Schütz S. et al. 2000. Sensors and Actuators. Vol. B65: 291–295.

SENSITIVE MICROMETHODS FOR QUANTIFYING INSECT PHEROMONES EMITTED FROM VARIOUS DISPENSER MATERIALS IN THE LOW NANOGRAM RANGE: A BLESSING FOR CHEMICAL ECOLOGY

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Quality control guided manufacturing and application of dispensers for insect pheromones is a demanding and ambitious task that occupied skilful analytical chemists for several decades (1, 2, 3). Tandem chromatographic-spectrometric methods for the low micro- to nanogram level play an important role, but other non-chromatographic tools are also employed for facilitating quantification and independently verifying GC results.

Employed were wind tunnel studies with gravimetric evaluation of pheromone loss; thermogravimetric studies for quantifying pheromone loss kinetics; scanning microscopic investigations for evaluating changes of nanofiber morphology with time; tandem CLSA-GLC-FID (4) and field bioassay studies with the Doyé-type field cage (5). All these studies were combined to arrive at a coherent picture of release rates which are necessary for appraising from on-line results if and for how long the spatial and temporal pheromone distribution in the field will fulfil the commonly agreed test criterion, the release level of 1 mg/day/ha/source, at 500 sources/ha. The grapevine moth *Lobesia botrana* pheromone (*E,Z*)-7,9-dodecadienyl acetate served as a well investigated model compound in vineyards at Freiburg, Germany.

So far, we compared commercially available ISONET®, RAK®, and non-commercially produced organic ECOFLEX®-nanofiber dispensers of our own design ((6), patents pending). These dispensers fulfil the above test criteria for 185, 70, and 49 days, respectively.

The task of working with ever more sensitive and sophisti-cated analytical methods so far is only partly solved. But solutions are in high demand from application engineers, insect pest managers, and economists searching for mechanizable ways of pest control with sustainable and biocompatible methods. In the near future, we will also field test a specialized, portable GLC-FID-MS-EAD apparatus (7) in an attempt to obtain a streamlined chain of actions. These reach from nanogram scale sample acquisition to quantifiable pheromone determination with several independent and simultaneous detectors, and are followed by practical interpretation of results. The ultimate goal is an on-line tandem method for communication disruption studies and instrument guided pest management decisions in vineyards and other field crops.

References

1. Leonhardt B. A., Beroza M. 1982. ACS Symposium Series.
2. Hummel H. E., Miller T. A. 1984. Techniques in Pheromone Research. Springer.
3. Millar J. G., Haynes K. F. 1998. Methods in Chemical Ecology. Kluwer Academic Publ.
4. Lindner I. et al. 2012. Comm. Gent. Vol. 76(4): 819–830.
5. Doyé E. 2006. PhD-Thesis, TU Kaiserslautern, Dept. of Biology.
6. Greiner A., Wendorff J. H. 2007. Angew. Chem. Int. Ed. Vol. 46: 5670.
7. Schott et al. 2012. HTC-12 Bruges, Abstract.

ORGANIC NANOFIBERS: A NEWLY DEVELOPING PARADIGM IN PHEROMONE DISPENSER TECHNOLOGY

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Pheromones as natural products occupy a privileged position in agriculture and viticulture. Their environmental compatibility, their high specificity and efficiency are, among others, their most prominent and attractive features.

Much less favorably developed is the aspect of suitable dispensers, in spite of nearly 50 years of effort. Economic entomologists and application engineers dearly wish they had the "smart, intelligent and ideal dispenser". Commercially available dispensers fall short of meeting all demands. Their field development should be mechanizable and be accomplished by one (or very few) application runs. Required dispensers, like the pheromones they emit, should be biodegradable, biocompatible, sustainably applicable, and they should be based on renewable resources.

Here we present first results of a novel organic, electrospun nanofiber dispenser with dimensions in the upper nanometer to low micrometer range. Its load of pheromone can be adjusted to be sufficient for 7 weeks of constant disruptive action in vineyards and can be directed against the European grape vine moth *Lobesia botrana* (Lepidoptera: Tortricidae), whose mating disruption is well studied by previous authors. Equally, nanofiber production by electrospinning (1) is well known and already has numerous applications in filtration technology, air conditioning, and medical wound dressing. The challenge was to bring together and successfully mate these (partly incompatible) technologies via technical tricks. We still must double the lifetime of currently available nanofibers to last for one growing season. Another challenge is the mechanical distribution of the fibers in the vineyards by suitable machinery currently under construction and development. Nanofibers of the Ecoflex® type (an aliphatic aromatic copolyester, investigated by (2)) are fully biodegradable within half a year of environmental exposure and, according to Julius Kühn Institute, the German independent federal authority. They kindly monitor in numerous test systems the compatibility of organic nanofibers for their effects on human, animal, and environmental health. As of today, organic nanofiber technology was found to be fully compatible with environmental health regulations. In essence, organic nanofibers emerge as a novel tool suitable for mating disruption studies in major insect pests.

Acknowledgements

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References

1. Greiner & Wendorff Angew. 2007. Chem. Vol. 46: 5670–5703.
2. Witt et al. 2001. Chemosphere. Vol. 36: 570–583.

COULD MATING DISRUPTION BORDER TREATMENTS AND BARRIERS PROTECT STONE FRUIT BLOCKS FROM INVASION OF ORIENTAL FRUIT MOTH *GRAPHOLITA MOLESTA* BUSCK. (LEPIDOPTERA, TORTRICIDAE)?

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Pheromone-mediated mating disruption (MD) is a major tool of sustainable, effective and environmentally-friendly Integrated Pest Management (IPM) systems in Australian orchards. Oriental fruit moth (OFM) (*Grapholita molesta* Busck, Lepidoptera: Tortricidae) is the most important pest of commercial stone fruit orchards in Victoria, Australia. OFM could migrate within orchard between blocks, as well as between distant orchards. MD applied in stone fruit blocks within orchard successfully controlled OFM populations for many years, but damage to shoot tips and fruit at the border of peach blocks located adjacent to pear blocks under insecticide treatments has become problematic. To improve protection of stone fruit against border damage and invasion of OFM from neighbouring pome fruit blocks within the orchard, MD was applied as 30 and 60 meters barrier treatments of neighbouring pears adjacent to MD peaches. Detailed monitoring of the OFM population under MD with kairomone traps, shoot tip and fruit damage assessments indicated that application of MD barriers on pears during two consecutive seasons provided improved control of OFM on MD peaches. Such MD barrier treatment was able to reduce the number of OFM caught in all experimental peach blocks, with damage to shoot tips and fruit giving similar results to MD treatment of the whole neighbouring pear block. Extending the MD treated area for 10 tree rows into the neighbouring pear block significantly reduced the border damage in MD treated peaches in the first season and almost eliminated OFM damage in the second season.

EVIDENCE OF A FEMALE PRODUCED PHEROMONE IN A CETONIIN CHAFER, *EPICOMETIS HIRTA* (COLEOPTERA, SCARABAEIDAE)

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Adults of the blossom chafer, *Epicometis hirta* Poda (Coleoptera: Scarabaeidae: Cetoniinae), are economically important horticultural pests in Central and Southern Europe, damaging the generative parts of plants. Although a funnel trap exploiting visual (light blue colour) and olfactory (synthetic floral blend) cues is available for detection and monitoring (1), joint application of the sex pheromone of *E. hirta* would presumably enhance trap efficacy. Here, we report on the first results of studies towards the identification of a pheromone in *E. hirta*.

Preliminary field observations on the behaviour of the day-flying *E. hirta* suggested that males are attracted to females sitting on flowers, predominantly dandelions (*Taraxacum officinale* Weber, Compositae). In order to quantify male attraction to females, *E. hirta* were first field collected in the early part of the flight period in large numbers using colour traps baited with floral volatiles. Beetles were separated into males and females by outer morphological characteristics. Ten individuals of each sex were put into cages with a piece of apple (*Malus domestica* Borkh.), a food source previously found to be suitable for keeping *E. hirta* alive for a long period. Apple pieces in cages served as control treatments. Funnel traps were baited with the cages, adding another piece of apple in the catch container to keep captured insects alive.

Males were attracted to female + apple baited traps, but not to either the male + apple or apple baited traps ($P < 0.000$ and $P < 0.0001$) at two experimental sites. Females were not attracted to either sex or to apple pieces alone; however, simultaneously operating colour traps baited with floral volatiles, caught significant numbers.

Based on the present results, the existence of a female produced sex pheromone is highly probable for *E. hirta*. A further proof of this is that stimuli from extracts of female full body washes and female air entrainment samples evoked EAG responses from male antennae which were significantly stronger than the solvent control ($P = 0.013$).

Acknowledgements

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References

1. Zeitschrift für Naturforschung C. 2004. Vol. 59: 288–292.

TRAPS BAITED WITH PEAR ESTER AND ACETIC ACID ATTRACTING BOTH SEXES OF THE GREEN BUDWORM MOTH, *HEDYA NUBIFERANA* (LEPIDOPTERA: TORTRICIDAE)

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When testing the attractiveness of pear ester [PE; ethyl (*E,Z*)-2,4-decadienoate] plus acetic acid (AA) lures to codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in Hungary, significant catches of the tortricid, *Hedya nubiferana* (Haworth) (Lepidoptera: Tortricidae), were also recorded. In all cases, traps baited with the PEAA lure caught far more than traps baited with either of the constituents presented alone. When comparing the activity of PEAA lures containing different amounts of PE, very broad dosage window found to be attractive for *H. nubiferana*. The importance of PE in the chemical communication of *H. nubiferana* is underlined by the outstandingly high EAG responses evoked from the antennae of females. PEAA lures were attractive to *H. nubiferana* no matter whether the two compounds were provided in separate dispensers or mixed together in a single one, and a large percentage (up to 45%) of trap catch were females. Traps with PEAA lures in some tests caught (females and males together) up to ca. 70% of the catch in traps baited with the synthetic green budworm moth sex pheromone (all males). The seasonal flight patterns recorded with the PEAA baited traps were similar to those with the sex pheromone. Consequently, the PEAA lure shows potential for future practical applications as a female-targeted lure for *H. nubiferana*. To our knowledge, this is the first report of the attractiveness of a lure containing pear ester and acetic acid for a tortricid species other than codling moth.

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PLANT COMPOUNDS MODIFY BEHAVIOURS OF BOTH *FRANKLINIELLA OCCIDENTALIS* ADULTS AND LARVAE

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Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), the western flower thrips, is one of the most harmful insect pests on agricultural and horticultural crops world-wide. Adults as well as immature stages of this highly polyphagous species both feed on plant tissue by penetrating plant cells and sucking out the cell sap. The search for bioactive plant compound for use in thrips control strategies previously revealed the monoterpenoid phenol carvacrol applied to crop plants to act as feeding and oviposition deterrent against *F. occidentalis* adult females.

Because thrips larvae settled on leaves will likely get in contact with a deterrent compound that is applied to a crop plant, we compared behavioural responses of second instar larvae and thrips females to direct contact with carvacrol at different concentrations applied to bean leaves. Both *F. occidentalis* life stages clearly avoided direct contact with a carvacrol-treated leaf surface, and the feeding activity of thrips larvae was significantly reduced by a carvacrol application. Determination of the DC_{50}, i. e. the concentration of carvacrol required to produce 50% feeding deterrence, in choice bioassays showed that thrips larvae respond to a 6-fold lower concentration of carvacrol than adult females. To our knowledge this is the first study on effects of plant compounds on behaviours of thrips larvae. Bioactive plant compounds that modify the behaviour of different life stages of *F. occidentalis* might be used as synergists for various biological or chemical control measures in conventional, integrated or organic farming systems.

BEHAVIOURAL AND CHEMICAL CORRELATES OF REPRODUCTIVE HIERARCHIES IN THE QUEENLESS ANT *DINOPONERA GIGANTEA* (FORMICIDAE, PONERINAE)

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About 100 species of ants in the subfamily Ponerinae have lost the morphologically specialized queen castes and in these species workers can mate and lay fertilized eggs. Workers are identical but in each colony only a few of them can reproduce sexually, becoming what has been called gamergates. Reproductive division of labour between the totipotent workers of queenless species is usually regulated through aggressive interactions which lead to the set-up of dominance hierarchies. Subordinates workers generally have undeveloped ovaries whereas high-ranking workers can lay eggs. The genus *Dinoponera* is composed of six monogynous queenless species with small colonies of less than 200 workers. In these species, only the top-ranking worker mates and becomes a gamergate able to lay fertilized female eggs. Other workers in the hierarchy can only lay unfertilized male eggs. Younger individuals usually join the high-ranking workers because of a higher potential fertility and therefore each emergence triggers an increase in agonistic and ritualized behaviour until the hierarchy is stabilized. In *D. quadriceps*, the gamergate exhibits a specific cuticular hydrocarbon profile and the hierarchy is maintained through specific ritualized behaviour such as gaster rubbing, blocking and immobilization. Here we studied a closely related species, *D. gigantea*, which has never been studied before in this context. We investigate whether a similar combination of pheromones and behaviour is at the basis of the reproductive division of labor in this species by monitoring 10 colonies collected in Belém, Pará, Brazil. We studied the hierarchy between high-ranking workers and analysed the cuticular profile of young, high and low-ranking individuals by SPME and GC-MS to correlate behaviour and rank with potential signalling compounds.

THE COST OF VENGEANCE: HERBIVORE-ATTACK IMPROVES RESISTANCE AND REDUCES FITNESS OF REGROWING SHOOTS

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When *Manduca sexta* feeds from *Nicotiana attenuata* leaves, systemic signals are deployed and trigger a reconfiguration of the root metabolism (1, 2). This reconfiguration includes the induction of costly alkaloids (3) as well as the sequestration of photo assimilates for tolerance purposes (4). Until today, it remains unclear whether these two counteracting processes increase or decrease the overall regrowth capacity of *N. attenuata* after herbivore defoliation. To test this question, we induced plants with real and simulated *Manduca sexta* feeding, removed their leaves and stems and measured regrowth and resistance of the newly forming shoots. We found that *Manduca sexta* attack strongly impairs the regrowth capacity of *N. attenuata*, resulting in reduced biomass and flower numbers. This effect could not be explained by a reduction of root biomass, or the removal of leaf-photosynthetic tissue and meristems. On the other hand, non-targeted metabolomics (5) revealed strong metabolic changes in the roots of leaf-attacked plants, and we found that the regrowing leaves were significantly more resistant against *M. sexta*. We are currently testing transgenic *N. attenuata* lines that are silenced in the production or perception of major stress signaling molecules to evaluate whether the reduction in regrowth is due to the increased defensive investment of attacked plants. We hypothesize that this approach will make it possible to evaluate the systemic costs of induced defenses and may reveal the presence of root-mediated tolerance processes.

References

1. Wu and Baldwin. 2010. Annu. Rev. Genet. Vol. 44: 1–24.
2. Steinbrenner et al. 2011. J Chem Ecol. Vol. 37: 1294–1303.
3. Baldwin. 1998. Proc. Natl. Acad. Sci. USA. Vol. 95: 8113–8118.
4. Schwachtje et al. 2006. PNAS. Vol. 103(34): 12935–12940.
5. Gaquerel et al. 2010. J. Agric. Food. Chem. Vol. 58: 9418–9427.

BEHAVIORAL STUDIES WITH OVIPOSITION PHEROMONE IN *CULEX* MOSQUITOES AND STUDY OF THE OBP1 GENE EXPRESSION IN ADULT MALES AND FEMALES

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In species with aquatic larvae, maternal reproductive success depends on the selection of a suitable site for depositing their eggs. This selection determines the continuation of such a species since, once in a hostile environment, immature stages are unable to move to another more suitable habitat and will perish (1). Female *Culex* mosquitoes deposit their eggs in the form of egg rafts on the water surface. Some species form a droplet at the apex of each egg in the egg raft which affects the oviposition behavior of conspecific gravid females (2). Insect attractants and traps are useful tools for monitoring insect populations and determining the need for control. Except blood meals, a complete oviposition cycle is required for the transmission of a disease after pathogen acquisition (3). Therefore, screening gravid female mosquitoes for virus infection provides a suitable tool for the estimation of the infective population and for virus surveillance (3, 4). Odorant binding proteins (OBPs) expressed in the female's antennae play a crucial role, albeit imperfectly understood, in sensing oviposition cues (5, 6). This study explored behavioural and gene expression aspects of odorant detection in *Culex pipiens* biotype *molestus*. The response of caged female and male mosquitoes to the oviposition pheromone was studied in relation to mating status and the results indicate that virgin and gravid females are equally attracted to the substance. Moreover, the role of the pheromone as an oviposition stimulant was confirmed in this study, as the number of eggs laid was significantly enhanced in the presence of the oviposition pheromone. The expression of the OBP1 gene, encoding a well-characterised odorant binding protein, was compared with qPCR among male and female, virgin, mated and post-oviposition mosquitoes. OBP1 transcript levels were overall higher in males, both among virgin and mated mosquitoes. Mating was shown to increase the expression of the gene in females and reduce it in males. Oviposition did not appear to affect OBP1 expression in female mosquitoes.

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References

1. Austral Ecology. 2003. Vol. 28: 33–37.
2. Hellenic Plant Protection Journal. 2010. Vol. 3: 33–56.
3. Journal of Vector Ecology. 2007. Vol. 32: 83–89.
4. Journal of Vector Ecology. 2007. Vol. 32: 285–291.
5. Journal of Chemical Ecology. 2002. Vol. 28: 867–871.
6. PLoS ONE. 2008. Vol. 3(8): e3045.

(E)-b-CARYOPHYLLENE AS PHEROMONE SYNERGISTS FOR RED PALM WEEVIL (RPW) *RHYNCHOPHORUS FERRUGINEUS* (OLIVIER) (COLEOPTERA: CURCULIONIDAE)

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The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is one of the most destructive pests of the palm trees in many parts of the world. Coupled gas chromatographic-electroantennographic detection (GC-EAD) and GC-MS analyses of Tenax TA volatiles released by male inflorescences of *Phoenix canariensis* and *P. dactiliphera* revealed a main EAD-active component, which was identified as (*E*)-β-Caryophyllene. This sesquiterpene elicited significant electroantennogram (EAG) activity from both *R. ferrugineus* male and female antennae. In field experiments, traps baited with ferrugineol plus (E)-β-Caryophyllene caught more weevils than did pheromone-sugarcane baited traps and significant differences could be found in rates of captures between males and females (1:3.2). Our data suggest that the use of (*E*)-β-Caryophyllene increase the attraction of aggregation pheromone and could be a good alternative to using standard pheromone-food baited traps, mainly because the kairomone dispensers can be easily replaced with new ones and do not require a tedious and continuous replacement.

ASSESSMENT OF SYNTHETIC CHEMICALS FOR DISRUPTION OF *RHYNCHOPHORUS FERRUGINEUS* ATTRACTION

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The red palm weevil (RPW), *Rhynchophorus ferrugineus*, Olivier (Coleoptera: Curculionidae), is currently one of the most severe pests of Canary palms in urban areas of Mediterranean countries and Date palms in cultivated areas of the Middle East. RPW aggregation pheromone can be implemented for both monitoring and mass trapping, however risks of RPW "spillover" onto palms is of great 313concern. Aiming to discover repellent semiochemicals for safer RPW population management, we conducted electroantennographic (EAG) screenings of 17 commercially available synthetic compounds. These compounds represent three groups of plant volatiles (isoprenoids, phenyl propanoid derivatives and fatty acid derivatives) and known for their repellent effects toward insects. These tests were followed by behavioral tests in olfactometer and field trap-based screenings of EAG-active compounds. In particular, menthone, α-pinene and methyl salicylate, singly and in combination, were selected for field studies under urban conditions using pheromone baited traps. RPW antennae of both sexes showed positive dose-dependent responses to 13 of the 17 synthetic chemicals in EAG bioassays. In field trapping experiments, conducted in the city of Palermo, Italy from weeks 31 to 38 in 2010 and 2011, α-pinene, tested singly or in combination with methyl salicylate (2010) or menthone (2011), found to significantly interfered with the attraction of both sexes of RPW to its aggregation pheromone reducing catches in pheromone and kairomone baited trap by about 30 to 40%. When tested alone methyl salicylate and menthone alone did not affect the number of adults captured compared to the control. These results indicate α-pinene as promising RPW repellent that could be included in semiochemical-based strategies of this pest, such as the "push and pull" techniques.

ELECTRICAL ACTIVITY AND SENSORY ADAPTATION OF THE ANTENNAE OF *BLATTELLA GERMANICA* IN RESPONSE TO THE INSECT REPELLENT DEET

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N, N-diethyl-3-methyl benzamide (DEET) is an insect repellent used worldwide. Its effectiveness has been proved in a number of insect species, including haematophagous and non-haematophagous. There are two contrasting hypothesis regarding the mode of action of DEET: the more classical hypothesis proposes that DEET interferes with the detection of odors, in particular host odors in haematophagous, instead of having a repellent effect. On the other hand, recent works demonstrated that DEET acts as an odorant molecule and elicits a behavioral response in the absence of other stimuli. In this work we show with electrophysiological recordings that the antennae of *Blattella germanica* respond to DEET and become adapted when stimulated with long pulses of the same substance.

We also observed the behavioral response of cockroaches to DEET repellent (results not shown). To determine whether continuous stimulation with DEET decrease the response of the antennae to the same compound, we delivered a long pulse of DEET and recorded the response of the antennae before and after the stimulation. We found that a stimulation of 6 or 60 seconds with DEET produced a decrease of 58% in the amplitude of the response to the same compound registered after stimulation. These results are in agreement with the role of DEET as an odor molecule, since it produces electrical response of the antennae of *B. germanica* and it is possible to adapt this response with continuous stimulation of the antennae.

Poster P086

EMPLOYING FLORAL BAITED COLOR TRAPS FOR DETECTION AND SEASONAL MONITORING OF SCARAB PESTS (COLEOPTERA: SCARABAEOIDEA) IN BULGARIA

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Large-scale field investigations for proving the potential and species-specificity of floral baited color traps as a new tool for detection and seasonal monitoring of some scarab pests were organized at eight sites in different regions in Bulgaria in 2009–2010. Three types of trap / bait combinations, commercial product of Plant Protection Institute, Budapest, Hungary, for four species were used: VARb3k traps with a light blue upper funnel for *Tropinota* (*Epicometis*) *hirta* (Poda) and *Cetonia aurata* L. / *Potosia cuprea* (Fabricius), and VARb3z traps with a fluorescent yeallow upper funnel for *Oxythyrea funesta* (Poda). Information about the occurrence in Bulgaria was obtained for all four species and information about seasonal appearance was obtained for the species with the most numerous catches: *T. hirta, C. aurata and O. funesta*. The data allow also making some conclusions concerning species-selectivity of the traps used. Catches of two not target pests: *Valgus henipterus* L. and *Blitopertha lineolata* (Fischer von Waldheim) were also recorded in the traps in almost all the sites. For both species information about their occurrence and seasonal appearance in Bulgaria was obtained.

Our results confirmed earlier data (1) that the traps developed for *T. hirta, O. funesta* and *C. aurata* /*P. cuprea* are very effective in catching the target pests and could be used successfully for their detection and seasonal monitoring. However, the selectivity of the traps is not enough high and in sites where more of one target species is present cross catching could occur.

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References

1. Crop Protection. 2010. Vol. 29: 1177–1183.

(8*E*,10*Z*)-8,10-DODECADIENAL: A MIMETIC OF THE SEX PHEROMONE OF THE HORSE CHESTNUT LEAFMINER, (*CAMERARIA OHRIDELLA*) (LEPIDOPTERA, GRACILLARIIDAE), OR A POSSIBLE NEW MINOR COMPONENT?

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The horse chestnut leafminer (*Cameraria ohridella*) (Lepidoptera: Gracillariidae) utilizes (8*E*,10*Z*)-8,10-tetradecadienal (E8Z10-14:Ald) as its sex pheromone, which is produced by females in picogram quantities. In order to search for compounds which would modify the biological activity of E8Z10-14:Ald, a series of structurally related compounds was designed and synthesized.

Purity of synthesized samples was greater than 96%, as checked by NMR and GC. Biological activity was field tested, using sticky delta traps (Csalomon® RAG) equipped with red rubber dispensers (TAURUS, Budapest, Hungary MSZ 9691/6), and funnel traps (Csalomon® VARL+) equipped by Wheaton sleeve stopper dispensers (Millville, NJ), both trap bodies produced by Plant Prot. Inst., Budapest, Hungary). Electrophysiological activity was tested by means of EAG, using glass electrodes, MP15 micromanipulator and IDAC 232 amplifier (Syntech, Hilversum, the Netherlands).

Results of field tests showed that on its own (8*E*,10*Z*)-8,10-dodecadienal (E8Z10-12:Ald), a homolog being shorter by two carbon atoms as the natural sex pheromone, attracted conspecific males to traps at high doses and in much lower numbers. The mean weekly captures ± S.E of E8Z10-12:Ald at a 100 μg dose and the natural sex pheromone at 3 μg were 355.5 ± 25.5 and 151.5.6 ± 46.1, respectively. The mean weekly captures of unbaited traps were 12.3 ± 9.7 (May 10 – June 1, 2005, Nagykovácsi, Kastélypark, Hungary, sticky traps, 2 traps / treatments, 5 recordings). When added to the sex pheromone in over threefold amounts (10 ug analog to 3 ug pheromone), the combination attracted significantly more males than the natural pheromone alone at the same dose level (4293 ± 1173 vs. 855 ± 707 mean weekly captures ± S.E) (July 6–20, 2005, Érd-Elvira, Hungary, funnel trap type, 4 traps / treatments, 4 recordings).

Results of the EAG test showed that E8Z10-12:Ald on its own did not evoke significantly larger responses than solvent control. Interestingly, however, a 1:1 mixture of E8Z10-12:Ald and E8Z10-14:Ald (10 μg each) evoked significantly larger responses than E8Z10-14:Ald on its own at 10 μg dose level: 502.5 ± 63.3 versus 321.4 ± 31.6% (a response to 10 μg of E8-14Ac, used as a reference standard, was taken as 100%). No such significant differences were found at lower dose levels.

Significance of these findings in the better understanding of peripheral pheromone perception, as well as of chemical communication of this species at behavioral level is discussed.

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PROGRESS IN DEVELOPMENT OF AN OPTIMAL COMBINATION OF LURE AND TRAP DESIGN FOR THE GREY CORN WEEVIL, *TANYMECUS (EPISOMECUS) DILATICOLLIS* GYLLENHAL, 1834 (CURCULIONIDAE)

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For development of an optimised lure and trap design as an effective tool for detection and seasonal monitoring of grey corn weevil, *Tanymecus (Episomecus) dilaticollis,* several field experiments were undertaken. These included comparisons of different lures, different types of dispensers and different trap designs. The most attractive lures were the two-component mixture of compounds already reported as attractants for this species and the five-component mixture containing volatile organic compounds (VOCs) identified from *T. dilaticollis* adults. No significant difference in mean catches of the target species in traps with different dispensers was found, whilst catches in modified sticky pitfall traps were more than four times higher than catches in the rest of the traps.

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IDENTIFICATION AND SYNTHESIS OF COMPOUND FOUND IN *KLADOTHRIPS* EXTRACT

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Thrips (order Thysanoptera) are tiny insects with fringed wings and the size of insects is ranging from 0.5 to 14 mm. Other common names include thunder bugs, storm flies and thunder flies. Thrips are eusocial, meaning that they have a high level of social organization; just like ants, bees and wasp.

Thrips have different food sources including animals and plants, but many of the thrips species are regarded as pests since they feed on plants of great economical value. It is not always easy to find good management but one possibility might be to use semiochemicals. One major benefit with semiochemical management is that it is possible to target a specific insect with a small amount of the chemical, basically the concentrations and ratios that the insect of interest use. This type of management reduces usage of environmentally unfriendly pesticides.

In this study we have investigated *Kladothrips nicolsoni* larvae extracts. The extract has been subjected to chemical analyses such as LC-QTOF, GC-MS and NMR. The structure of the isolated compound has also been verified by synthesis and by retention time comparisons on polar and non-polar GC-columns. We are now investigating the biological activity of extracts and of the synthetic compound.

HONEY BEES SMELL EXPLOSIVES

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Classical olfactory conditioning of the proboscis extension reflex was used to reveal the ability of honey bees, *Apis mellifera* L., to recognise explosive substances often found in explosives.

The ability of honey bees to recognise and learn as well as to generalize different doses of either trinitrotoluene (TNT), ethylene glycol dinitrate (EGDN) or glyceryl trinitrate (NG) was tested. The learning was performed with EGDN and NG at a dose of 10 mg and TNT at a dose of 100 mg. The study results show that worker bees were able to learn and recognise all the three stimuli tested. However, the acquisition rate was different. Bees learnt to detect EGDN the fastest. They needed more learning trials to recognise NG and TNT when compared to EGDN.

After-conditioning tests revealed that honey bees were able to generalize at least two doses of TNT (10 mg ir 100 mg), and three doses of both EGDN (0.1 mg, 1 mg and 10 mg) and NG (0.1 mg, 1 mg and 10 mg).

The current results demonstrate that honey bees can be used as biosensors in developing technologies for the detection of explosives.

LASER VIBROMETER FOR PRECISION RECORDING OF SUBSTRATE BORNE ACOUSTIC SIGNALS

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Recording substrate-transmitted vibratory signals in insects has been difficult and hindered by the lack of a reliable and sensitive measuring device. In the past, various equipment has been used to record substrate-transmitted vibratory signals, such as bamboo membrane and microphone, microphone, and gramophone pickup. Such equipment can be difficult to operate, prone to noise and tend to distort measurements because of the signal detectors mass. Recently a new non-invasive device was developed for precise recording of substrate-transmitted vibratory signals. In this device a red laser pointer beam (~630 nm, ~2 mm Ø) is reflected by a retro-reflective foil with prism spacing in the order of 80 μm, and the reflected light generates interference speckle patterns on surfaces around the laser beam. At ~1 m from the reflector, the speckles are spaced 1–3 mm. If the reflector is vibrated, the speckle pattern moves rapidly. The concomitant modulation of the illuminance of a photodiode is used to detect the movements of the speckles and hence the vibration generated by insects. The new device enables precise measurement and characterization of substrate-transmitted vibratory signals involved in mating and courtship in many insects. Characterization of vibratory mating signals will enable more sophisticated studies of the interactions of semiochemicals and acoustic signals in pest insects.

ATTRACTION OF FEMALE FLATHEAD OAK BORERS *COROEBUS UNDATUS* (COLEOPTERA: BUPRESTIDAE) TO SEMIOCHEMICAL-BAITED TRAPS

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The flathead oak borer (FOB) *Coroebus undatus* Fabricius (Coleoptera: Buprestidae) is one of the primary pests of the cork oak *Quercus suber*, endemic tree of the occidental Mediterranean and only producer of cork. FOB females lay their eggs individually or in small groups in bark flaps and fissures of the trunk. The larvae perforate successive layers of the cork, penetrate into the bark and construct large galleries around the trunk (up to 1.8 m long and 3–4 mm wide), downgrading the cork quality and causing important economic losses. Knowledge of the cues that FOB adults use to locate host trees or sex partners could enhance the current goal to control this pest.

In this communication, we present studies directed to develop a suitable combination of trap type (visual cue) and lure (olfactory cue) to capture FOB adults. Among different types of purple colored traps, prism traps were the most effective in capturing FOB females, significantly more than single panel and Lindgren funnel traps. Single panel traps were superior to Lindgren traps indicating that this type, often used for trapping wood boring and bark beetles, is not appropriate to catch FOB. In addition, prism traps combined with host-plant green leaf volatiles (GLVs) caught more insects than traps baited with other lures tested, suggesting that GLVs play an important role in the host and / or mate finding behavior of FOB females. These data are the first to demonstrate attraction of the genus *Coroebus* to a semiochemical-based trapping method, and set the basis for future studies addressed to monitoring and control of this important species.

SEASONAL VARIATION OF PHENOLIC COMPOUNDS IN BLACK POPLAR (*POPULUS NIGRA*)

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Plants within the family of the Salicaceae produce a variety of phenol derived compounds, such as salicinoids, flavanols and flavonoids. Many of these phenolics counteract deleterious biotic and abiotic influences, e. g. from herbivores, pathogens or photooxidative stress. Under natural conditions plants encounter these stresses simultaneously, yet most studies investigating the role of phenolic compounds in Salicaceae have been performed under controlled conditions with only one particular stress applied.

In this study we investigated the trajectory of phenolic compounds in trees within a natural population of black poplar (*Populus nigra*) in the course of one year. Three different kinds of phenol derived compounds (salicinoids, flavan-3-ols and flavonol glycosides) were investigated in leaves, bark and buds. The phenolic concentrations were strongly dependent on the tree genotype. Moreover, the phenolic content in the leaves was subjected to strong seasonal patterns but was relatively constant in the bark. We argue that the seasonal variation of foliar phenolic concentrations may have a great influence on biotic interactions (e. g. arthropod community, biotrophic fungi) in black poplar.

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