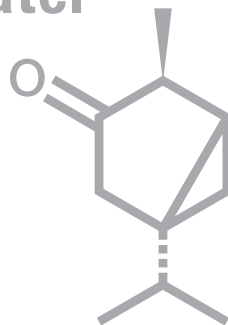
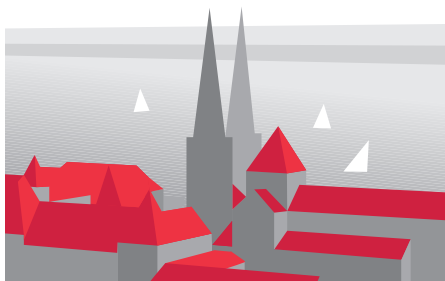
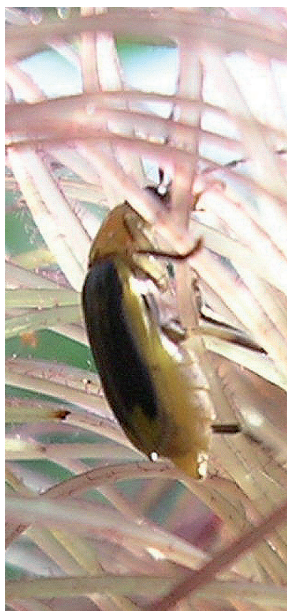


25th Annual Meeting Neuchâtel 2009



International Society of Chemical Ecology

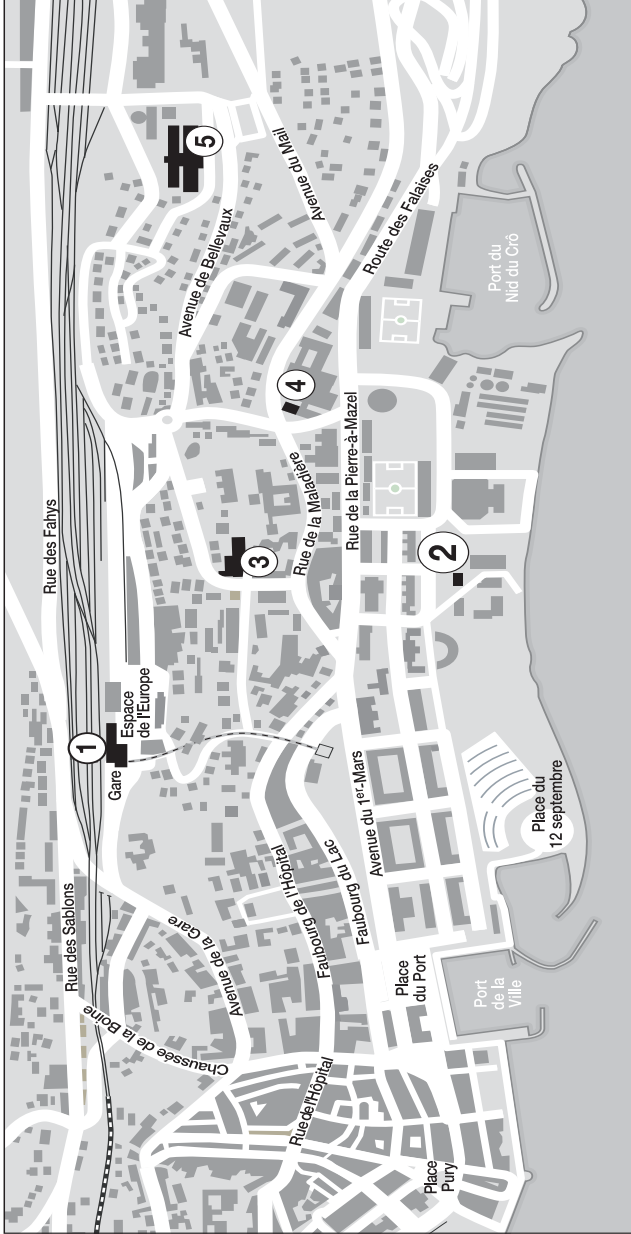


August 23-27, 2009





Where to go ?



Neuchâtel City Map

- 1. Gare CFF / Railway Station
- 2. Aula des Jeunes-Rives
- 3. Cité universitaire
- 4. Restaurant Le Romarin
- 5. Chambres d'hôtes UniMail



Meeting Overview

Sunday, 23 August

Executive committee meeting, 12h30-14h00 (committee members only)
Registration, Aula des Jeunes-Rives, 13h00-17h00
Official welcome, Aula des Jeunes-Rives, 15h00
Special lecture: 25-years ISCE meetings, Aula des Jeunes-Rives, 15h15
Student travel award ceremony, 16h00
Welcoming reception on boat, 17h30-19h00

Monday, 24 August

Symposium 1 - The Chemical Ecology of Vertebrates, Including Humans
Session 1.1: Aula des Jeunes-Rives, 09h00-10h30
Session 1.2: Aula des Jeunes-Rives, 11h00-11h30

Symposium 2 - Pheromones: Synthesis and Perception
Session 2.1: Aula des Jeunes-Rives, 11h30-12h30
Session 2.2: Aula des Jeunes-Rives, 14h30-16h00

Parallel sessions

Symposium 1 - Vertebrates (cont.)	Symposium 2 - Pheromones (cont.)
Session 1.3: room 02, 14h30-18h30	Session 2.3: Jeunes-Rives, 16h30-18h00

Silverstein-Simeone Award lecture, Aula des Jeunes-Rives, 18h10-19h00

Tuesday, 25 August

Symposium 3 - Plant Defense: Mechanisms, Ecology and Application
Session 3.1: Aula des Jeunes-Rives, 09h00-10h30
Session 3.2: Aula des Jeunes-Rives, 11h00-12h30

Parallel sessions

Symposium 2 - Pheromones (cont.)	Symposium 3 - Plant defense (cont.)
Session 2.4: room 02, 14h30-16h00	Session 3.3: Jeunes-Rives, 14h30-16h00
Session 2.5: room 02, 16h30-18h00	Session 3.4: Jeunes-Rives, 16h30-18h00

Silver Medal Award lecture, Aula des Jeunes-Rives, 18h10-19h00

Wednesday, 26 August

Symposium 4 - The Chemical Ecology of Disease Vectors
Session 4.1: Aula des Jeunes-Rives, 09h00-10h30
Session 4.2: Aula des Jeunes-Rives, 11h00-12h30

EXCURSIONS all afternoon (buses in front of hotel Beaulac)

Thursday, 27 August

Symposium 5 - The Chemical Ecology of Pollination

Session 5.1: Aula des Jeunes-Rives, 09h00-10h30

Session 5.2: Aula des Jeunes-Rives, 11h00-12h30

Parallel sessions

Symposium 5 - Pollination (cont.)

Session 5.3: room 02, 14h30-16h00

Symposium 2 - Pheromones (cont.)

Session 2.6: room 02, 16h30-18h00

Symposium 3 - Plant defense (cont.)

Session 3.5: Jeunes-Rives, 14h30-16h00

Session 3.6: Jeunes-Rives, 16h30-18h00


ISCE business meeting, Aula des Jeunes-Rives, 18h10-19h00

Banquet with social lecture, Cité universitaire, 19h00-midnight



Main Program

* = invited speaker

 = oral presentation being judged for an award

Sunday, 23 August

12h30 -
14h30

ISCE Executive meeting (committee members only)

13h00 -
17h00

Registration

Aula des Jeunes-Rives

Registered participants can pick up their printed program, abstract book and name tags

Poster hanging

15h00

Official Welcome

Aula des Jeunes-Rives
(Rahier M, Turlings TCJ)

15h15

25-years ISCE meetings

(McNeil J)

Aula des Jeunes-Rives

17h30 -
19h00

Welcoming reception on boat

Neuchâtel harbour

Monday, 24 August

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Symposium

Aula des Jeunes-Rives

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10h00 - 10h30	Starkenmann C * Analytical Challenges in Understanding Gender-Specific Differences in Human Body Odors	39
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10h30 - 11h00	Coffee break	
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Session 1.2 - The chemical ecology of vertebrates, including humans

Symposium

Aula des Jeunes-Rives

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Symposium

Aula des Jeunes-Rives

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Session 2.2 - Pheromones - Synthesis and Perception

Symposium

Aula des Jeunes-Rives

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17h15 - 17h30	Müller CT Otter Scent Communication; Development of a Novel Monitoring Technique	44
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Session 2.3 - Pheromones - Synthesis and Perception

Parallel sessions

Aula des Jeunes-Rives

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Symposium

Aula des Jeunes-Rives

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10h00 The Roles of Volatile Terpenes in Direct and Indirect Defenses of Plants

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10h30 - **Coffee break**
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Session 3.2 - Plant Defense: Mechanisms, Ecology and Application

Symposium

Aula des Jeunes-Rives

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11h30 The Ups and Downs of Insect-Plant-Insect Interactions

11h30 - **Heil M** 84
11h45 Damaged Self-Recognition in Plant Anti-Herbivore Defence

11h45 - **van Dam NM** 85
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Parallel sessions

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Parallel sessions

Aula des Jeunes-Rives

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16h45 - 17h00	Allmann S Lipoxygenases – a Nexus of Plant Defense Signaling	95

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Session 4.1 - The chemical ecology of disease vectors

Symposium

Aula des Jeunes-Rives

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09h30 Hypotheses for Developing New Controls for Animal and Human Disease Vectors

09h30 - **Takken W *** 116
10h00 Development of Push-Pull Strategies for Management of Malaria Mosquitoes

10h00 - **Torto B *** 117
10h30 Chemical Ecology of Afrotropical Disease Vectors: Lessons Learned and Future Challenges

10h30 - **Coffee break**
11h00

Session 4.2 - The Chemical Ecology of Disease Vectors

Symposium

Aula des Jeunes-Rives

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11h30 - **Gurba A** 119
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Symposium

Aula des Jeunes-Rives

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09h30 - **Kuhlemeier C ***
10h00 Speciation Genes in the Genus *Petunia* 134

10h00 - **Baldwin IT ***
10h30 How Plants Get What They Want from Floral Visitors 135

10h30 - **Coffee break**
11h00

Session 5.2 - The Chemical Ecology of Pollination

Symposium

Aula des Jeunes-Rives

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11h30 Molecular Examination of Variations in Floral Scent Biosynthesis in Natural Populations 136

11h30 - **Troncoso AJ**
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Oral Presentations Abstracts



Symposium 1
the chemical ecology of vertebrates, including humans

Individual Odor Signatures: Do MHC Genes Play a Role?

Penn DJ

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Individuals may have their own characteristic odor signature, shaped by genetics, commensal microflora, or both. We tested these ideas in human subjects in a village in the Austrian Alps. We conducted an olfactory assay in which 24 adults assessed the similarity of axillary sweat samples from 34 subjects (triangle test). Most individuals were found to have a distinctive scent, despite changes that occur over time, and we found evidence that MHC genes influence distinctiveness. We chemically analyzed the volatiles in the axillary sweat (GC-MS) and microbiota (PCR-DGGE) from 197 adults over 10 weeks. We found individual and sex-specific signatures in both, the volatiles and microflora, and identified the chemical structures of many of the compounds. Most but not all individuals showed distinctive signatures in their axillary sweat and microflora, even for subjects within the same family. Finally, we found that individual microbiota composition influences GC-MS signatures, though only when we analyzed subjects that followed certain rules during the sampling period. Our next aim is to determine whether MHC genes influence individual volatiles, microbiota, or both.

The Biochemistry of Human Body Odor: Genetic Effects on Odor Precursor Secretion and Enzymes of the Skin Microflora Involved in Odor Release

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Glands in the axillary region make a key contribution to the human body odors, and this skin region has been termed the human 'scent organ'. Three classes of odorants were described in axilla secretions: Steroids, short chain branched acids and sulfanylalcohols. We have identified, synthesized and sensorially characterized a number of new structures in the latter two chemical classes. The human nose can detect very low concentrations of these odorants, indicating that evolution has selected for high affinity receptors. On the other hand, for some compounds a high frequency of selective anosmia is found which may indicate that body odors have lost importance as a sensorial signal in recent human evolution. The odorants are secreted as precursors by the axillary glands: The acids as Glutamine-conjugates and the sulfanylalcohols mainly linked to the dipeptide Cys-Gly. The cleavage of the Gln-conjugates is catalyzed by an aminoacylase cloned from the skin bacterium *Corynebacterium striatum*, and the Cys-Gly-conjugates are cleaved by the sequential action of a dipeptidase and a β -lyase both isolated from the same skin bacterium. These specific enzymes from a commensal human skin bacterium recognizing the physiological odor precursors point to an interesting case of co-evolution. In detailed recent studies, the genetic influence on the pattern of odor precursor secretion was investigated. A study on monozygotic human twins indicated a strong genetic influence, with twins sharing a common and stable pattern of precursors of odorant acids. In mice, patterns of carboxylic acids in urine appear to be influenced by genes of the Major Histocompatibility locus (MHC). To study this effect in humans, siblings were analysed in parallel for their HLA-type and for the patterns of odorant acids. The first results will be presented strongly suggesting that in humans no influence of the HLA-loci on these specific odorants could be disclosed, so that other genetic factors appear to confer these genetically determined odor patterns.

Analytical Challenges in Understanding Gender-Specific Differences in Human Body Odors

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The challenge for the perfumery industry is to prevent the formation of human odors or to mask them with perfumes. At *Firmenich*, we have taken a pragmatic approach by testing our perfume in the presence of formulations that mimic human odor. These formulations of synthetic body odors were based on analytical data, but they were missing authenticity. The human sweat-like odor of Sclarimol®, 1-methoxyhexane-3-thiol, discovered in *Salvia sclarea* L.¹, guided us to search for sulfur compounds in fermented human sweat. Using GC-olfaction and affinity chromatography allowed us to discover Transpirol™ (R/S)-3-mercapto-3-methyl-1-hexanol². The structure of the precursor, [1-(2-hydroxyethyl)-1-methylbutyl]-(L)-cysteinylglycine³, was also elucidated by using liquid chromatography techniques guided by fermentation steps with *Staphylococcus haemolyticus*. This presentation discusses the analytical challenges leading to the discovery of thiols, the dosage of non-volatile precursors in male and female sweat, and the statistical differences observed⁴.

References

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2. Chem. Biodiv.1:1022-1035, 2004
3. Chem. Biodiv.2:705-716, 2005
4. Chem. Senses. 34:203-210, 2009

Innate versus Learned Olfactory Behaviors: Molecular Basis of Odor Perception in the Mouse

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In the mammalian olfactory system, odorants are detected with ~1000 odorant receptor (OR) genes expressed by olfactory sensory neurons (OSNs) in the olfactory epithelium (OE). Since the discovery of OR genes, it has remained entirely elusive how each OSN expresses only one functional OR gene, and how OSNs expressing the same type of OR converge their axons to a specific set of glomeruli in the olfactory bulb (OB). We have previously reported that singular OR gene choice is ensured by the combination of a rate-limiting enhancer-promoter interaction and negative-feedback regulation by OR proteins¹. For the OR-instructed axonal projection, OR-derived cAMP signals determine the anterior-posterior (A-P) topography². The dorsal-ventral (D-V) arrangement of glomeruli is determined by anatomical locations of OSNs in the OE³. After axons are guided to approximate destinations in the OB, axon termini are further sorted based on the expressed OR species in an activity-dependent manner⁴. The mouse olfactory system mediates various responses, including aversive behaviors to spoiled foods and fear responses to predator odors. In the OB, each glomerulus represents a single species of ORs. Since a single odorant can interact with many different OR species, the odor information received in the OE is converted to a topographic map of multiple glomeruli activated in distinct areas in the OB. In order to study how the odor map is interpreted in the brain, we generated mutant mice in which OSNs in a specific area of the OE are ablated by targeted expression of the diphtheria toxin gene⁵. In the dorsal zone-ablated mice, the dorsal domain of the OB was devoid of glomerular structures. The mutant mice lacked innate responses to aversive odorants, even though they were capable of detecting them and could be conditioned for aversion with remaining glomeruli. The mutant mice also failed to demonstrate the male-male aggressive behavior and male-female sexual behavior. It was thought that glomeruli in the OB would contribute equally to the processing of odor information in the glomerular map. However, our study indicates that the mouse main-olfactory system is composed of two functional modules: one for innate odor responses, and the other for memory-based associative learning. The mouse olfactory system provides insightful information as to how the neural circuits are formed and how they direct olfactory behaviors⁶.

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New Approach to Study Poison Frog Secretions Reveals Novel Frog Skin Chemistry

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Old World *Mantella* and Neotropical *Dendrobates* poison frogs are known to sequester alkaloids from their diet¹, but no other skin chemistry has been documented from these frog taxa. Over the past 50 years *unfractionated* skin secretions from poison frogs have not been analyzed by NMR². Using a 5mm 600 MHz Bruker cryoprobe and NMR spectrometer at AMRIS, 2D NMR experiments (TOCSY, COSY, HSQC, HMBC, NOESY) were performed on unfractionated skin secretions collected in Madagascar from individual *Mantella* poison frogs—the sensitivity of this instrument has proven sufficient for single frog analysis. NMR spectra will be presented to reveal both novel and common chemical structures never before reported from the skin of any amphibian. These studies, which are in preparation for publication, demonstrate that combining a different, nonlethal approach of skin secretion collection with modern NMR technology can lead to novel compounds, even for animals that have already been studied in detail and using limited amounts of natural samples.

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Amphibian Chemical Defense: Repelling *Batrachochytridium dendrobatidis* with the Metabolites of Cutaneous Bacteria

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Amphibians worldwide are facing extinction at an alarming rate. The decline has been attributed to deforestation, pollution, global climate change, and recently, emerging disease. Chytridiomycosis, an amphibian disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* has been linked to a number of extinctions. Some cutaneous bacteria, found naturally on amphibians, can inhibit the fungus via antifungal metabolites. *Lysobacter gummosus* produces 2,4-diacetylphloroglucinol, an antifungal compound with a minimum inhibitory concentration (MIC) of 136 μ M. *Janthinobacterium lividum* produces indole-3-carboxaldehyde (MIC = 69 μ M) and violacein (MIC = 2 μ M). In addition, red back salamanders from the wild have been observed to have detectable amounts of violacein in their mucus. These concentrations have been above the MIC of violacein. Since the antifungal metabolites from *J. lividum* are so potent, the bacterium was used in a bioaugmentation experiment with frogs (*Rana muscosa*) and salamanders (*Plethodon cinereus*). The bacterium was able to protect all frogs and most salamanders exposed to the fungus. These results indicate that bioaugmentation with antifungal bacteria is an effective way of protecting individuals from mortality associated with chytridiomycosis. Further research into this and other antifungal bacterial species is necessary to develop this method for probiotic application to wild species.

Epidermal Lipids of Reptiles – Potential Chemical Signals in Crocodylia and Rhynchocephalia?

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Reptiles are currently divided into five orders, Testudines (turtles and tortoises), Crocodylia (alligators etc.), Squamata (snakes and lizards), Rhynchocephalia (the tuatara), and Aves (birds). While there have been some reports on the use of skin associated compounds as chemical signals in lizards, snakes, and testudines¹, similar reports in the other orders are scarce. We analyzed the content of cloacal glands of the tuatara and paracloacal glands of crocodylians. Both glands have been suspected to secrete pheromones, although this function has not been demonstrated so far. The secretion of the tuatara consists of unique glycerides with up to 150 different components². These glycerides are composed of up to 12 short and medium chain carboxylic acids, attached to glycerol in a combinatorial way. The secretion proved to be specific for each individual. The paracloacal glands of several caimans contain no glycerides, but a complex, species specific mixture of terpenes as well as aliphatic compounds as esters and alcohols³. Furthermore, some species contain unique, ethyl branched, volatile ketones, diketones, and aldehydes, some of which proved to be highly sticky on surfaces. Biotests with these reptiles are difficult for obvious reasons, but sampling from wild populations of the tuatara will give more insight into their chemical communication system.

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Otter Scent Communication; Development of a Novel Monitoring Technique

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Standard monitoring for otter *Lutra lutra* identifies otter presence but does not provide information about population structure. DNA analysis of spraints can identify individual otters, but is costly, time-consuming and has low success rates. A novel method is needed to better monitor populations and assess conservation efforts. It is thought that otters largely communicate using scent, so chemical profiling of otter spraint has the potential to provide much needed information such as sex, age, reproductive status and individual identity. Sampling using headspace SPME (solid phase micro extraction) and analysis using GCMS (gas chromatography mass spectrometry) have been used to distinguish volatiles from scent glands taken at post mortem. In this paper preliminary results are presented distinguishing otters by sex and age group. Multivariate statistical techniques are employed to analyse these complex mixtures and some individual compounds, indole, 2-pentyl furan and dimethyl disulfide, differ in relative concentrations between groups. Analysis of more scent glands from our considerable archive will be used to develop a predictive model with the aim to discriminate otter identity from spraints collected in the wild.

African Wild Dog BioBoundaries – Identifying the Messenger Compounds in *Lycaon pictus* Territorial Scent Marks

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African wild dogs (*Lycaon pictus*) are very susceptible to edge effects in even the largest conservation areas, because they have low population densities and very large home ranges. Because most conservation area perimeters are permeable, loss of dogs to predator control measures around conservation areas is a sink that threatens the sustainability of core populations. The dogs mark their pack territories with urine; overmarks by the dominant pair are especially socially potent, and packs rarely trespass. We are developing BioBoundaries; artificial territorial boundaries of synthetic scent marks that will deter dogs from leaving conservation areas by mimicking the presence of territorial neighbours in the danger zone outside. Gas chromatography – mass spectrometry identification of volatile components of wild dog urine and faeces has revealed the presence of a series of aliphatic acids and aldehydes, benzoic acid, dimethyl sulfone, squalene, phenolics, and cholesterol and steroid metabolites among many others. The challenge now is to determine which components send the territorial “keep out” message, and to formulate them into a synthetic scent mark.

Olfaction as a Mechanism Guiding Kin Recognition and Mate Choice in a Primate Species

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Sexual selection research has provided growing empirical evidence that the competitive sex advertises his genetic quality to potential opponents to minimize intrasexual contest and the choosy sex relies on these honest indicators to select an appropriate mate. The accurate assessment of genetic characteristics also may be critical for directing nepotism towards relatives or for minimizing inbreeding in offspring. Researchers have focused on visual or auditory modes of signal transmission; however, the importance of olfactory indicators is gaining recognition. Combining chemical, genetic, and behavioral analyses in 17 female and 19 male ring-tailed lemurs (*Lemur catta*), we provide evidence relating olfactory cues to individual genome-wide heterozygosity in males and to the genetic distance between individuals in both sexes. These relationships between semiochemical profiles and genetic characteristics are apparent only during the highly competitive and stressful breeding season when such signals are likely to be strongly selected. As heterozygosity accurately predicts health and survivorship in this population, we first identify olfactory cues as honest indicators of male quality, with relevance to both sexes. We further suggest that signal convergence between the sexes may reflect strong selective pressure on kin recognition, whereas signal convergence within the sexes may arise as its by-product or function independently to prevent competition between relatives. We also suggest that the link between an individual's genome and its olfactory signals could be mediated by biosynthetic pathways producing polymorphic semiochemicals or by carrier proteins modifying the individual bouquet of olfactory cues. Finally, we showed that male and female lemurs behaviorally respond to odors according to these genetic characteristics during behavioral bioassays. In conclusion, we unveil a possible olfactory mechanism guiding kin recognition and mate choice that has specific relevance to understanding inbreeding avoidance and nepotistic behavior observed in free-ranging primates, and broader relevance to understanding the mechanisms of vertebrate olfactory communication.



Symposium 2

pheromones: synthesis and perception

Moderator

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To Smell or Not to Smell: Plasticity in the Insect Brain

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Sex pheromones are essential in many insects to find a mating partner. In Lepidoptera, males detect female-emitted pheromones over large distances. Peripheral detection and sex pheromone processing within the central nervous system have been well studied in moths. However, sex pheromone-guided behaviour might change as a function of the physiological state of an insect or with experience. We study the neurobiological mechanisms underlying this plasticity in two noctuid moth model systems. In the migratory moth *Agrotis ipsilon*, males are not sexually mature at emergence and take a few days before they respond to the sex pheromone. After mating, they immediately cease to respond to sex pheromone, but are highly responsive again the next day. The antennal detection system does not change its sensitivity during adult maturation and after mating, whereas neurons within the primary olfactory centre, the antennal lobe, change their responses to sex pheromone profoundly within a very short time after mating and more slowly during adult maturation. Juvenile hormone and biogenic amines are involved in behavioural and central nervous changes during maturation. Changes in pheromone coding properties of neurons in the antennal lobe after mating indicate that the central circuitry is modified, but the underlying mechanisms are still unknown. In the moth *Spodoptera littoralis*, males have innately a high sensitivity to the female-produced sex pheromone. Behavioural responses to this pheromone can, however, be further increased by brief pre-exposure to the sex pheromone. In parallel, an increase in sensitivity of neurons within the antennal lobe was observed 24 h after pre-exposure with pheromone. We showed that this form of long-term sensitization for the sex pheromone, observed at the behavioural and central nervous level, can also be elicited by other behaviourally meaningful sensory stimuli such as predator sound or attractive/aversive gustatory stimuli.

Neural Processing of Sex-Pheromonal Information in the Brains of Moths

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Natural olfactory stimuli typically are mixtures of volatiles with characteristic chemical compositions and proportions. Many animals use information about proportions in addition to chemical composition to discriminate between an important odor source, such as a conspecific mate, and unrewarding or inappropriate one, such as an individual of another species. The sex-pheromone system of the moth *Manduca sexta* offers an experimentally favorable model of mixture processing, as only two pheromone components, released by the female at a consistent 2:1 ratio, are required to attract conspecific males. Each of those components activates receptor input to one of a pair of large male-specific glomeruli in the antennal lobe (AL), the primary olfactory center in the brain. Neural connections within and between those glomeruli form circuitry for primary processing of sensory information about the pheromone, and glomerular projection (output) neurons (PNs) convey the processed information to downstream targets in the brain as patterns of action potentials («spikes»). Chemical information is represented in the identity of responsive PNs, and stimulus intensity and spatio-temporal features are encoded in the patterns of PN spiking. In behavioral experiments in a wind-tunnel, the 2:1 pheromone mixture elicits robust mating behavior, but altering the ratio reduces its effectiveness. We recorded responses of pairs of PNs to mixtures of the two pheromone components at the natural (2:1) and altered ratios. We hypothesized that a “stronger” signal, received by neurons postsynaptic to AL outputs, would consist of either increased firing rate or increased firing synchrony. Although the 2:1 mixture did not consistently elicit higher firing rates than mixtures with other ratios, synchrony between spikes of simultaneously recorded output neurons in the same glomerulus was highest for the 2:1 mixture. Increased synchrony of spikes in AL output neurons therefore may underlie the behavioral selectivity for the natural ratio.

Recent Advances in Cerambycid Beetle Pheromones Only Lead to More Questions

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The cerambycid beetles are a large insect family, with ~35,000 described species. Some species are of major economic importance, particularly invasive species that have been introduced into new areas of the world. Despite their importance, little is known about their semiochemistry, and until five years ago, pheromones had been reported for only ~10 species. Rapid progress has been made since then, with sex or aggregation pheromone blends or components identified from >40 additional species, including the first examples of female-produced sex pheromones. This presentation will summarize recent progress in identification and synthesis of pheromones for these insects. It will also address several unusual aspects of cerambycid semiochemistry and biology. For example, we have found that the common pheromone component (*R*)-3-hydroxyhexan-2-one attracts several sympatric species simultaneously, including species which apparently do not produce this compound. Thus, the pheromone may be exploited as a kairomone or synomone by other members of the arthropod community. Second, several species have been found to produce large amounts of (*R*)-3-hydroxyhexan-2-one or related "pheromone-like" compounds, but we have not been able to discover role of the compound(s), because they have no apparent effect on the behaviors of either sex. Third, males can produce pheromones in large amounts (e.g., >100 µg/hr), so that pheromone lures must release 5-10 milligrams of pheromone per day. Thus, release devices must be designed for these high release rates, and pheromone syntheses must be carried out on relatively large scale. Overall, recent progress has shown that cerambycid beetles have a rich semiochemistry, both for study and for exploitation for practical applications.

Pheromones at 50: Celebration and Remaining Ambiguities

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This year is the 50th anniversary of both the coining of the word 'pheromone' by Karlson and Lüscher^{1,2} and the first chemical identification of a pheromone, Bombykol, by Butenandt's lab. Pheromones have turned out to be ubiquitous across the animal kingdom³ but there is still one area of ambiguity, about highly variable odours in mammals and social insects and whether these should be considered as pheromones. The problem continues to be the distinction between pheromones and signature mixtures (≡ mosaic signals⁴). Pheromones are molecules that are evolved signals, in defined ratios in the case of multiple component pheromones, and usually species specific. Signature mixtures (used for recognition and identity) are highly variable mixtures of molecules and are useful for this reason as they give a basis for distinguishing individuals or colonies. A key difference between pheromones and signature mixtures is that in all taxa so far investigated it seems that signature mixtures need to be learnt³. Clear parallels across the animal kingdom are emerging in the evolution and characteristics of signature mixtures and pheromones in a broad range of taxa including mammals (about which the debate is most heated) and social insects. It is likely that pheromones and signature mixtures are processed differently by the sensory system and CNS in most animals.

www.zoo.ox.ac.uk/groups/pheromones

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Focusing of Pheromone Molecules by Trichoid Sensillar Cuticular Lipid Coatings

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The lipid coatings on trichoid sensilla of male moths may be involved in both the selective adsorption of pheromone molecules from the air, as well as focusing these molecules onto sensillar pores. If the lipids on trichoid sensilla were better at adsorbing pheromone-type molecules than other surfaces of the antennae, pheromone molecules would be selectively focused onto the trichoid hairs. Secondly, different regions of the sensilla could be coated heterogeneously with different forms or orientations of lipids to expedite the movement of adsorbed pheromone molecules down the pores to the binding proteins and then to the receptor neurons in the sensillum lumen. We found that in *Helicoverpa zea* moths, there were in fact significant differences in the lipids on male antennae compared to those of females¹ and these could be attributed to the trichoid sensilla that exist in huge numbers on male antennae but not on female antennae. Thus at the macro-level we found evidence for differential coating of male trichoid sensillar surfaces that might selectively adsorb pheromone molecules. We then used atomic force microscopy (AFM) on the sensilla to determine their individual nano-terrains, followed by chemical force microscopy (CFM) to contact the surfaces and probe for differences in chemical bonding forces that could indicate differences in lipid coatings on different local sensillar regions. We found that *H. zea* trichoid sensilla exhibited heterogeneity in their lipid coatings, with the multitudes of ridges on each sensillum possessing more hydrophobic coatings than the pores. These consistent differences between the chemical compositions coating the ridges versus the pores suggests that there is a lipid-based nano-focusing of the aldehyde pheromone molecules into the pores from non-pore-containing areas of the sensilla. A second species, *Utethesia ornatrix*, that uses hydrocarbon pheromone components, not aldehydes, exhibited no lipid heterogeneity on its trichoid sensillar surfaces.

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Broad Interspecific and Intergeneric Recognition of a Host Marking Pheromone within *Anastrepha* and *Toxotrypana* Flies (Diptera: Tephritidae): Evolutionary and Practical Implications

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Females of most derived species within *Anastrepha* (Diptera: Tephritidae) deposit a host marking pheromone (HMP) after ovipositing into a fruit. Interestingly the few studied primitive species do not mark fruit. Depending upon the concentration, the HMP elicits further ovipositional activity or deters it (a process strongly influenced by fruit size). The pheromone is found in the feces of flies and can therefore be easily extracted and purified. Here, we investigated if there was cross-species recognition of the HMP of seven *Anastrepha* species. We also tested crude methanolic pheromone extracts of *Toxotrypana curvicauda*, a species phylogenetically close to *Anastrepha* (i.e., sister group), and of *Ceratitis capitata* and *Rhagoletis pomonella* and *R. cerasi* (all Diptera: Tephritidae). Results from electrophysiological studies using crude HMP extracts and various compounds separated along the process of HMP structure elucidation showed that cross-recognition was very broad within *Anastrepha*. We also found that a crude HMP extract from feces of a basal *Anastrepha* species (*A. bezzii*) or a closely related one (*T. curvicauda*), elicited a strong electrophysiological response in more derived *Anastrepha* species. Based on these results, we tested crude HMP extracts in field cages into which *A. ludens*, *A. obliqua* and *A. serpentina* females were released singly and allowed to forage for fruit that had either been artificially treated with crude HMP extracts or was clean. The cross-recognition pattern discovered in the electropysiological tests was confirmed. Rejection of treated fruit was almost complete when compared to untreated fruit. Finally, we applied crude extracts and synthetic derivatives thereof of the *A. ludens* HMP under field conditions to determine if it would lower the infestation of *A. obliqua* in tropical plums and mangoes. In both cases, a significant reduction in fruit infestation was recorded. We discuss these findings with respect to their evolutionary implications and also their applied ones, given that our discovery represents potentially a biorational management alternative in the case of pestiferous *Anastrepha* species.

Quantity Matters: Mate Assessment by *Nasonia vitripennis* Females Using the Male Attractant

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In many species there is a sexual conflict concerning the mating frequency with promiscuous males and females that not seldom mate only once in their lifetime. Therefore, females are choosy and often well adapted to chemical cues and signals indicating the quality of potential mates. One crucial quality parameter for females is the male sperm status. Mate assessment often requires contact of the choosing sex with the potential mate. Here we show for *Nasonia vitripennis*, a wasp with haplodiploid sex determination and female biased sex ratios, that females discriminate against sperm limited males already from a distance by orienting along gradients of the male sex attractant. Thereby, they decrease the risk of getting constrained to produce suboptimal offspring sex ratios, i.e. more sons than necessary. We found sperm limitation in freshly emerged and multi-mated males (\geq seven previous matings) which was reflected by clearly reduced pheromone titres. In behavioural bioassays females preferred not only higher doses of the synthetic pheromone but also scent marks deposited by males with a full sperm load over those from sperm limited males. Remote discrimination by females of males according to their sperm status adds another facet to the plethora of pheromone mediated mechanisms driving sexual selection.

Pheromones and Biodiversity - Monitoring Change in a Changing World

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Monitoring the abundance of species in space and time is crucial to determine the conservation status of potentially threatened species. Insects deserve special attention in this regard as the largest and most diverse group of organisms, with a disproportionately high number of threatened species. Insects are also excellent potential indicators of environmental and climate change, as many species have high dispersal ability and respond quickly to change. Assessing their threat status can be a formidable task, however, due to taxonomic diversity and the difficulty of obtaining population estimates and accurate geographical distributions. We are developing methods to facilitate monitoring of rare and threatened species that are otherwise largely inaccessible with other methods. The legendary attractiveness of sex pheromones makes them an ideal tool for studying many insect species occurring at very low population densities. We present model systems from saproxylic hollow tree and dead wood habitats that are likely the first to exploit pheromones in the context of conservation and biodiversity: 1) The sex pheromone-kairomone system of the threatened saproxylic scarab beetle *Osmoderma eremita* and its predator, the click beetle *Elatер ferrugineus*; 2) Monitoring a whole range of tineid and clearwing moths in fragmented forest landscapes, using only a few single compounds from their phylogenetically restricted pheromone systems. Our investigations illustrate the versatility of pheromones for precise monitoring in space and time and include estimates of absolute population size by capture-recapture studies; estimates of presence/absence and population density on landscape and regional scales; monitoring of population fluctuations over several years, and studies of dispersal frequency and dispersal distance.

From Ecology to Biomolecules: Smart Utilization of Methyl Eugenol, a Plant-Derived Potent Attractant by a Male Oriental Fruit Fly, *Bactrocera dorsalis*

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The male Oriental fruit fly *Bactrocera dorsalis* is strongly attracted to feed voraciously on methyl eugenol (ME), a naturally-occurring phenylpropanoid found both as a plant secondary metabolite, and a component of essential oils found in over 200 species of plants from 46 families. It is now widely acknowledged that the central role this important attractant plays in the interrelationships between ME-sensitive fruit fly species with their host plants and predators. Consumption of this phenylpropanoid is known to confer male mating advantage and protection against vertebrate predators. Further, ME and its derivatives have also been shown to be involved in the pollination of certain *Bulbophyllum* orchids by *Bactrocera* fruit flies. These findings highlight the fascinating interactions between insects and their non-host plants in the natural ecosystems. We have previously demonstrated that ME-fed male *B. dorsalis* produced chemical cues that were more attractive to conspecific females compared with ME-deprived male. Upon consumption, the male fly biotransforms ME into sex pheromonal components, 2-allyl-4,5-dimethoxyphenol and (*E*)-coniferyl alcohol in the crop organ. The rectal gland then sequesters these phenylpropanoids, prior to their release during dusk. A novel assay was developed using sexually mature *B. dorsalis* males as biodetectors for the pheromonal components that were also male attractants. Those components were detected in the haemolymph. Further, physiological experiments such as parabiosis further demonstrated the haemolymph transport of the pheromonal components from the crop to the rectal gland. Bioactive fractions containing the ME-derived sex pheromonal components from the hemolymph have been separated and identified using biodetection and GC-MS analyses. We have also been able to detect the appearance of peptide bands in the fractions using the SDS-PAGE technique. These findings suggest the role of the bioactive peptides as pheromone-binding peptides in the transport of the attractant-derived sex pheromonal components.

Chemical Compound Transfers between Mates during *Drosophila* Copulatory Behaviour

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Most living organisms use pheromones for interindividual communication. In *Drosophila melanogaster* flies, several pheromones perceived either by contact / at a short distance (cuticular hydrocarbons, CHs), or at a longer distance (cis-vaccenyl acetate, cVA), affect courtship and mating behaviours. We combined SPME with GC-MS to precisely identify all potential pheromonal compounds and simultaneously monitor their variation on a time scale. This allowed us (i) to identify 59 compounds — including 17 newly described CHs — on the cuticle of control flies, (ii) to precisely quantify their amounts readily available to the sex partner, and (iii) to measure their variation related both to aging and mating in individual males and females. If aging induced sex-specific variations, mating affects cuticular compounds with three possible patterns. The variation was either (1) reciprocal between sex partners, as expected for a passive mechanical transfer during mating, (2) parallel in both sexes, such as for cVA which only appeared during mating, or (3) unilateral, suggesting that this rapid change in one sex directly resulted from the sexual interaction and favoured mating propensity in the sex partner.

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Role of Pheromones in *Drosophila*

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Drosophila melanogaster produces sexually dimorphic pheromones, with C23 and C25 monoenes produced in males and C27 and C29 dienes produced in females. Two genes, *desatF* and *eloF*, specific to females, are responsible for female diene pheromone biosynthesis. Other species, such as *D. simulans*, produce only C23 and C25 monoenes in both sexes. In this species, *desatF* and *eloF* are present in the genome, but not transcribed. As *Drosophila* male courtship is largely dependent on female pheromones, we investigated the role of *desatF* and *eloF* in courtship behaviour, using RNAi knock-down in oenocytes. Results show that inactivation of either *desatF* or *eloF* resulted in a dramatic loss of pheromones and a moderate inhibition of courtship behaviour. Inactivation of both genes led to a *D. simulans* hydrocarbon profile and complete inhibition of courtship behaviour. Pheromone and courtship data suggest that *desatF* and *eloF* are crucial enzymes for courtship behaviour and might have played determinant role in sexual isolation leading to speciation.

Antifeedants Produced by Pine Weevil Gut Bacteria

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Pine weevils, *Hylobius abietis*, are considered a major pest to the conifer forest industry in Sweden. They feed on bark and are a major threat to planted seedlings which they frequently girdle. The females lay their eggs in gnawed out cavities in the root bark of dead or dying trees. During ovipositioning the females regularly deposit feces adjacent to each egg. The cavity is sealed afterwards by feces and a plug of bark fragments. Bark areas which contain egg cavities with feces are avoided as food by pine weevils. In previous studies substances extracted from feces has been identified and tested in feeding bioassays and some of these substances were found to have strong antifeedant properties e.g. 2-metoxyphe¹ 2³ The biochemical origin of the compounds is not known. They may be produced by the female herself and/or from bacteria and fungi enclosed in the feces. Three bacteria strains have been cultivated on either NB-media, the phloem of pine twigs or on ¹³C-labelled phenylalanine. Volatile compounds were collected by Solid Phase Microextraction (SPME) and separated and identified by Gas chromatography - Mass spectrometry (GC-MS). Bacteria isolates emitted several compounds with strong antifeedant effect on *Hylobius abietis*.

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Context-Dependent Communication in a Non-Social Insect

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Communication is the basis of social interactions. It involves sending and receiving signals and a decision of the receiver as to which action to undertake. Signals may be sent in a fixed form (e.g. sex-pheromones) or in a form that varies in relation to the context (e.g. signals conveying information on the quality of a food source). Because danger may come from a variety of sources and these sources vary in the risk they impose, it is hypothesized that alarm signals are context-dependent. Indeed, the literature on vocal alarm in vertebrates lends support for this hypothesis, but the literature on chemical alarm signals, largely pertaining to invertebrates, lacks a critical test and seems to suggest that alarm pheromones do not vary within species. We have tested for context-dependence of an alarm pheromone of the Western Flower Thrips, *Frankliniella occidentalis*. In the presence of danger, thrips larvae excrete anal droplets containing an alarm pheromone that is supposed to consist of decyl acetate and dodecyl acetate. Although it is known that the ratio of the two alarm chemicals change with the age of the thrips larvae, it is not known whether it can change with the type of predator imposing death risk to thrips larvae. Our experiments provide preliminary evidence that signal-sending thrips larvae modify the ratio of the two alarm chemicals depending on whether they face the risk of being eaten by a predatory mite (low risk) or by a predatory bug (high risk) and that the alarm pheromone induces a response in the signal-receiving thrips larvae that depends on the context perceived by the sender. In future tests we hope to assess whether thrips larvae communicate the nature of danger to kin and non-kin larvae.

Exploring Genomic and Molecular Causes of Pheromone Divergence in Small Ermine Moths

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Moth pheromone biosynthesis entails a cascade of genes, with the encoded products together establishing the final structure and ratio of each component in a pheromone mixture. Pheromone gland-specific acyl-CoA $\Delta 11$ -desaturases evolved before the ditrysian radiation^{1,2} and have been accredited as being key players in the catalysis of most long-chain unsaturated fatty acyl moieties described in moths to date. Nine species of small ermine moths (Lepidoptera: Yponomeutidae) distributed in the west European Palaeartic region use $\Delta 11$ -desaturated acetates and alcohols as pheromone components. The rare exception is *Yponomeuta rorellus*, which differs dramatically in pheromone composition, having a simple bouquet composed of saturated 14:OAc as major component. This suggests that the emergence of this species was associated with the evolution of genetic or regulatory mechanisms that led to the loss of unsaturated pheromone components³. Our functional investigations on fatty-acyl-CoA reductases (FARs) in *Yponomeuta* spp. evidenced that in *Y. rorellus*, an active FAR enzyme with broad substrate range has maintained its ability to reduce unsaturated precursors *in vitro*, thereby strongly supporting that regulation occurs at an earlier stage in the biosynthesis. In order to validate the previously proposed biosynthetic scheme⁴ and using a similar functional approach, we characterized and established the precise biochemical activity *in vitro* of two homologous $\Delta 11$ -desaturases active in producing the respective $\Delta 11$ -unsaturated intermediates in *Y. padellus* and *Y. evonymellus*. Using a GenomeWalker approach and a gene-specific primer walking strategy we screened genomic DNA libraries in *Y. rorellus* to query for a $\Delta 11$ -desaturase like homolog gene and explore its putative adjacent 5' regulatory region. We aim at pin pointing genomic elements that could highlight the potential mechanistic causes implied in the observed genetic outbreak that led to colonization of a new ecological niche through divergence in mate communication.

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An Unexpected “Appeasement Signal”: the Role of Sesquiterpenes in the Chemical Profile of Tropical Stingless Bees

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Stingless bees (Apidae: Meliponini) of Borneo appear to be unique in having species-specific compositions of terpenoid compounds in their chemical profiles, which are likely derived from plant resins¹. Whole groups of terpenes (e.g. sesquiterpenes) can be found in the chemical profile of some species, but are missing in the profile of others. We investigated the role of sesquiterpenes in inter- and intraspecific aggression. Sesquiterpenes, abundant in the chemical profile of several bees, were added to the chemical profile of *Tetragonula melanocephala*, a species that does not express sesquiterpenes in its own profile. Such modified profiles significantly reduced aggression in *T. melanocephala* in intercolonial encounters, suggesting that cuticular sesquiterpenes confuse *T. melanocephala* bees by “masking” their actual profile. This mechanism may explain why bee species without sesquiterpenes in their chemical profile barely show aggression towards species with sesquiterpenes. Sesquiterpenes may promote aggregated nesting in Bornean stingless bees, particularly between species with and without sesquiterpenes. Moreover, terpenes and their influence on aggression may provide a potential explanation for the widespread occurrence of aggregated nesting in paleotropical stingless bees, which seems to be absent among their highly aggressive neotropical relatives².

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Functional and Genomic Analyses of Reductase Alleles Determining Specific Sex Pheromones in European Corn Borer Females

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When others show their long tails or sing, male and female moths rely on pheromones to find each other. Most moth species use a given blend of compounds to constitute a private communication channel. When two groups eventually diverge and give rise to two distinct species, the speciation process is usually accompanied by changes in the communication system. Since 1959 and the breakage of the code used by domesticated silk moth females to attract their partners, the code of more than a thousand species has been broken. However, our understanding of the molecular bases of pheromone production and especially the source of variations is still largely superficial. Our subject species the European corn borer *Ostrinia nubilalis* has been examined extensively from a chemical ecology perspective and is polymorphic regarding pheromone production: the so-called E race uses a 98:2 blend of (*E*)- and (*Z*)-11-tetradecenyl acetate whereas the so-called Z race uses a 3:97 E/Z mixture. The inheritance patterns for sex pheromone production in females has been demonstrated previously to be primarily controlled by a single autosomal factor involving one pair of alleles. We assessed the plausibility of the reduction step, the reduction of fatty acyl precursors to corresponding alcohols, to be the key to explain this drastic difference in pheromone blend. Both the results of our functional assays and the segregation patterns seen in pure and hybrid progenies support this hypothesis, ultimately revealing that the autosomal factor encodes a fatty acyl-coA reductase. The significance of our results on an evolutionary view-point will be discussed.

Simulation of Mating Disruption and Mass Trapping Using the Effective Attraction Radius in Two and Three Dimensions

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A pheromone source in a trap that catches insects at a certain rate has an effective attraction radius (EAR) that is the radius of a spherical trap that would intercept the same number of insects flying at random. The EAR was used as a key parameter in a recent simulation model of mating disruption and mass trapping with competitive attraction and camouflage¹. The EAR can be adjusted in size to catch at a rate equivalent to any active-space plume model of arbitrary complexity or size². Simulations were used to explore variation of key model parameters such as lure and female EAR dimensions and densities on efficacies of mating disruption and mass trapping of insects, especially moths. Male moth movement was simulated as a "correlated random walk" and encounters with EAR of lures of variable radius and density. Calling female moths were in competition with lures in attracting males that oriented in the EAR for various periods. When male orienting time was constant regardless of EAR, there was no difference in mating disruption efficacy between either a higher density of dispensers with smaller EAR, or a lower density of dispensers with a compensating larger EAR. However, when the orienting time was increased in proportion to dispenser EAR, then fewer dispensers with larger EAR were more effective in reducing female mating than were more numerous ones with smaller EAR. When costs of pheromone are substantial, more numerous dispensers of smaller EAR are more economical since dose-response curves in previous studies indicate release rate must increase exponentially to achieve a linear increase in EAR. Use of the spherical EAR in two-dimensional encounter rate models requires a conversion formula as supported by simulations of flying insects in three-dimensions in normally distributed populations³. The models are useful in understanding and developing successful mating disruption and mass trapping programs.

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Sex Pheromone of *Cryptoblabes gnidiella* (Lepidoptera: Pyralidae): Synthesis and Biological Activity of the Main Natural Components and their Formate Mimics

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The honeydew moth, *Cryptoblabes gnidiella* (Lepidoptera: Pyralidae), is a Mediterranean moth that has become well established in South America during the past decade. In Uruguay and Southern Brazil, *C. gnidiella* has developed into a severe pest in vineyards, both due to direct damages as well as an increased occurrence of the fungus *Botrytis cinerea* in attacked grapes. The sex pheromone of *C. gnidiella* has been described as a 1:1 mixture of Z-11-hexadecenal and Z-13-octadecenal, and these compounds have been used for monitoring purposes. In order to test the best formulation to monitor local populations of *C. gnidiella*, we synthesized and evaluated different mixtures (1:1; 1:9 and 9:1) and dosages (1; 0,5 and 0,1 mg/septa) of the natural pheromone components in a vineyard in Uruguay. In addition, given that aldehydes are unsuitable for the mating disruption technique, we synthesized and tested the electrophysiological activity of formate mimics of both pheromone components, in search of alternatives for the development of a control strategy based on the disruption of sexual communication. We found that a 1:1 mixture of Z-11-hexadecenal and Z-13-octadecenal at a dose of 0,5 mg/septa is suitable for the monitoring of *C. gnidiella* males for over 4 weeks, performing slightly better than commercial dispensers. Interestingly, both pheromone mimics, Z-9-tetradecenyl formate and Z-11-hexadecenyl formate, elicited GC-EAD responses comparable to those of the natural pheromone components. Finally, we will present electrophysiological and behavioural (wind tunnel) data from current experiments concerning the response of *C. gnidiella* males to the natural pheromone components, after pre-exposure to the pheromone mimics.

Diversity of Putative Odorant-Degrading Enzymes Revealed by Expressed Sequence Tags of Antennae from the Moth *Spodoptera littoralis*

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By bioinformatic screening of a male antennal EST library from the moth *Spodoptera littoralis*, we have identified a high diversity of putative Odorant-Degrading Enzymes belonging to various families (cytochrome P450, esterases, UGT, GST). In particular, we have isolated 19 sequences of putative antennal carboxylesterases. This family is of great interest because *S. littoralis* uses a mix of esters as sex pheromone. Phylogenetic analysis on these sequences showed that these genes were distributed into the various clades of carboxylesterases previously defined, in agreement with presumptively different cellular and subcellular localisation. One esterase gene, SICXE7, was only expressed in antennae and over expressed in males, suggesting a potential function in sex pheromone degradation. Preliminary results revealed that recombinant SICXE7 can indeed degrade the major component of the pheromonal blend. Analyses are now in progress to precise SICXE7 catalytic properties. These numerous antennal esterases could potentially act on a wide range of endogenous or exogenous compounds, odorants or xenobiotics. This diversity shed a new light on the multiple metabolic pathways using esterases that may coexist in this highly specialized tissue.

Aphid-Ant Mutualism: How Do Aphids Focus Ant Foraging?

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The mutualistic relationships that occur between myrmecophilous aphids and ants are based on the rich food supply that honeydew represents for ants and on the protection they provide against aphid natural enemies. While aphid predators and parasitoids actively forage for oviposition sites by using aphid semiochemicals, scouts of aphid-tending ant species would also benefit from locating honeydew resources by orienting toward aphid pheromone sources. The present study aims to identify the chemical factors that attract ants and that maintain their mutualistic relationships with aphids. The perception and behavioral impact of *Aphis fabae* alarm pheromone, namely (E)- β -farnesene, on *Lasius niger* were firstly investigated using electroantennography and a four-arm olfactometer. *Aphis fabae* honeydew sugar composition was subsequently analyzed while the foraging and recruiting behaviour of *L. niger* scouts towards each of the identified sugars was studied. Clear electrical depolarisations were observed from *L. niger* scout antennae to stimulations of *A. fabae* alarm pheromone. Scouts were significantly attracted toward (E)- β -farnesene in the four-arm olfactometer, suggesting for the first time that the latter compound is a key chemical in the establishment of the mutualism. *Aphis fabae* honeydew consisted of 9 identified mono-, di- and tri-saccharides and 8 hydrocarbons that could not be identified. The main identified sugars were sucrose, fructose, glucose and melezitose. *L. niger* scouts showed the following drinking preferences for the tested sugars: melezitose = sucrose = raffinose > glucose = fructose > maltose = trehalose = melibiose = xylose, with a recruitment launched toward the first three sugars. Therefore, ant scouts may use aphid semiochemicals to locate at distance an aphid colony and subsequently estimate honeydew quality by tasting it before recruiting conspecifics and establishing a mutualistic relationship.

Chemical Signals Involved in the Attraction and Courtship Behavior of the Brown Spider *Loxosceles intermedia*

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Loxosceles intermedia is the most abundant species of brown spider in Curitiba, state of Paraná, Brazil. This spider is responsible for the highest number of loxoscelic incidents in the world, particularly those recorded in Paraná. The aim of this study was to evaluate the importance of chemical communication in the sexual behavior of *L. intermedia*, which was accomplished through two experiments. In one of the experiments, males and females were placed in contact with a piece of filter paper containing extracts prepared by washing the bodies of the spiders, and their behavior was evaluated. Females showed motor patterns related to species recognition, when in contact with extracts from the bodies of males ($\chi^2_{(1)} = 10$; $P < 0.01$) and females ($\chi^2_{(1)} = 22.5$; $P < 0.01$). Males showed these motor patterns only when in contact with extracts from the bodies of females ($\chi^2_{(4)} = 56.3$; $P < 0.01$). GC-MS analysis of the extracts showed the presence of several compounds in common, except for two that were present only in the extracts from females. These two compounds were also obtained by aeration of the females. The structures of the compounds in question were determined by GC-MS as geranyl acetone and geranyl acetate. Experiments carried out with the two compounds in glass arenas indicated that they attract males from a long distance, but do not exercise the same attraction on females ($\chi^2_{(1)} = 26.6$; $P < 0.01$). The data from the present study suggest that the extracts from the bodies of males and females, which had most of the compounds identified by GC-MS, elicit species-recognition behavior over a short distance, and that the two compounds that are present only in the extracts from females are involved in long-distance communication.

Characterisation of *Bombyx mori* Odorant-Binding Proteins Reveals that a 'General Odorant-Binding Protein' Discriminates Between Sex Pheromone Components

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In many insect species odorant-binding proteins (OBPs) are thought to be responsible for the transport of pheromones and other semiochemicals across the sensillum lymph to the olfactory receptors (ORs) within the antennal sensilla. In the silkworm *Bombyx mori* the OBPs are subdivided into three main subfamilies, pheromone-binding proteins (PBPs), general odorant-binding proteins (GOBPs) and antennal binding proteins (ABPs). We used the 'MotifSearch' algorithm to search for genes encoding putative OBPs in *B. mori* and found 13, many fewer than are found in the genomes of fruitflies and mosquitoes. The 13 genes include seven new ABP-like OBPs as well as the previously identified PBPs (three), GOBPs (two) and ABPx. Quantitative examination of transcript levels showed that *BmorPBP1*, *BmorGOBP1*, *BmorGOBP2* and *BmorABPx* are expressed at very high levels in the antennae and so could be involved in olfaction. A new two-phase binding assay, along with other established assays, showed that *BmorPBP1*, *BmorPBP2*, *BmorGOBP2* and *BmorABPx* all bind to the *B. mori* sex pheromone component (10E,12Z)-hexadecadien-1-ol (bombykol). *BmorPBP1*, *BmorPBP2* and *BmorABPx* also bind the pheromone component (10E,12Z)-hexadecadienal (bombykal) equally well, whereas *BmorGOBP2* can discriminate between bombykol and bombykal. X-ray structures show that when bombykol is bound to *BmorGOBP2* it adopts a different conformation from that found when it binds to *BmorPBP1*. Binding to *BmorGOBP2* involves hydrogen bonding to Arg110 rather than to the Ser56 as found for *BmorPBP1*.

Trans-Sexual Antennal Transplants Alter Sex-Specific Olfactory Behavior in a Moth

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The primary olfactory centers of the sphinx moth *Manduca sexta*, the antennal lobes, contain a small number of sexually dimorphic glomeruli: the male-specific macroglomerular complex and the large female glomeruli. These glomeruli play important roles in sex-specific behaviors, such as the location of conspecific females and the selection of appropriate host-plants for oviposition. The development of sexually dimorphic glomeruli depends strictly on the ingrowth of sex-specific olfactory receptor cell afferents. Here we tested the role of female-specific olfactory receptor cells in mediating female-specific host-plant approach behavior and in determining the response of downstream antennal lobe neurons. We generated male gynandromorphs by excising one imaginal disk from a male larva and replacing it with the antennal imaginal disk from a female donor. Most male gynandromorphs had an apparently normal female antenna and a feminized antennal lobe. These male gynandromorphs were tested for flight responses in a wind-tunnel towards tomato plants, a preferred host-plant for oviposition in *M. sexta*. Male gynandromorphs landed on host-plants as often as normal females, demonstrating that the presence of the induced female-specific olfactory glomeruli was necessary and sufficient to produce female-like, odor-oriented behavior. We also characterized the physiological and morphological properties of antennal lobe neurons of male gynandromorphs. We found that projection neurons with arborizations in the induced large female glomeruli showed physiological responses akin to those of female-specific projection neurons in normal females. These results therefore indicate that olfactory receptor cells confer specific odor tuning to their glomerular targets and furthermore, instruct odor-specific behavior.

Caste Specific Gene Expression in the Honeybees Pheromone Glands

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Caste specific pheromones are the hallmark of social insects. In the honeybee these pheromones regulate a plethora of behaviors, include the absolute reproductive dominance of the queen. These include the mandibular gland pheromones that mediates reproductive dominance of the queen and/or of egg laying workers under queenless conditions, and the Dufour's gland pheromone, the ester component of which signals reproductive capacity. The advent in honeybee sociogenomics has paved the way for studying genetic basis of such differential pheromone expression. The queen mandibular pheromone provides an excellent study system because caste-specific pheromone biosynthesis appears to be reduced to a single chemical functionalization step. At least with respect to the hydroxyfatty acids, queens are typified by ω -1 hydroxylation of stearic acid, as opposed to the worker-typical ω hydroxylation. We hypothesized that this bifurcation is the consequence of differential catalysis of hydroxylation enzymes from the P450 family, and that this reflects caste-specific gene expression. A bioinformatics study disclosed two candidate genes *CYP4AA1* and *CYP18A1* the expression of which was studied in the mandibular gland of queens, queenright (QR) workers and queenless (QL) workers. The RT-PCR results revealed that *CYP4AA1* (ω hydroxylation) was expressed at high levels in QR and in QL workers, whereas in queens its expression was negligible. The expression of *CYP18A1* (ω -1 hydroxylation), on the other hand, was high in queen's gland and negligible in QR workers. In QL workers, however, the expression of *CYP18A1* was significantly greater than that of QR workers. The fact that queen pheromone plasticity can be tracked all the way to gene expression provides a new insight on the process of caste differentiation and the accompanying social communication.


Sterility Signaling in Worker Bumble Bees, *Bombus terrestris*, as means to Reduce Aggression during Reproductive Competition

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Reproductive conflicts within insect societies are regulated largely by behavior and chemical signaling. In a recent study we demonstrated that in the bumblebee *Bombus terrestris* workers produce a series of octyl esters that are correlated with both young age and sterility. In this study we further present an association between the sterility signal and aggressive behavior in young queenless workers. We used callow workers to construct paired bees under 4 regimes of physical contact (freely interacting, separated by a single mesh, separated by a double mesh and complete isolation), and recorded their behavior during the establishment of hierarchy. In the freely interacting pairs, dominance hierarchy was established by the third day of pair's formation, first through overt aggression and later through threat gestures such as wing movements. The behaviorally dominant workers had higher ovary development and lower amounts of esters in their Dufour gland compared to their mates. The high ester amount in the subordinate workers seems to act as pacifier because it was negatively correlated with the aggression they received. Since these esters honestly indicate sterility, workers with higher amounts of esters are recognized as less threatening and as a result – less aggressed. The establishment of hierarchy was dependent on direct contact between the paired bees because in all other situation the bees' reproductive development was similar to that of bees kept in isolation. Accordingly, all had high amounts of octyl esters in their Dufour's gland secretion. These results confirm the hypothesis that ester production is a default phenomenon in workers, and reaffirm their role in reducing aggression during the establishment of hierarchy. Moreover, their persistence in the bees is regulated by aggressive behavior.



Symposium 3

plant defense: mechanisms, ecology and application

Moderators

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Elucidating the Role of Terpene Resin in Conifer Defense

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The terpene oleoresin of conifers has long been thought to play a role in plant defense because resin synthesis and exudation is induced by herbivore and pathogen attack, and individual terpene components of the resin are toxic or repellent to attackers in *in vitro* bioassays. However, to prove such a defensive role requires experiments with living plants because it is very difficult to realistically simulate the physical compartmentation and exudation of resin in *in vitro* studies. We increased the level of resin in Norway spruce (*Picea abies*) 2-3 fold by application of methyl jasmonate, a treatment which increased resistance against the bark beetle (*Ips typographus*) and the associated fungus (*Ceratocystis polonica*). However, since methyl jasmonate stimulates many defense reactions in plants, the increase in resin may not be responsible for the increased resistance, but is only correlated with it. To manipulate terpene levels in conifers more precisely, we developed an *Agrobacterium tumefaciens*-mediated genetic transformation system for Norway spruce. Transformation of embryonic tissue of *P. abies* was undertaken with a short-chain isoprenyl diphosphate synthase gene that encodes a protein producing C₁₀ precursors for monoterpene biosynthesis and C₂₀ precursors for diterpenes. Transformation led to plant lines with stable increases in resin monoterpenes, such as (+)- α -pinene, and diterpene resin acids, such as levopimaric acid, in their stems. Transgenic lines were tested with the pine weevil, *Hylobius abietis*, one of the most common pests of young *P. abies* seedlings in northern Europe. Transgenic seedlings with elevated terpene resin levels suffered significantly less damage from *H. abietis* feeding than wild-type controls, confirming that resin does serve as an insect defense in intact *P. abies*.

The Roles of Volatile Terpenes in Direct and Indirect Defenses of Plants

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Despite the remarkable abundance and diversity of terpenoid secondary metabolites in plants, there are still large gaps in our knowledge of their function in plant defense. While only few plants like conifer trees and Lamiaceae species produce and store large amounts of terpenes in specialized organs, most others only produce low levels of volatile terpenes in their vegetative parts. An analysis of maize revealed complex patterns of low concentrations of mono- and sesquiterpenes throughout the plant. To characterize the functional roles of these terpene blends in their respective tissues, we identified the biosynthetic enzymes responsible for the production of these compounds. The key step of terpene biosynthesis is catalyzed by the enzyme class of terpene synthases which can form multiple products with different carbon skeletons from only one prenyl diphosphate substrate. The differential expression of terpene synthases in plant tissues controls their respective terpene content. The terpene synthases TPS10 and TPS23 are induced in leaves after damage by lepidopteran larvae and produce a blend of sesquiterpene olefins while roots attacked by *Diabrotica virgifera virgifera* only induce to TPS23 which forms (-)-(E)- β -caryophyllene. The terpene synthases TPS4, TPS5, TPS6, TPS8 and TPS11 are not induced by herbivory but expressed constitutively in different tissues of the plant. Overexpression of the maize terpene synthases in transgenic *Arabidopsis thaliana* allowed to test the biological function of subsets of the maize terpenes in bioassays. The leaf volatiles of TPS10 and TPS23 attract natural enemies of the herbivores and thereby constitute a signal important for the indirect defense of the plant. In roots, the (E)- β -caryophyllene produced by TPS23 attracts natural enemies of the beetle. In contrast, the complex product blends of the other terpene synthases appear to directly affect attacking herbivores and microbial pathogens.

The Use of Herbivore-Induced Plant Volatiles in Pest Management

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In response to damage by herbivorous arthropods, plants emit a blend of volatiles referred to as herbivore-induced plant volatiles (HIPVs). Several studies have reported that herbivore species-specific HIPVs attracted specific natural enemies of the damaging herbivores. However, despite accumulating laboratory-based studies on the specific interactions between infested plants and carnivores mediated by HIPVs, no studies have reported on the possibility of exploiting specific HIPV-mediated interactions for the control of target pest species. We investigated the potential of such an application by using a tritrophic system of crucifer plants, diamondback moth (DBM) larvae (*Plutella xylostella*), and specialist parasitic wasps *Cotesia vestalis*. We identified volatiles emitted from cabbage plants infested by DBM larvae that attract *C. vestalis* under both laboratory and field conditions. In 2006 and 2008, we conducted field experiments in Miyama, Kyoto, Japan, focusing on the damage caused by DBM in greenhouses in which cruciferous vegetables were grown. In both years, we were able to successfully control the DBM by setting the synthetic attractants together with food devices in greenhouses.

A Semiochemical-Based Push-Pull Management Strategy for Pepper Weevil

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The pepper weevil *Anthonomus eugenii* is a serious pest on peppers in the southern United States, Mexico, Central America, and the Caribbean islands. Weevils lay their eggs in flower buds and immature fruit and the larvae feed internally on the developing seeds, making it difficult to control infestations with pesticide applications. The male-produced aggregation pheromone of the weevil was identified several years ago and was shown to attract male and female weevils in a laboratory environment. In the field, however, the weevils appear to be more attracted to pepper plants and largely ignore the pheromone traps that therefore consistently have failed to work even for population monitoring purposes. We found that in Y-tube and wind tunnel assays both male and female weevils were strongly attracted to volatiles released by pepper plants with active feeding and the weevils were especially attracted to volatiles released by feeding-damaged fruiting plants. Thus, a combination of feeding-induced plant volatiles and the aggregation pheromone has the potential to be a strong attractant. Furthermore, we have shown that after depositing an egg, female weevils secrete an oviposition plug that, when perceived by other females, reduces subsequent oviposition in the same fruit by more than half. In the laboratory, oviposition plug extracts as well as fractions thereof mimics this oviposition deterring effect. The goal for this project is to develop a semiochemical-based trapping and oviposition deterring system for the pepper weevil that can be used to effectively monitor populations and ultimately also to control this important pest. In this presentation our progress in isolation and characterization of the most potent volatile attractants as well as the oviposition deterring pheromone will be presented.

The Ups and Downs of Insect-Plant-Insect Interactions

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Plants, by growing both above and below ground, are important mediators between otherwise only loosely connected food webs. The systemic changes upon root herbivory for instance can profoundly influence leaf-feeders and vice versa. Yet, until now, the physiological basis of these interactions is poorly understood¹. We therefore conducted a series of experiments to study the changes in physiology and resistance of *Zea mays* shoots upon root attack by larvae of the beetle *Diabrotica virgifera* and the changes in the roots upon leaf attack by larvae of the noctuid moth *Spodoptera littoralis*. Our results show that the systemic changes in the leaves upon root attack are largely caused by a shift in the plant's water balance. This leads to the induction of ABA-dependent and ABA-independent defenses and increased resistance against *Spodoptera littoralis*^{2,3}. By manipulating the plants water supply and its capacity to synthesize ABA, we show that the reduction of leaf water, but not the induction of ABA is the causal factor of the increase in early resistance against *S. littoralis* after root herbivory by *D. virgifera*. The physiological changes in the roots upon leaf attack are possibly even more dramatic. Using whole-genome arrays, we demonstrate that the systemic transcriptional reprogramming in the roots upon leaf-attack by *S. littoralis* is more pronounced than the local response. Neither jasmonic acid nor any other common phytohormone seems to be responsible for this phenomenon, suggesting a specific root-response caused by a yet unknown signal. Roots of plants attacked in the leaves become considerably more resistant against *D. virgifera*, demonstrating the possible ecological importance of this phenomenon.

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Damaged Self-Recognition in Plant Anti-Herbivore Defence

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Feeding by herbivores induces plant defences, but many signals that mediate this response remain to be discovered. We argue that a general principle is 'damaged-self recognition', that is, the perception of motifs that indicate disintegrated plant cells¹. Most defence-inducing molecules are (or contain) plant-derived motifs or disintegrate plant cells and thereby release defence elicitors. For example, fatty acid-amino acid conjugates have been isolated from insect oral secretions, but their fatty acid portion derives from the plant. Plant-derived molecules occurring outside the intact cell and fragments of plant-derived molecules are commonly described defence elicitors and comprise extracellular sugars and nucleotides as well as fragments of plant macromolecules such as include pectines, oligosaccharides and peptides. All these fragments are released by hydrolytic enzymes from plant-derived precursors. The same mechanism underlies the release of green-leaf volatiles from damaged plant tissue and the rapid, wound-induced synthesis of jasmonic acid. By perceiving the 'damaged self', plants can retain evolutionary control over their interactions with herbivores, rather than depending on insect-derived molecules and thereby allow herbivores to dominate the interaction. Damaged-self signals should be taxonomically widespread, elicited by generalist herbivores and induce resistance against many types of herbivores. The concept of 'damaged-self recognition' provides a paradigm for plant responses to herbivory and helps the search for the as yet unknown elicitors of those defence responses that have only been described at the phenotypic level.

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Time-Resolved Proton-Transfer Mass-Spectrometry (PTR-MS) Analyses of Volatile Emissions from *Brassica* Plants Infested with Cabbage Root Fly Larvae

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Cabbage root fly (*Delia radicum*) is a serious pest, feeding roots of wild and cultivated crucifer species. Previous studies analyzing head-space of entire *Brassica nigra* plants revealed that specific volatile organic compounds (VOCs) are emitted when plants are damaged by root flies¹. One of these compounds, dimethyldisulfide (DMDS), was shown to attract natural enemies of *D. radicum* in the field². However, DMDS is also an oviposition stimulant for female root flies and deters parasitoids of shoot herbivores¹. These insect behaviours were all dose-dependent. Therefore, we performed a time-resolved analysis of root-fly induced VOC emissions to understand their dynamics as well as the effects these VOCs may have on aboveground and belowground multitrophic interactions associated with plants. On-line PTR-MS VOC analysis showed that DMDS and dimethylsulfide (DMS) emissions from the roots indeed increased significantly 12-16 hours after root fly infestation. Additionally, infested plants showed increased emission rates of mass 60 (compound m/z 59, as PTR-MS measures mass + 1). The emission of mass 60 was indicative for the presence of actively feeding larvae, because it started within 4-6 h of infestation and ceased when larvae pupated or died. Artificial wounding of the roots briefly increased the emission rates of mass 60 as well. This suggests that mass 60 represents a product of glucosinolate conversion by myrosinase. The identity of the mass 60 compound is currently elucidated by GC-MS analysis. Compounds typical for leaf wounding ("green leaf volatiles") or monoterpenes were not induced by root fly larvae. Our results show that specific VOC are emitted when crucifers are damaged by root fly larvae, and these VOCs may have different dynamics in time. These results are valuable for plant breeders aiming to select crops with enhanced attractiveness to natural enemies of *D. radicum* as well as of shoot feeding herbivores.

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Regulation of Plant Photosynthesis by Growth Promoting Soil bacteria

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Plant growth-promoting rhizobacteria are naturally occurring soil microorganisms that colonize roots and stimulate plant growth¹. Although such beneficial bacteria have been applied to a wide range of agricultural crops for the purpose of growth enhancement, the biochemical complexity of such plant-microbe interactions have limited our mechanistic understanding of bacterial-induced plant growth promotion. Using a simplified experimental design in which the bacterial stimulus is limited to volatile components, we have begun to characterize initial chemical signals from the commercial strain *Bacillus subtilis* GB03 as well as down-stream molecular and physiological plant responses involved in inducible growth promotion in *Arabidopsis*^{2,3}. Here we provide biochemical evidence that GB03 regulates photosynthesis through modulation of endogenous sugar/ABA-signaling and iron acquisition to increase photosynthetic efficiency as well as chlorophyll content thereby establishing a regulatory role for soil symbionts in plant acquisition of energy⁴.

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Redundancy between Belowground Direct and Indirect Defenses in Common Milkweed *A. syriaca*

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Upon herbivore attack, plants rely on a matrix-like type of responses, generally involving direct and indirect defenses. Direct defenses can act by physical or chemical means, via toxic or antinutritive effects; indirect defenses typically act by providing rewards or cues for predators or parasitoids to locate their host herbivores (e.g., volatile organic compounds or extrafloral nectar). Belowground, the release of terpenoid volatiles in the soil has been shown to attract entomopathogenic nematodes foraging for herbivorous insect larvae. In the milkweeds (*Asclepias* spp.), plants are predominantly defended in the shoots by the production of cardenolides and latex, whereas belowground mainly by cardenolides. Despite the fact that larvae of the specialist beetle *Tetraopes tetraophthalmus* are major herbivores of *Asclepias syriaca*, little was previously known about root defenses in the common milkweed. In common garden experiments, we tested whether insect damaged *A. syriaca* roots attract more nematodes than undamaged plants. We then analyzed volatile organic compounds from roots of damaged and undamaged plants. Finally, by screening and selecting milkweed genotypes for high and low root cardenolides, we examined the relationship between allocation to cardenolides and indirect defense in the roots. Results show that entomopathogenic nematodes (*Heterorhabditis bacteriophora*) are extremely efficient in killing beetle larvae, and that attacked plants are more attractive than healthy plants. We also found higher level of volatiles emitting from damaged roots. Field results show that *T. tetraophthalmus* larvae survive better on low cardenolide genotypes of *A. syriaca* compared to high cardenolide genotypes, but the presence of nematodes in the soil reduces larval survival to similar levels in both high and low cardenolides genotypes. Thus, we conclude that direct and indirect defenses act redundantly in *A. syriaca* roots to reduce the impact of a specialist herbivore in the field.

Are Flower-Feeders Fitter – and Flowers Thus at Risk?

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The qualitative and quantitative metabolite composition varies highly within plants between different tissues. Specialist insects may prefer tissue with higher concentrations of the plant's characteristic defence metabolites. Most herbivorous larvae of butterflies or sawflies are considered to be folivores. However, recently it was shown that caterpillars of the cabbage white, *Pieris brassicae* (Lepidoptera: Pieridae), which are specialists of Brassicaceae, move during larval development from the leaves to the flowers. Feeding on flowers sustained an increased growth rate compared to feeding on leaves in these caterpillars¹. We investigated, whether the turnip sawfly, *Athalia rosae*, might behave in a similar way, as it can moreover sequester the plant-characteristic glucosinolates in its haemolymph. Larvae moved from leaves to flowers of *Sinapis alba* for feeding from the third larval stage onwards. Flower-fed individuals reached higher body masses and developed in shorter time than larvae fed with young leaves. Larvae fed exclusively with old leaves showed the poorest performance. Flowers had three times higher myrosinase activities than young leaves and ten times higher activity than old leaves, whereas the total glucosinolate concentration of flowers and young leaves was equally high. For this specialist herbivore tissue with high myrosinase and glucosinolate levels seems to be thus a highly valuable feeding source, likely in addition to high levels of sucrose. However, the larvae did not actively choose the flowers but rather moved upwards on the plant, regardless on how plants were orientated (straight or upside-down). Plants clearly run a high risk of exploration by specialists by increasing putative defence levels in their most valuable tissue.

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Parasitoids Use Chemical Footprints to Track Down Caterpillars

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Parasitoid wasps in search for plant-feeding hosts display typical patterns of behavior. The braconid *Cotesia marginiventris*, which parasitizes young caterpillars, is guided by herbivore-induced plant volatiles to an infested plant. On the plant, the female wasp searches for further chemical residues (kairomones) originating directly from the host. We showed that caterpillars leave minute amounts of treacherous chemical footprints while walking over a plant surface¹. Female wasps are able to detect these residues for up to two days after their hosts have left the site. Analyses of the caterpillar footprints revealed that these consisted of linear and monomethyl-branched alkanes as well as few minor unidentified compounds. A reconstructed blend of the major footprint compounds, consisting of linear C21-C32 alkanes, induced characteristic antennation behavior. However, the artificial blend was less attractive than the original one, suggesting a role for additional minor compounds in recognizing former caterpillar presence. Previous investigations using wax mutants of barley showed that the physico-chemical traits of the epicuticular leaf wax can modulate the parasitoids' response to host footprints². We hypothesize that long-chain hydrocarbons of insect and plant cuticular origin are important mediators of insect-plant interactions. Their role in modulating trophic cascades still awaits full appreciation.

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Responses of *Myzus persicae* to Volatile Organic Compounds of *Nicotiana benthamiana* Plants Infected with Wild-Type *Potato leaf roll virus* (PLRV) and PLRV with Point Mutations within the Viral Gene *P4*

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The basis for changes in volatile organic compounds (VOC) released from virus-infected plants, and aphid responses to these VOC, were examined using *Nicotiana benthamiana*, a species widely employed for studies of plant virus interactions¹. The study specifically examined the effect of point mutations within the gene *P4* within *Potato leaf roll virus* (PLRV) on the release of VOC by *N. benthamiana*, and on the responses to these VOC by the aphid, *Myzus persicae*, the principal vector of PLRV. Individual mutations in the *P4* gene were generated by site-directed mutagenesis, and were designed to preserve the overlapping P3/P5 gene coding region. Six such mutations were compared with the original wild-type PLRV. Infection of experimental plants was achieved using agroinoculation of *N. benthamiana* plants with full-length, infectious cDNA clones of each PLRV. The accumulation of PLRV was assessed by detection of viral coat protein (TAS-ELISA) or by RT-PCR; sequencing confirmed that the mutations remained intact. Approximately 3 weeks after inoculation, responses of apterous *M. persicae* to the infected plants, inoculated and non-inoculated controls were assessed. In a dual choice test (method from 2), in which the aphids could contact leaf surfaces of test plants, apterous *M. persicae* preferentially settled on wild-type PLRV inoculated plants as compared with agro controls. In an emigration bioassay that isolated aphid responses to VOC from the test plants (method from 3), aphid responses did not differ between wild-type PLRV-infected plants and controls, but plants infected by several PLRV clones with mutations were more arrestant than wild-type. Analysis of headspace revealed PLRV-induced increases in some VOC, including the predominant VOC, nicotine. No single component in the headspace VOC is consistently associated with responses by the aphids. The result indicates that differences in the genetic sequence of ORF 4 influences virus-induced VOC and aphid responses to infected plants.

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Metabolomics in the Quest for New Wound Biomarkers and a Global Survey of their Dynamics in Plants

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In recent years, metabolomics has become a valuable technology to study plant responses to a variety of stress conditions¹. Indeed, metabolomics represents an efficient way to identify different key compounds that are induced in response to environmental stresses such as signaling molecules or phytoalexins. Among the different analytical tools used in metabolome analysis², the most used are mass spectrometry and nuclear magnetic resonance, associated to advanced data mining strategies. These techniques of choice are complementary and present inherent advantages and limitations. In the selected example, significant metabolome variations related to stress caused by wounding in the model plant *Arabidopsis thaliana* (Brassicaceae) were analysed by ultra-high pressure liquid chromatography coupled to time-of-flight mass spectrometry (UHPLC/TOFMS) and capillary nuclear magnetic resonance (CapNMR). Extensive research on the wound response has brought important information on the defence mechanisms against herbivores and different bioactive oxygenated fatty acids of the jasmonate family were found responsible for the expression of defence genes³. These compounds were mainly studied by targeted GC-MS approaches⁴. Our approach was based on the following steps: 1. Rapid UHPLC/TOFMS metabolite fingerprinting on numerous wounded and unwounded specimens for group discrimination and determination of ions (m/z) responsible for the main differences after adequate data treatments. 2. High resolution metabolite profiling and selected MS/MS experiments for localization and preliminary identification of the biomarkers. 3. Targeted LC-MS triggered microfractionation of the biomarkers of interest at the semi-preparative level based on computed LC conditions from UHPLC gradients. 4. Complete structural determination at the microgram scale of the unknown biomarkers based on MS/MS and CapNMR experiments. Thanks to this strategy, a broad survey of wound biomarkers with various physicochemical properties was obtained in *Arabidopsis*. Besides known signalling molecules, original oxylipins and related products were identified and a global view of their dynamics was obtained.

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Why Harm Yourself? Induction of Toxin Releasing β -Glucosidases in Specialist and Generalist Lepidoptera on Iridoid Glycoside Containing Food Plants

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Iridoid glycosides (IGs) are secondary plant compounds which have deterrent, growth reducing or even toxic effects on non-adapted herbivorous insects, while some specialized species are well adapted to IGs and even sequester them as chemical defense against predators. To exert negative effects, IGs have to be hydrolyzed by β -glucosidases, ubiquitous enzymes found in plants, as well as in animals and bacteria. So far, midgut β -glucosidases of various insect species were shown to be induced or reduced in reaction to plant glycosides in their diet. To test the hypothesis that β -glucosidases play a key role in tolerating and adapting to IG containing plants, we reared polyphagous arctiid species and the monophagous IG specialist *Junonia coenia* on two plantain species that differed in their IG concentration, or on the IG-free dandelion. For larvae of all species and treatments, the midgut β -glucosidase activity towards a standard β -glucosidase substrate (4-nitrophenyl- β -D-glucopyranoside) and towards the IG aucubin were compared. Kinetic analyses revealed Michaelis-Menten constants that were comparable to the β -glucosidases of other lepidopteran species, while heat inactivation experiments could prove the existence of more than one β -glucosidase. Contrary to our expectations, a significant induction of aucubin hydrolyzing β -glucosidase activity could be detected in all species when reared on *P. lanceolata*, the plant with the highest IG concentration. While this does not seem to represent an adaptive response of the insects to host plant toxins, it might be evidence for the presence of symbiotic gut bacteria like *Bacillus* species that possess β -glucosidases and could thus use the plant IG as a glucose and carbon source.

Oviposition Affects the Transcription of Sesquiterpene Synthases in Pine Species and Results in Behavioural Changes of Egg Parasitoids: How Species-Specific are these Effects?

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Volatile compounds, especially terpenoids, often mediate direct and indirect defence mechanisms in plants. Indirect plant defence mechanisms are frequently species-specific and highly chemically defined. Scots pine (*Pinus sylvestris*; Pinaceae) is known to change its terpenoid volatile blend after egg deposition by the herbivorous sawfly (*Diprion pini*, Diprionidae) after 72 h egg deposition, which results in the attraction of a specialist egg parasitoid. Previous studies showed that neither oviposition of *D. pini* on a closely related pine species (*P. nigra*) nor oviposition by the sawfly species *Gilpinia pallida* (Diprionidae) on *P. sylvestris* does attract the parasitoid *Chrysonotomyia ruforum* (Eulophidae) at that time point. In contrast oviposition by the sawfly species *Neodiprion sertifer* (Diprionidae) on *P. sylvestris* does attract the egg parasitoid after 72 h egg deposition¹. The focus in this study was to investigate with molecular tools indirect defence mechanisms in two coniferous species after egg deposition by three different sawfly species when looking at two additional time points (48 h and 96 h) of ovipositional induction. Based on the finding of previous studies, that *P. sylvestris* terpene synthases genes (*PsTPS 1*, *PsTPS 2*) are induced 72 h after *D. pini* egg deposition², we examined here the transcript levels of *PsTPS 1*, *PsTPS 2*, *PsTPS 4* and *PsTPS 5* under the same experimental conditions but additional induction times. *PsTPS 4* and *PsTPS 5* are two new genes isolated from *P. sylvestris*. Whereas *PsTPS 4* could not be functionally described so far, *PsTPS 5* produces - when heterologously expressed in *Escherichia coli* (*E*)- β -farnesene - the terpene that has exclusivity crucial impact on the parasitoid attraction. Surprisingly *PsTPS 5* does not show enhanced transcription at any time point and treatment. However transcript level analyses of *PsTPS 1*, *PsTPS 2* and *PsTPS 4* show higher expression level only in plant material attractive (after 72 h induction) for the parasitoid, in non-attractive plant material this increase could not be seen (48 h and 96 h after induction). We support these findings with results of behavioural biotests.

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Alteration of Glucosinolates Profiles in Induced *Brassica* Plant Reveals a Strong Paradox between Preference and Performance in *D. radicum*

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Plants are not passive victims of phytophagous insects. During the evolutionary process, they have developed chemical weapons to defend themselves against herbivores. Glucosinolates and their breakdown products in *Brassica* plants are well-known to be involved in direct defenses processes against herbivores, leading in numerous cases to a decrease of both performances and preferences. The present study examined the induced responses in terms of glucosinolate profiles of two cultivated species of *Brassica* (broccoli, *Brassica oleracea* and turnip, *Brassica rapae*) submitted to several root treatments and their consequences on the cabbage root fly, *Delia radicum* preferences and performances. Results show that previous infestation and applications of jasmonic acid lead to modifications of *D. radicum* performances and oviposition preference but also a change in glucosinolate profiles. Jasmonic acid stimulated oviposition but interestingly caused a strong decrease of the cabbage root fly survival rate in broccoli. Conversely it induced an increase of survival rate and seemed to have no influence on oviposition behaviour in turnip. Previous infestation influenced neither performance nor oviposition of *D. radicum* in broccoli, while in turnip it did not have any effect in *D. radicum* egg-laying behavior but increased survival of larvae. When looking at glucosinolate profiles, it appeared that both the local and systemic production may contribute to explain the strong paradox observed between *D. radicum* performances and preferences particularly in JA-induced plants. In addition, although both species are closely related, this study shows that induced responses exhibited by turnips and broccolis are completely different. In an agronomical context, a strategy based on the stimulation of plant defenses should necessarily takes into account the specific features of induced responses for different species and even different cultivars.

Lipoxygenases – a Nexus of Plant Defense Signaling

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Plants have diverse metabolic means of defending themselves against attack from herbivores. Often, the same signaling pathway regulates the expression of different defenses. Lipoxygenases (LOXs) are the key enzymes of the oxylipin signaling pathway which regulates the biosynthesis of a diversity of distinctly different metabolites used for defense. LOXs catalyze the formation of fatty acid hydroperoxides (HPs) through the deoxygenation of fatty acids liberated from membranes by wounding. These HPs can in turn be further metabolized via two distinct pathways, one via the enzyme allene oxide synthase (AOS) the other via the enzyme hydroperoxide lyase (HPL). The AOS branch leads to the formation of jasmonic acid (JA), the central plant defense hormone, while the HPL branch leads to the formation of C6-volatiles or 'green leaf volatiles' (GLVs). GLVs are rapidly released after wounding and herbivory and are known to play a role as signaling molecules in tritrophic interactions. Although AOS and HPL both use HPs, recent data from *Nicotiana attenuata* suggests that the LOX that supplies AOS with HPs might be encoded by a different gene than the one that supplies HPL with HPs. In order to investigate the impact of each LOX on both pathways we used two different isogenic lines of plants that were silenced in the expression of either *NaLOX3* or *NaLOX2*. Silencing the expression of *NaLOX3* strongly reduced JA biosynthesis but barely influenced the wound-induced release of GLVs. In contrast, silencing *NaLOX2* strongly decreased the plant's ability to produce GLVs but barely influenced JA biosynthesis. These results demonstrate that in *N. attenuata* the flux of HPs is channeled by the activity of different LOXs to supply these two important mediators of direct and indirect defenses.

Metabolic Profiling Analysis: a New Tool in the Prediction of Host-Specificity in Classical Biological Control?

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The aim of the study is to use metabolic profiling analysis i) to understand the disjunct host range of the potential weed biological control agent *Ceutorhynchus cardariae* Korotyaev (Coleoptera: Curculionidae) for the invasive Brassicaceae plant *Lepidium draba* L., and ii) ultimately, to assess the usefulness of these analytical techniques as an additional tool in classical biological control to predict the host range of potential weed biological control agents. Current host-specificity testing for the selection of an environmentally safe biological weed control agent is based on the phylogenetic relationships of the weed¹. According to the centrifugal phylogenetic method², non-target species closely related to a target weed should be at greater risk of attack by a biological control agent than distantly related plant species. This prediction, however, is not always confirmed. In our model system, for example, several distantly related species within Brassicaceae are attacked by *C. cardariae* under no-choice conditions, resulting in the observed disjunct host range. We suggest that phenotypic characteristics such as plant chemistry and morphology are important host affiliation predictors and that variation in these characteristics departs sufficiently from phylogeny to account for the observed disjunct host range. Thus, a method based on metabolic profiling is likely to be a useful additional tool for assessing risk of nontarget attack. As a first step towards developing such an approach, we characterized the glucosinolate profile of 23 plant species/populations, differing in susceptibility to attack by *C. cardariae* as determined by feeding and oviposition trials. The glucosinolate profiles were used to generate a chemical similarity tree for these species. The relative conservatism of susceptibility to *C. cardariae* within this tree was compared with that observed to occur within the phylogenetic tree for the test species³, using a modification of the measure of phylogenetic correlation⁴.

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Volatile-Mediated Impact of Bacteria on Plant Growth

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Bacteria are known to produce a wide spectrum of volatile organic compounds (VOCs). Recently, it has been discovered that these VOCs can lead to a significant plant growth promotion¹. In this study, we used a divided Petri dish assay to screen 42 soil-borne bacterial strains grown on four different culture media for their volatile-mediated effects on the growth of the model plant *Arabidopsis thaliana*. The results show that the VOCs-mediated influence of bacteria on plant growth is a widespread phenomenon: all 42 strains led to a significant growth modification in at least one of the culture media. Effects were striking, ranging from six-fold growth promotion to plant death, and highly medium-dependent. Interestingly, VOCs-mediated growth promotion was also observed for phytopathogenic bacteria, whereas the VOCs of well-known plant growth-promoting rhizobacteria (PGPRs) could lead to plant death. One particular strain, belonging to the species *Burkholderia pyrocinia*, promoted plant growth independently of the culture medium used. Additional experiments revealed that the density of the bacterial inoculum plays an important role, with growth promotion induced by low inoculum densities and plant death by high inoculum densities. Furthermore, first results indicate a role of quorum sensing (QS, the ability of bacteria to sense their density and adapt their behavior accordingly) in the VOCs-mediated effects of specific bacterial strains, with QS-impaired mutants having a reduced impact on plant growth than the wild-type. The identity of the VOCs responsible for the observed effects is being currently elucidated using GC/MS analysis and *in vitro* tests of pure chemicals.

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Specificity in Plant Herbivore Interactions: Plant-Mediated Conversion of Insect

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In the ongoing evolutionary arms race between plants and insects, compounds derived from insect oral secretions are important mediators of plant-based insect recognition. However, there is so far no unequivocal proof for the mode of action of these elicitors, and in the decade since the discovery of the first insect elicitor, a few mechanisms have been proposed. None of these however, have addressed the possibility of plant-based conversion of insect elicitors. We dissected the direct interaction between the tobacco hornworm *Manduca sexta* and coyote tobacco, *Nicotiana attenuata*, and focussed on the metabolism of one of the main elicitors in the insects' oral secretions, 18:3-glu. The results show that the metabolism of 18:3-glu is extremely rapid, as soon as it comes into contact with wounded leaf tissue enzymatic metabolism takes place immediately. Using mass spectrometry, several different derivatives could be identified which all appeared in the same timeframe as 18:3-glu disappeared from the leaf surface. We discuss this metabolism in the light of specificity in plant-insect interactions, and hypothesize about its role in fine-tuning the defense response.

8-Hydroxyquinoline-2-Carboxylic Acid (HQA) from the Insect Gut Impacts Bacterial Growth via Iron Chelation

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Insects developed a variety of mechanisms to prevent bacterial infections in the gut. One aspect is the harsh gut condition which includes pH extremes and diverse digestive enzymes. Furthermore the occurrence of reactive oxygen species and antimicrobial peptides repress the survival of bacteria¹. In the regurgitant of Lepidoptera larvae, the 8-hydroxyquinoline-2-carboxylic acid (HQA) was identified² in remarkably high concentrations (0,5-5 mM). Quinolinic derivatives are well-known from various environments and very distinct organisms (insects, plants, bacteria)³⁻⁵. HQA was first detected in the defence secretion of the water beetle *Ilybius fenestratus*⁶. However, there was not a specific biological function attributed to this his compound. HQA derives from the tryptophane metabolism⁷ and is able to form complexes with several bivalent metal ions, including Ni(II), Co(II) and Cu(II)⁸. HQA occurs in the gut *Spodoptera* and *Heliothis* species and it could be shown that the compound is formed by the larvae themselves. Their gut bacteria were not able to form HQA. In contrast it was observed that the substance inhibits their growth. It could be shown that this molecule works as iron chelator and consequently inhibits important iron-regulated enzymes like the cytochrome oxidase or the superoxide dismutase. Thus, iron-depletion could be an additional instrument of the larvae controlling their intestinal microbiota.

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Identification of Regulators of FAC-Mediated Responses in *N. attenuata*

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Nicotiana attenuata tunes its defense responses against *Manduca sexta* larva by recognizing elicitors present in its oral secretion (OS). In particular, fatty acid-amino acid conjugates (FAC) are necessary and sufficient to enhance jasmonic acid and ethylene production among other specific responses in this plant species. How plants perceive insect specific elicitors such as FACs and trigger downstream signaling cascades to induce insect-specific responses is largely unknown. In an attempt to unravel early mechanisms elicited by FACs, we combined a SuperSAGE approach for in-depth gene expression profiling with viral induced gene silencing (VIGS) and biological/biochemical assays for screening of gene function. We have identified several potential regulators of FAC-mediated responses. One of them encodes for a putative membrane-localized protein kinase involved in the FAC-mediated activation of the ethylene burst.

Leaf Epicuticular Waxes Effects Egg Parasitoid's Response To Host Adult Footprint Contact Kairomone

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Chemical footprints left behind by true bugs are perceived as a contact kairomones by scelionid egg parasitoids. In the system *Nezara viridula* and its egg parasitoid *Trissolcus basalus*, previous studies have shown that the kairomone mediating such behavior is part of *N. viridula*'s cuticular hydrocarbons^{1, 2}. In this study, the effect of epicuticular waxes of leaves of broad bean, *Vicia faba*, on wasp response to footprints of *N. viridula* females are investigated. Plants with intact waxes layer were used (a) without host chemical contamination, or (b) contaminated by host female footprints. Mechanically de-waxed plants were tested (c) without host chemical contamination, or (d) de-waxed a few minutes after being contaminated, or (e) de-waxed about 30 min before being contaminated by host female footprints. And, finally, female wasps were tested on the side of the gum arabic film that had been appressed to (f) the adaxial leaf surface of plants without host chemical contamination, and (g) to plants contaminated by host footprints. Scanning electron microscopy revealed that the epicuticular waxes occurred as a film densely crystallized as irregularly shaped platelets with spherical granules randomly distributed. These findings demonstrated that epicuticular waxes of broad bean leaves can mediate the foraging behaviour of *T. basalus* females by absorbing host adult footprints contact kairomone.

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Rapid Phosphorylation of the Flagellin Receptor FLS2 and its Associated Kinase BAK1 Activates Plant Immune Responses

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The first line of plant defense against invading microbes is elicited by the recognition of microbe-associated molecular patterns (MAMPs). These are perceived by highly specific receptors at the plasma membrane, such as the flagellin receptor FLS2 (Flagellin sensing 2)¹. We have previously found that FLS2 associates with BAK1 ("BRI1 Associated Kinase 1", originally described as signaling partner of the brassinosteroid receptor BRI1) upon stimulation with the peptide flg22, the characteristic epitope of bacterial flagellin². While FLS2 is responsible for ligand binding, the kinase domains of both FLS2 and BAK1 are believed to be activated leading to cellular signal transduction. Using in vivo pulse-labeling with [³³P]phosphate, we now characterize de novo phosphorylation events on FLS2 and BAK1 and followed the stability of the phosphorylated proteins over time. In Arabidopsis cell cultures both, FLS2 and BAK1, are phosphorylated within 15 s of treatment with flg22. Additionally, we provide evidence for the interaction and subsequent phosphorylation of BAK1 with several other receptor kinases. This allows us to broaden our observations with FLS2 and BAK1 to a general principle of transmembrane receptor activation in plants.

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Characterising a Plant C-glycosyltransferase from the Biosynthesis of C-Glycosylflavones, Allelochemicals that Control *Striga* Parasitic Weeds in Africa

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Parasitism of maize by *Striga hermonthica* is a major problem in East Africa infecting around 40% of arable land on the African savannah¹. Intercropping maize (*Zea mays*) with the cattle legume *Desmodium uncinatum*, commonly known as desmodium, significantly protects the cereal from *Striga* parasitism. This has led to successful implementation of the strategy in farming practices. The mechanism of protection has been defined as allelopathic with desmodium stimulating germination of the weed and interfering with subsequent development, as shown by inhibition of radicle growth *in vitro*². A range of biologically active plant secondary metabolites have been isolated and identified from desmodium root extracts³. So far all biologically active compounds have been identified as flavonoids. One compound that demonstrated high radicle growth inhibition at low concentrations was the di-C- glycosylflavone isoschaftoside which has also been isolated from hydroponic root exudates in biologically active concentrations. The pathway by which C-glycosylflavones are biosynthesised is currently unclear. 2-Hydroxynaringenin has been identified as the substrate for C-glycosylation in buckwheat, *Fagopyrum esculentum* and more recently in wheat, *Triticum aestivum* and rice, *Oryza sativa*^{4, 5}. Using synthetic ²H₄ labelled putative biosynthetic intermediates and ESMS analysis we have identified the pathway by which C-glycosylflavones are biosynthesized in desmodium by protein extracts from leaf tissue and identified the flavanone 2-hydroxynaringenin as the first committed step in C-glycosylflavone biosynthesis. There have been numerous reports of C-glycosylflavones in plants in the literature but little data is available for the enzymes responsible. Purification of a C-glycosyltransferase from desmodium is now underway. Identification of enzymes responsible for isoschaftoside biosynthesis has the potential to allow edible legumes to be identified as alternative intercrops to the cattle forage legume either through screening, breeding or genetic manipulation.

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Chemical Structures and Elicitor Activities of Fatty Acid Amino Acid Conjugates: the Strategies between Herbivore and Plants

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17-Hydroxylinolenoyl-glutamine (17OH-volicitin), which is a well-known herbivore-produced elicitor identified from *Spodoptera exigua* regurgitants, is the most active elicitor of corn plant volatile emissions of all fatty acid-amino acid conjugates (FACs)¹. The previous reports that linolenoyl-glutamine was only 30% as active as volicitin and that 17(R)- and 17(S)-volicitin showed no differences in a corn seedling assay suggest the hydroxylation on the 17th position is essential but the chirality does not affect its elicitor activity². Interestingly, 18-hydroxylinolenoyl-glutamine (18OH-volicitin), newly identified from *Manduca sexta* regurgitants, is no more active than linolenoyl-glutamine in the corn assay while 18OH-volicitin is as active as 17OH-volicitin when treated on solanaceous plants such as tobacco or egg plants, host plants of *M. sexta*. However, these glutamine type FACs including 18OH-volicitin are present in extremely small amounts in *M. sexta*. Instead the larvae synthesize large amounts of glutamic acid type FACs as main components, which are negligibly active elicitors for both corn and egg plants. These results suggest that plants have developed a defense system customized to their pest insects and that herbivores also have developed different FAC components as a result of counter-adaptation.

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A Metabolomic Approach to Study Secondary Plant Compounds Involved in Thrips Resistance

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Western flower thrips (*Frankliniella occidentalis*) has become a key insect pest of agricultural and horticultural crops worldwide. Little is known about host plant resistance to thrips. We applied a metabolomic approach to compare the metabolomic profiles of thrips resistant and susceptible plants. As study systems we used F_2 hybrids of *Senecio jacobaea* and *Senecio aquaticus* as a wild plant and chrysanthemums as an ornamental plant. We identified thrips resistant plants applying bioassays. Subsequently, nuclear magnet resonance spectroscopy (NMR) was applied. NMR facilitates a wide-range coverage of the metabolome, making NMR especially suitable if there is no *a-priori* knowledge of the herbivore defense compounds. We show that thrips resistant and susceptible plants can be discriminated on basis of their metabolomic profiles. Thrips resistant *Senecio* hybrids contained higher amounts of the pyrrolizidine alkaloids (PA), jacobine and jaconine. Also, a flavanoid, kaempferol glucoside, accumulated in the resistant plants. Both, PAs and kaempferol, are known for their inhibitory effect on herbivores and pathogens¹. Thrips resistant chrysanthemums contained higher amounts of the phenylpropanoids chlorogenic acid and feruloyl quinic acid. Both phenylpropanoids are known for their inhibitory effect on herbivores as well as pathogens². The effect of chlorogenic acid on thrips was further studied in bioassays with artificial diets. These experiments confirmed the negative effects on thrips. Our results prove NMR to be an important tool to identify metabolites involved in thrips resistance. It constitutes a significant advance in the study of plant–insect relationships, providing key information on the implementation of herbivore resistance breeding strategies in plants. Most of the compounds identified also affect pathogens. This may form the basis of a multi-resistance breeding program. Besides their negative effect on thrips kaempferol, jacaranone and the phenylpropanoids are investigated for their positive effect on human health preventing cancer development. This unique combination makes them the candidates of choice to improve host plant resistance.

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Bioactivities of Abietane-Type Diterpenes from *Taxodium distichum* Cones

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Taxodium distichum Rich. (Taxodiaceae), commonly known as “bald” or “swamp” cypress, is well known as an extant deciduous, living fossil conifer indigenous to the southern part of North America. The cones produced by the conifers are essential parts for self-propagation. Consequently, the potential for having antifeedant, antifungal, as well as phytochemical activities against external influences are strongly suggested¹. From the *n*-hexane extract of air-dried *T. distichum* cones, eight known abietane-type diterpenes were isolated. They were identified as 6,7-dehydroroyleanone, taxodal², taxodione, salvinolone, 14-deoxycoleon U, 5,6-dehydrosugiol, sandaracopimaric acid, and xanthoperol. We investigated the bioactivities of these compounds against subterranean termite (*Reticulitermes speratus* Kolbe)³ and white (*Trametes versicolor*: NBRC30340) and brown (*Fomitopsis palustris*: NBRC30339) rot fungi. In addition, these activities of three major abietane-type diterpenes, ferruginol, 6,7-dehydroferruginol, and sugiol were evaluated together. 6,7-Dehydroroyleanone and taxodione showed potent termiticidal activity, and 14-deoxycoleon U and xanthoperol showed potent antifeedant activity. 6,7-Dehydroroyleanone was found to be one of the representative termiticidal compounds in the *n*-hexane extract of *T. distichum* cones. Also, taxodione, salvinolone, and 14-deoxycoleon U showed potent antifungal activities against both wood rot fungi. The inhibition rates of taxodione and 14-deoxycoleon U were twice as strong as that of ferruginol. From these results, we concluded that *T. distichum* cones contain strong bioactive compounds. These findings suggested that the various forms of the abietane-type structure due to oxidation reflect their various bioactivities. Therefore, the presence of various oxidation forms of the abietane-type structure reflects their various bioactivities. As several bioactive compounds against subterranean termite and wood rot fungi were discovered, these compounds could also be related to the self-defense of *T. distichum*.

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cis-Jasmone as an Activator of Plant Defence: Understanding Underlying Mechanisms and Towards Field Deployment

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Seminal studies on the interaction of aphids, and their natural enemies, with host plants showed that the interaction is modified by the activation of defence signaling by *cis*-jasmone, a volatile plant secondary metabolite¹. Subsequent studies showed that for field plots of wheat, *Triticum aestivum*, aphid populations are significantly reduced by a single spray application of *cis*-jasmone². The mechanisms underlying activation of defence by *cis*-jasmone have been subsequently studied in the model plant, *Arabidopsis thaliana*³ and in wheat, *T. aestivum*⁴. For wheat, liquid-phase extraction and vapour-phase extraction approaches showed that levels of allelopathic compounds, *i.e.* benzoxazinoids and phenolic acids, were elevated in *cis*-jasmone treated wheat compared to levels for control plants⁴. The potential for using *cis*-jasmone as a practical tool for activation of defence in other major world crops has been explored. Early studies show that stress signalling in cotton, *Gossypium hirsutum*, is activated by *cis*-jasmone, primarily the production of (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, resulting in repulsion of the cotton aphid, *Aphis gossypii* (Homoptera: Aphididae). Defence pathways have also been shown to be induced in soybean, leading to the attraction of natural enemies of stink bugs (Heteroptera: Pentatomidae)⁵, and in tomatoes, leading to reduced infection by plant parasitic nematodes. These results demonstrate activation of defence in a range of crop plants through application of *cis*-jasmone, and offer real promise for the use of small lipophilic molecules as natural plant activators in the sustainable control of aphid, stinkbug and nematode pests.

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Do Plants Distinguish Herbivores on Neighboring Plants?

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When attacked by herbivores, plants emit a specific blend of volatile organic compounds (VOCs) that attract carnivorous natural enemies of the herbivores. Furthermore, neighboring plants use such volatiles as information about the presence of “danger” and increase their defense levels prior to the herbivores’ attack. However, the levels of danger depend on herbivores’ food preference between the neighboring uninfested and currently infested plant species. This would affect the likelihood of the invasion. Therefore, we tested whether plants are able to distinguish VOCs from neighboring plants infested by different herbivore species with different preferences for the currently infested plants and neighboring plants were different. We focused on the production of extrafloral nectar (EFN), an indirect defense of plants against herbivores that functions by offering alternative food to carnivores, in lima bean plants. Lima bean plants were exposed to VOCs emitted from cabbage plants infested by either diamondback moth (*Plutella xylostella*) larvae, common cutworm (*Spodoptera litura*) larvae, or two-spotted spider mite (*Tetranychus urticae*) females. Lima bean plants are a suitable food for *S. litura* larvae and *T. urticae* but not for *P. xylostella* larvae, while cabbage plants are a suitable food for *S. litura* and *P. xylostella* larvae but not for *T. urticae*. We expected that lima bean plants would increase EFN when they were exposed to VOCs from cabbage plants infested by either *S. litura* larvae or *T. urticae* females. However, lima bean plants increased the amount of EFN only when they are exposed to VOCs from cabbage plants infested by *T. urticae*. *T. urticae* was more likely to move from cabbage plants to lima bean plants relative to the other two herbivore species. Thus, lima bean plants distinguish between VOCs from plants infested by herbivores with different propensities to invade lima bean plants.

Using Radiotracers to Study Changes in the Short-Term Kinetic Fluxes of Intra- and Extracellular Sugars into Cellulose in Response to Jasmonate and Isoxaben

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The sophisticated defense mechanisms of higher plants often produce signal molecules such as jasmonate (JA) that have been implicated in cell-wall modification. Yet, little is known about the coordination of these signal pathways or their mechanisms of action. Using radioactive ¹¹C (t_{1/2} 20 m), administered to leaves as ¹¹CO₂, and ¹⁸F (t_{1/2} 110 m), administered to roots as the glucose analog, 2-deoxy-2-[¹⁸F]fluoro-D-glucose (¹⁸FDG), we measured the flux of tracer into cell-wall cellulose exploring the roles of intracellular (¹¹C) and extracellular (¹⁸F) sugars in cellulose production. We used these tools in combination with pharmacological treatments of isoxaben (ISX), a benzamizole pre-emergence herbicide and JA, both administered topically to intact mature leaves (L2) of *Nicotiana tabacum* L. (cv Samsun) and testing responses 1 h later. Both treatments inhibit cellulose, while mediating increased lignin. However, unlike JA, ISX inhibits sugar transport, while JA promotes it. We hypothesized that the different physiological responses to treatments should manifest in differences in the cellulose kinetic fluxes reflecting the demand cellular metabolism places on sugar trafficking. In the ISX studies, treatment significantly reduced the flux of ¹⁸F into cellulose relative to controls, and it slightly increased the flux of ¹¹C into cellulose over the 1 h timeline. These data suggest that cell wall construction requires large import of sugars, and when that supply is impeded, the cell reprograms its partitioning of recent carbon in order to sustain the cellulose synthesis machinery. Furthermore, JA treatment significantly reduced fluxes of both ¹⁸F and ¹¹C into cellulose relative to controls suggesting that even though there is a healthy supply of sugars to sustain the cellulose metabolic machinery, that process is greatly inhibited, likely through direct action of the hormone on the cellulose synthase enzyme. Research was supported by the US DOE and by Deutscher Akademischer Austauschdienst.

How to Explain Structural Diversity of Secondary Metabolites: Do We Need a New Raison d'Être?

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Secondary metabolites are produced by many pro- and eukaryotic organisms. Originally considered as waste products, today the prevailing notion is that secondary metabolites function as defense of the producing organism against all kinds of predators. The terms “chemical defense” and “biological functions”, however, are metaphors used in a too teleological context. The structurally diverse secondary metabolites entail also many inactive compounds or compounds with unknown functions. By assuming that specific structural properties are required to bind as ligand to a receptor, the number of successful molecules is dramatically reduced. In terms of costs of chemical defense, the question still remains unanswered why nature maintains the luxury of so many structurally diverse secondary metabolites. The evolution of photosynthesis and respiration involves the transport of electrons from water to oxygen and back. Disturbance causes the formation of reactive oxygen species (ROS) which are involved in the regulation of many cellular processes in low concentration levels. If the concentration of free radicals increases during abiotic and biotic stress, secondary metabolites may prove as beneficial by reducing them to water as antioxidants. Conversely, the reducing power of the same molecule may lead to reduction of molecular oxygen to ROS, a pro-oxidative effect. Furthermore, ions of transitory metals such as iron and copper can function as additional donators of electrons that result in the formation of hydroxyl radicals, the most reactive and destructive free radicals. If we accept the chemical feature of participation in electron transfer reactions as fundamental constraint for the evolution of secondary metabolites, then the structural diversity may be explained as follows: presence and absence of specific functional groups in a molecule act as higher constraint than the specific structure of the molecule itself. As a consequence, the carbon skeleton of secondary metabolites may vary. Examples and assays to explore this hypothesis will be presented.

Chemical Study of Leaf-Movement of *Albizzia saman*

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Albizzia plants close their leaves in the evening, as if to sleep, and open them in the morning according to the circadian rhythm. Potassium β-D-glucopyranosyl-12-hydroxyjasmonate **1** was isolated as leaf-closing factor (LCF) of *Albizzia saman*. We developed molecular probes consisting of modified LCF **1** in order to identify its mode of action. We have already demonstrated that a specific binding protein is involved in the motor cell of *A. saman*¹. We synthesized natural-type photoaffinity probe and biologically inactive enantiomer-type probe. We utilized them for photoaffinity labeling of the target protein for LCF **1**. By using protoplasts of motor cell, we found membrane protein of 38 kDa which strictly recognizes the stereochemistry of **1**, and it is highly likely that the protein is the target protein for LCF **1**. Recently, we observed that LCF shrank motor cell protoplasts prepared from *A. saman*. And comparing the results of several bioassay using glucosyl jasmonate-type LCF and jasmonic acid, it is also interesting that the mode of action of LCF is completely different with that of jasmonate.

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Symposium 4

the chemical ecology of disease vectors

Moderator

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Hypotheses for Developing New Controls for Animal and Human Disease Vectors

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Toxicants may no longer be acceptable for control of disease vectors, but the insect nervous system remains a major target for exploitation. Behavioural control by means of semiochemicals can be exploited by developing repellents based on hypotheses relating to evolution of repellency. These fall into three classes: (i) botanicals (ii) non-host species (iii) host derived repellents. (i) *Botanicals*. In conveying a strong plant cue, botanicals interfere with host location by carnivorous and haematophagous biting flies and other arthropods. Where plant essential oils are involved, these are highly volatile and can be readily lost. These agents can also cause dermatitic problems. Nepetalactones currently under development may avoid some of these problems. However, as with the use of synthetic repellents derived from structure-activity studies, recently shown to act on the same olfactory receptors⁵, hosts can still be located. (ii) *Non-host species*. Although the response to compounds from related non-host species is more deeply embedded in the behavioural ecology of arthropod vectors than for botanically derived repellents, it is still possible for hosts to be detected in the presence of non-host species. For example, human beings are easily located by anthropophilic mosquitoes such as *Anopheles gambiae* s.s., even when surrounded by cattle. However, compounds derived from waterbuck, a non-host for tsetse fly, repel tsetse flies, and compounds derived from the African hunting dog, a non-host for ticks, are potentially useful against ticks. The hypotheses that underpin this work have allowed development of repellents in aquatic ecosystems, for example against fish lice, *Lepeophtheirus salmonis*³. (iii) *Host derived repellents*. Individuals within an animal population, e.g. bovine or human, can be extremely unattractive even to arthropods highly adapted to these species². We have proved this to be caused by the presence of additional compounds which can themselves be used as repellents on "attractive" human beings^{1,4,6}. Host derived attractants and attractant pheromones. Host derived attractants and attractant pheromones can be further exploited by combined use of trapping with repellents in a push-pull system. Examples of new host derived attractants will be given and an approach to providing, economically, the more sophisticated attractant pheromone components described.

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Development of Push-Pull Strategies for Management of Malaria Mosquitoes

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Malaria mosquitoes locate their blood hosts mainly by chemical cues derived from the host. Several African malaria vectors (*Anopheles* spp.) are anthropophilic, and have evolved a close association with humans, using human dwellings as their favourite feeding and resting site. This principle is now being used to develop synthetic odour baits for the manipulation of mosquito behaviour. Blends of chemicals naturally present in human emanations cause physiological and behavioural responses in mosquitoes, which are similar to behaviours when the mosquitoes are exposed to human hosts. While several human volatiles are kairomones, others cause a repellent effect by contrast, particularly when offered in selected concentrations. Recently, it was shown that the Afro-tropical malaria vector *Anopheles gambiae* can be lured to a house from a distance by a synthetic odour blend that is competitive with a human host *in-vivo* and that addition of a repellent to an attractive blend inhibits that attractive effect. It is therefore postulated that this knowledge can be exploited to manipulate the mosquito behaviour around a house and/or village by application of a push and pull technology. Strategic placement of attractant traps and repellent sources near human settlements may be a powerful strategy for the removal of mosquitoes from a malaria-endemic habitat. The application of this technology promises a novel strategy for the control of this ancient disease.

Chemical Ecology of Afrotropical Disease Vectors: Lessons Learned and Future Challenges

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This presentation focuses on lessons learned from studies carried out on the chemical ecology of some of the most notorious vectors of diseases in Africa. Special emphasis is given to work done for more than a decade at *icipe*, on two of these vectors; the tsetse fly, a vector of trypanosomiasis and mosquito vectors of malaria. The main lessons learned about tsetse flies are: 1) the savanna-group of tsetse flies *Glossina morsitans morsitans* and *G. pallidipes*, both respond differentially to kairomones from preferred vertebrate hosts, 2) the waterbuck is a nonpreferred host for *G. m. morsitans* and *G. pallidipes* because of the levels and presence of certain aldehydes, 2-ketones, a lactone and phenols released from its body odour, 3) among allomonal phenols, guaiacol is a mild repellent, but its 4-methyl synthetic analogue is more repellent to the two species of savanna-group of tsetse flies, reducing significantly the loads of *G. pallidipes* feeding on cattle in field trials, 4) kairomones identified for the savanna-group of tsetse flies are not effective for the riverine-group tsetse fly *G. fuscipes fuscipes*, which is a vector of the trypanosome causing Human and Animal Trypanosomiasis (HAT), 5) *G. f. fuscipes* is attracted to odors of monitor lizards. Lessons learned about mosquitoes are: 1) semiochemicals of microbial origin mediate oviposition site selection by *Anopheles gambiae sensu stricto*, 2) when in humans, the malaria parasites tend to modify the attractiveness of the host to the vector, and 3) like the savanna-group of tsetse flies, certain *Anopheles* subspecies respond differentially to human odors. While these new findings open promising ways for their exploitation, there are challenges for their effective deployment in vector control. Some of these challenges and those associated with other diseases, especially emerging infectious diseases in Africa, will be highlighted in this presentation.

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Olfaction: A Gateway for Reducing Vector-Borne Disease Transmission

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Environmentally friendly strategies for controlling *Culex* mosquito populations are sorely needed as they are vectors of pathogens causing human diseases throughout the world, including filariasis and various types of encephalitis. In the United States *Culex* mosquitoes spread West Nile Virus (WNV) while feeding on birds and humans. *Cx. p. quinquefasciatus* (= *Cx. quinquefasciatus*) is now emerging as a potential threat to the bed net programs for malaria control in Africa. There is growing evidence suggesting that in many villages in Africa users are abandoning bed net use because *Culex* bites mislead them to incorrectly conclude that impregnated nets are not fending off malaria mosquitoes. We have already demonstrated that an odorant-binding protein (OBP) from the Southern House mosquito *Cx. quinquefasciatus*, CquiOBP1, could be used as a molecular target for the development of environmentally friendly mosquito oviposition attractants. OBPs are involved in insect olfaction, specifically the transport of odorants to membrane-bound odorant receptors. We have demonstrated that CquiOBP1 is expressed in sharp tip trichoid sensilla on *Cx. quinquefasciatus* antennae, which detect both the mosquito oviposition pheromone (5*R*,6*S*)-6-acetoxy-5-hexadecanolide and 1-octen-3-ol. Binding assays showed that CquiOBP1 binds to the former, but not to the latter. 1-Octen-3-ol is also detected with very high sensitivity by peg sensilla on maxillary palps and yet they do not express CquiOBP1. Taking advantage of the genome sequence of *Culex pipiens quinquefasciatus* (*Culex* Genome Consortium), we have identified a multigenic family of 53 OBPs, including a suite of 13 OBPs specifically expressed in olfactory tissues ("true" OBPs). Investigation of the the role(s) of these newly identified OBPs in reception of semiochemicals is underway. Newly identified functional OBPs are being used as molecular targets in "reverse chemical ecology" to fish out novel attractants, which in turn are expected to lead to better trapping systems for reducing mosquito populations.

3D Recording of Tsetse Fly Responses to Chemical and Visual Stimuli in a Wind Tunnel

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Studies on how tsetse flies locate hosts can help in the development of better trapping devices. Recording and analyzing 3D motions of tsetse flies is technically challenging due to their small size, their speed of flight, their particular sensitivity to lighting conditions and, like all flying insects, their capacity to exploit three-dimensional space. Furthermore, the propensity of tsetse flies to fly is governed by daily cycles in environmental conditions. We have built a wind tunnel where it is possible to control critical environmental parameters that allow filming the flight of diurnal tsetse flies in 3D and in real time to quantify their flights to different treatments. We have recorded the flight of *Glossina brevipalpis*, a forest tsetse fly species, to human breath presented with and without a visual target in the wind. Flies make undulating upwind flights to breath alone. By contrast, flights are highly directed in the presence of the visual target. Whereas the turning rate during upwind flights is probably influenced by breath components, the predominating role of the visual target causes *G. brevipalpis* to fly within stricter limits of ground speed and angular velocity from takeoff and with increasing flight control as it approaches the visual target. These 3D recordings show how tsetse flies use different sensory modalities during host searching, undulating upwind to chemostimuli borne in the wind but switching to directed flight as soon a target is within the visual range.

Control of Pathogenic Diseases by Leaf Cutting Ants and Their Microbial Symbionts

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Leaf-cutting ants cultivate a symbiotic fungus (*Leucoagaricus gongylophorus*) in their nests which serves them as their major food source. The ants supply this fungus with pre-processed leaf material and weed their fungus garden to avoid infections. Still, for example, the specialised pathogenic fungus *Escovopsis weberi* can overcome *L. gongylophorus* and thus threaten the survival of the whole ant colony¹. Besides waste removal the ants use chemical treatment against pathogens. In addition symbiotic microorganisms contribute to the ants' defence against pathogens². Until recently, not a single antifungal compound from the microorganisms was known. From three *Acromyrmex* species, we isolated 19 microbial symbionts (*Pseudonocardia*, *Dermacoccus*, and *Streptomyces*). Because *Streptomyces* sp. Ao10 was highly active against the pathogen *Escovopsis*, we selected this strain for bioassay-guided isolation and identified the highly active candicidin macrolides³. At least one symbiont from each of the three leaf-cutting ant species analysed produced candicidin macrolides suggesting that it is widespread and plays an important role against pathogenic fungi.

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Cultured Skin Microbiota Attract Malaria Mosquitoes

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Host-seeking of the African malaria mosquito, *Anopheles gambiae* Giles *sensu stricto*, is guided by human odours. The precise nature of the odours, and the composition of attractive blends of volatiles, is still unknown. The skin microbiota plays an important role in the production of human body odours and without bacteria human sweat is odourless. Human eccrine sweat is attractive to *A. gambiae*, but only after incubation for one or two days suggesting that microorganisms on the skin are responsible for the conversion of fresh sweat into sweat attractive to *A. gambiae*. We hypothesize that host selection of this malaria vector is affected by the species composition, density, and metabolic activity of the skin microbiota. Here we present a study in which the production and constituency of volatile organic compounds (VOCs) by human skin microbiota is examined. Agar plates incubated with skin microbiota were attractive to *A. gambiae* in olfactometer and indoor trapping experiments, with a significant effect of incubation time and dilution of the skin microbiota. Analysis of entrained air collected from agar plates incubated with natural skin microbiota or with a reference strain of *Staphylococcus epidermidis* resulted in fourteen putative attractants. A synthetic blend of 10 of these was attractive to *A. gambiae*. The discovery that volatiles produced by human skin microorganisms *in vitro* mediate malaria mosquito behaviour provides new opportunities for the control of malaria.

Acknowledgements

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To Feed or Not to Feed: State-Dependency of Host-Seeking Behaviour in Blood-Sucking Bugs, Vectors of Chagas Diseases

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In haematophagous insects the frequency of feeding is an important factor determining the competence of disease vector for transmitting pathogens. This frequency is determined, among other factors, by the insect's motivation to respond to host cues, which depends in turn on its physiological state. Provided that hosts display defensive behaviour and can also play the role of predators, feeding on blood represents a risk and should be modulated in order to make insects feed only when necessary. Data available on the modulation of the motivation to feed only focused on a few mosquito species, mostly concerning the influence of feeding and reproduction. In contrast to mosquitoes, hemimetabolous blood-sucking insects like bed bugs or kissing bugs are obligatorily haematophagous during their whole life. They can acquire pathogen parasites early in life and transmit them each time they feed. In these insects, less is known about the importance of the physiological state on their host-seeking behaviour. We studied, for the first time in a hemimetabolous blood-sucking insect, the kissing bug *Rhodnius prolixus*, how the motivation to feed is modulated by the physiological state. We showed that different factors act: 1) During the first days following ecdysis insects do not respond to any stimuli. The ability to follow chemical and physical cues increases either gradually (heat) or step-wise (CO₂) with time. 2) The responsiveness to CO₂ and heat depends on the time elapsed since a blood meal; in the case of CO₂, the same concentration may attract or repel, depending on the moment. This is the first time that the same host-signal is shown to be either attractant or repellent for a haematophagous insect. We demonstrated that this modulation is under the control of haemolymph born factors and abdominal distension. These results will be discussed in an adaptive framework.

Chemically Mediated Interactions in a Plant-Insect Vector-Phytoplasma System

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Apple proliferation is one of the major plant diseases affecting apple growing in Europe. This disease is caused by *Candidatus Phytoplasma mali* (Bacteria: Mollicutes), which colonizes the phloem of infected trees. The main symptoms of this disease are dwarf sized fruits and the proliferation of axillary buds (witches brooms). Known vectors of this phytoplasma are the jumping plant lice *Cacopsylla picta* and *C. melanoneura* (Hemiptera: Psyllidae). We investigated the olfactory reactions of the insects to host plants used for reproduction and overwintering, and elucidated chemically mediated interactions between all players in this multitrophic system. While *C. melanoneura* was not influenced by the phytoplasma, complex interactions between *Malus domestica*, *C. picta*, and *Ca. P. mali* were investigated in laboratory and field. Results from Y-tube olfactometer trials showed that immature adults of *C. picta* differentiated between the odours of healthy and infected apple trees and preferred the odour of infected trees. GC-MS analysis of the headspaces revealed the induction of the sesquiterpene β -caryophyllene in infected trees. Y-tube olfactometer trials revealed β -caryophyllene to be an attractant for *C. picta*. Interestingly, the infection of apple by *Ca. P. mali* influences the fitness of the offspring of *C. picta* negatively: the nymphs developing on infected plants suffer higher mortality compared to nymphs developing on uninfected plants. But by avoiding oviposition on infected trees, *C. picta* females prevent detrimental effects to their offspring. In conclusion, the phytoplasma directly manipulates the plant physiology and indirectly the psyllid behaviour, leading to a better spread within its host plant population. In contrast, its vector *C. picta* evolved mechanisms to minimize harmful effects emanated by the phytoplasma. While infection by *Ca. P. mali* is tolerated by adults, detrimental effects to offspring are avoided by an adapted oviposition behaviour. Thus, the development of a new, vital vector generation is ensured.

Non-lethal Impacts of Natural Enemies in a Caterpillar

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Insects are often attacked by natural enemies, either parasitoids or predators. The main results of these attacks are usually death. However, insect preys can often escape from enemies' attacks or defend themselves. In these cases, even though death is avoided, the prey might suffer from the disturbance of these attacks. We investigated in the laboratory these non-lethal impacts of enemies for the caterpillar of the moth *Spodoptera littoralis* developing on cotton plants. The enemy used in this study was the mosquito *Aedes aegypti*. Mosquitoes usually feed on vertebrate hosts, mainly birds or mammals. However, when no vertebrate host is available, also insects – like large caterpillars – may provide a haemolymph meal¹. Although the importance of this event in natural conditions is unknown, it still gives us a way to evaluate how disturbance by natural enemies might affect the development of the caterpillars: although the caterpillar gets disturbed and stung, it still survives and develops. Larval weight, pupal weight and developmental time were compared with or without mosquitoes. These results help us understand how, even when they are not killing their prey/host, natural enemies can still affect them.

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Chemical Ecology of Stable Fly and its Future Practical Applications in Control

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Stable flies, *Stomoxys calcitrans* (L.), are one of the most serious livestock pests that feed mainly on cattle and cause significant economic loss in the cattle industry. Standard stable fly control involving insecticides and sanitation usually are costly and ineffective. The present study reports the discovery of catnip as an effective repellent, as well as its adulticidal activity on stable fly. The essential oil of catnip reduced the biting of stable flies by more than 96% in an *in vitro* bioassay system, when compared with other sesquiterpene-rich plant oils. Catnip was also a strong spatial repellent, and it reduced gravid stable fly egg-laying at a 98% inhibition-rate. Catnip demonstrated the most repellency against stable flies, with strong repellency observed from other insect repellents, including isolongifolenone, 2-methylpiperidinyl-3-cyclohexen-1-carboxamide and (1S,2'S)-2-methylpiperidinyl-3-cyclohexen-1-carboxamide as well. But, the repellency from most commonly used repellent, DEET, was relatively lower. In addition, adulticidal activity of catnip was also measured to have a knock-down time less than 6min and kill time of ~16min. The calculated LC₅₀ and LC₉₀ values were 3.7 mg and 19.57 mg per 20 cm², respectively. Compared to control, slow-release formulations containing 10% catnip oil showed at least 90% of repellency were observed in the cattle feedlot trials. The catnip repellency against other filth fly species, such as horn fly and face fly was also conducted. In addition, the exploration of potential filth fly attractants (including oviposition attractants) will be discussed, as well their future applications in fly management using a Push-Pull strategy.

Odour Blends that Attract the Malaria Mosquito *Anopheles gambiae* s.s.

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The host-seeking behaviour of the females of *Anopheles gambiae* Giles *sensu stricto*, which is the most important malaria vector in Africa, is guided by volatiles of human origin. In this study we aim to establish the components of human odour that are essential in the host-seeking process of this mosquito species. Ammonia, lactic acid and several carboxylic acids are known to be present in the human odour blend. We investigated the effect of these compounds on the behaviour of female mosquitoes using a dual-port olfactometer. Our experiments showed that ammonia is an attractant on its own, whereas lactic acid is not. Carboxylic acids, offered as a mixture of 12 compounds, were repellent. However, a synergistic effect was found when ammonia, lactic acid and the carboxylic acids were applied as a blend. In addition, the response to 16 individual carboxylic acids was examined in combination with ammonia and lactic acid. The results showed that seven carboxylic acids augmented the attractiveness of ammonia and lactic acid at certain concentrations. Subsequently, subtraction experiments revealed which of these carboxylic acids play a significant role in the attractiveness of the blend of ammonia, lactic acid and carboxylic acids. The most attractive blend will be used as the basis on which to further build a highly attractive synthetic mixture that can be applied in mosquito traps in malaria-endemic regions.

Prospects for the Development of Odour Baits to Control the Palpalis Group Species of Tsetse Flies, Vectors of Human African Trypanosomiasis, in West Africa

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African sleeping sickness, or Human African Trypanosomiasis (HAT), is endemic to 37 countries in sub-Saharan Africa, with 60 million people at risk, and is fatal if left untreated. More than 90% of HAT cases are caused by *T. brucei gambiense* transmitted by the Palpalis group species of tsetse flies (Diptera: Glossinidae) found in Central and West Africa. Populations of the Morsitans group species of tsetse, important vectors of African Animal Trypanosomiasis (AAT), have been effectively controlled in the past with the use of insecticide-treated targets and traps baited with synthetic blends of host odours. Our aim is to reduce the rates of transmission of trypanosomes causing HAT by identifying effective attractants which can be utilised in traps to control and monitor populations of the Palpalis group species of tsetse flies. Behavioural investigations were conducted in Burkina Faso to determine whether volatile chemicals, produced by potential vertebrate hosts (cattle, humans, pigs), are involved in the host location process. Human and cattle odours were shown to significantly enhance (>2x) trap catches of *Glossina palpalis gambiense*, whereas only cattle odour significantly increased the trap catch of *G. tachinoides* (~5x) when compared with unbaited traps. During the trapping experiments, volatile samples were collected from all hosts by air entrainment, providing liquid extracts for chemical analyses. Coupled gas chromatography–electroantennography (GC-EAG) with female antennae was used to locate electrophysiologically active peaks. EAG-active peaks were then identified tentatively by coupled GC-mass spectrometry (GC-MS) and confirmed by peak enhancement with authentic compounds on GC columns of different polarities. Ongoing field trials have revealed several host derived chemicals that significantly affect tsetse behaviour, suggesting that odour baited traps and insecticide treated targets could be used to monitor and control the Palpalis group species of tsetse flies in West Africa.

Single Sensillum Recordings of the Entire Antennal Olfactory System of the Common Bed Bug *Cimex lectularius*

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The common bed bug *Cimex lectularius* (Hemiptera; Cimicidae) is a human blood-feeding insect that is currently reinvading the developed countries due to increase of human travelling, long survival to starvation, resistance to insecticide and poor knowledge about its ecology¹. Nevertheless, like other haematophagous arthropods, host seeking and orientation in *C. lectularius* is partly achieved by the olfactory senses located at the distal tip of their antenna. In this study, we re-assessed the external morphology distribution of the olfactory sensilla on the antenna of *C. lectularius*² and correlated it with an electrophysiological characterization of the olfactory receptor neurons they housed. Using a relevant panel of molecules already known to be perceived by various haematophagous arthropods, we measured single sensillum recordings on the nine grooved pegs, the six smooth pegs and most of the hairs composing the entire antennal olfactory system of *C. lectularius*. Responses for eight of the 31 compounds tested were confirmed by dose response analysis and permitted to separate sensilla in distinct functional classes. This study has also an applied impact as it points out compounds that bed bugs are equipped to perceive and must use in their sensory ecology.

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Symposium 5

the chemical ecology of pollination

Moderator

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Evolution of Floral Scent: Preadaptations and Pollinator Mediated Selection

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Although floral scent is a key trait for pollinator attraction, little is known about the evolutionary process that shapes its chemical composition and variability. Whereas pollinators can impose selection on floral scent through innate preferences and learning, preadaptations are thought to strongly influence the outcome of the selection process. For example, biologically active compounds in a sexual deception system, alkenes of various chain lengths, have been shown to occur widely in related taxa of non-sexually deceptive orchids. This supports the assumption that while preferences of male insects for alkenes drive the evolution of this pollination systems, this was enabled by the pre-existence of these compounds in the respective orchid clade. Overall, the evolution of floral scent compounds is not independent from insect chemical communication, as a general associations exists between the commonness of volatile compounds in families of insects (as allomones and pheromones) and plant families (as floral scent). This suggests that pre-existing preferences in pollinators have driven the evolution of floral scent compounds through a sensory trap mechanism. Since many of the compounds produced by flowers do already occur in the evolutionarily older gymnosperms, we can assume that this pre-existence of biosynthetic pathways for producing scent compounds, serving primary functions such as herbivore deterrence, has enabled the quick diversification of floral scent in the angiosperms.

Speciation Genes in the Genus *Petunia*

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Animal-mediated pollination is essential in the reproductive biology of many flowering plants and tends to be associated with pollination syndromes, sets of floral traits that are adapted to particular groups of pollinators. The complexity and functional convergence of various traits within pollination syndromes are outstanding examples of biological adaptation, raising questions about their mechanisms and origins. Elucidation of the molecular-genetic basis of this interesting reproductive biology requires a model system that combines distinct pollination syndromes with excellent molecular and classical genetics. In the genus *Petunia*, complex pollination syndromes are found for nocturnal hawkmoths (*P. axillaris*), diurnal bees (*P. integrifolia*) and hummingbirds (*P. exserta*), with characteristic differences in petal color, corolla shape, reproductive organ morphology, nectar quantity, nectar quality and fragrance. We dissected the *Petunia* syndromes into their most important phenotypic and genetic components. Several quantitative trait loci were identified for each syndrome component. Using petal color as an example, we show that a polymorphism in a single gene can have a major effect on pollinator preference. Identification of such polymorphisms at the molecular level will help us understand how the evolution of pollination syndromes contributed to the restriction of gene flow during incipient speciation.

How Plants Get What They Want from Floral Visitors

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Plants enjoy the evolutionary benefits of sexual reproduction and outcrossing, but due to the sedentary habits of most sexually mature plants, they must rely on external biotic or abiotic agents to transport their gametes to potential mates. The biotic agents, consisting of a postal service of floral visitors, have their own evolutionary agendas, and plants appear to use a complicated mixture of chemical rewards and deterrents to manipulate the behavior of these floral visitors. Once a selection of gametes from potential mates has been delivered, plants appear to deploy a suite of pre- and post-zygotic means of selecting among the potential mates. This talk will describe recent progress in the use of genetic and ecological tools to understand how *Nicotiana attenuata* uses floral and nectar chemistry to enforce "good behavior" from the community of insects and birds that visit their flowers in its native habitat, the Great Basin desert. Particular emphasis will be given to how this plant solves a vexing but common problem that many plants have with their pollinators: that important pollinators can also be important herbivores during larval stages. A molecular tool box to study the potential for pre-zygotic mate choice in this species is also under development and will be discussed.

Molecular Examination of Variations in Floral Scent Biosynthesis in Natural Populations

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Scent compounds emitted from the flowers of many species play an important role in attracting specific pollinators. Different species have distinct floral scent bouquets and such differences can contribute to reproductive isolation. Genes encoding scent biosynthetic enzymes and transcription factors involved in the regulation of scent biosynthetic genes have now been identified in many plant species. However, research on genetic variations in scent biosynthesis within a species is lacking. I will present data on intraspecific variation in the synthesis of specific scent compounds in two species, *Clarkia breweri* and *Petunia axillaris*, and discuss the molecular mechanisms underlying those differences.

Host-Mediated Volatile Polymorphism in a Hemiparasitic Plant Influences its Attractiveness to Pollinators

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Parasitic plants are subjected to the chemical influence of their hosts, which in turn affects the parasites' interactions with other organisms. The present study reveals how a hemiparasitic plant on three host species produces distinct hemiparasite-host neighborhoods which differ in terms of volatile composition and pollinator attractiveness. The study was performed in a population of the hemiparasite *Tristerix verticillatus* (Loranthaceae) infecting three different species of hosts occurring in sympatry within a small area, thus exposing all individuals studied to similar abiotic conditions and to the same pollinator diversity; we assessed the effect of hosts on the hemiparasites' visual and olfactory cues for pollinator attraction. During the study period, the hemiparasite individuals were flowering but the hosts were passed their flowering stage. We georeferenced all parasitized host individuals in the area, collected volatile organic compounds from the hemiparasite and its hosts, measured floral patch characteristics and monitored bird and insect visitors to inflorescences of the hemiparasite, and measured the movement of fluorescent powder between hemiparasite-host systems as a proxy of pollen movement. We showed that: i) floral patches did not differ in terms of visual variables potentially involved in the attraction of pollinators, ii) hosts and hemiparasites on each host were discriminated as distinct chemical populations on the basis of their volatile chemical profiles; iii) insect visitation rates differed between hemiparasites parasitizing different hosts, iv) volatile compounds from the host and the hemiparasite affected the arrival of insects to inflorescences of the hemiparasite, and v) host species influenced the movement of fluorescent powder between hemiparasite-host systems. Overall, the study showed that volatile profiles of hemiparasite-host systems were associated with differential attractiveness of hemiparasite flowers towards insect visitors.

The Role of Chemistry in Reproductive Isolation and Speciation in Australian Sexually Deceptive Orchids

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Australia is one centre of diversity for the unusual orchid pollination strategy of sexual deception, with more than 150 species in multiple terrestrial genera securing pollination by the sexual attraction of male hymenoptera. Our breakthrough discovery of the semiochemicals responsible for attracting specific thynnine wasp pollinators to *Chiloglottis* orchids has uncovered a new class of natural products, 2,5-dialkylcyclohexan-1,3-diones, that we have called 'chiloglottones'^{1, 2}. Further research across multiple species indicates that pollinator attraction is achieved by single, paired or triplet combinations of these compounds. When there is more than one active compound, a specific ratio is required for biological activity. Although some allopatric orchids may share the same floral odour, co-flowering sympatric species are always characterised by a different floral odour that attract different pollinators. We have now developed innovative synthesis methods to produce these novel compounds. This has enabled the experimental evaluation of pollinator response to artificial blends and alternative chemical variants. The emerging evidence from pollinator choice experiments and our chemical and genetic analysis across species, indicate that these novel molecules play a key role in maintaining reproductive isolation among co-flowering sympatric species and have therefore likely played a key role in the evolution of these orchids.

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Insect Perception and the Recognition of Floral Odours

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The production and detection of floral scents has played a major role in the coevolution of insects and plants. Floral odours are unique blends of volatile chemicals that attract pollinators, but also act as signals to insect herbivores, which damage the plant through adult and larval feeding. Using the noctuid moth, *Helicoverpa armigera*, as a model species, we have conducted (i) laboratory based (wind tunnel) experiments using odour artificial blends and (ii) flight cage experiments, using genetically modified plants with differing odour profiles. Our work investigates how changes in odour blend composition influence the nectar foraging and egg laying behaviour of an important agricultural pest.

Host-Plant Recognition by the Oligolectic Bee *Osmia adunca* (Megachilidae)

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Solitary bees are important pollinators of angiosperms. They visit flowers mainly to collect pollen and nectar to provide their larvae with food. Oligolectic bees are specialized on few closely related plant species for pollen foraging. At the beginning of the flight season newly emerged, naïve females have to find and recognize their host-plants. To investigate the importance of floral cues for host-plant recognition in oligolectic bees, we chose *Osmia adunca* (Hymenoptera: Megachilidae), a solitary bee species which is highly specialized on the flowers of *Echium* spp. (Boraginaceae). We hypothesized that floral scent plays a major role for the attraction of *O. adunca* females and that they use *Echium*-specific signals to recognize their host-plants. To test the hypothesis we used a combination of chemical (GC/GC-MS) and electrophysiological (GC-EAD) analyses, spectral reflection measurements and bioassays. The bioassays revealed that visual cues of flowers are highly attractive to females. However, a combination of visual and olfactory cues was required for host-plant recognition. Our conclusion is that floral scent plays a major role for the specific attraction of newly emerged *O. adunca* females by *Echium* flowers and visual cues are only important for flower location. Furthermore, the bioassays showed that there was a change in foraging behaviour of the bees with increasing flower experience. Chemical analyses of the floral scent provided a distinctive odour profile that was genus-specific to *Echium*. Additionally, *Echium*-specific compounds elicited strong responses in *O. adunca* antennae (GC-EAD) which means that they are detectable by the bees. Presently, we are investigating the role of these chemicals in host-plant recognition by newly emerged females.

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How Cheating Affects Relationships

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Mutualisms are cooperative acts between individuals of unrelated species in which both participants derive a benefit. In a pollination mutualism, animals carry pollen from one plant a conspecific stigma, thereby fertilizing the latter and ensuring plant reproduction. The plant rewards the vector, often in terms of nectar. While the pollinator transports male gametes in a self-serving behavior, the plant has to invest costly resources for nectar production. A key question is what stabilizes plant-pollinator mutualisms and why cheating does not occur more frequently in angiosperms. We would like to assess whether hawkmoths are capable of discriminating between cheating and honest petunia plants and if their behavior limits reproductive success of cheating plants. In the past, plant-pollinator experiments with reduced nectar availability were conducted on manually manipulated flowers. However, this method neglects the costs involved in nectar production and can thus not be conclusive in respect to fitness. We chose to pursue a novel approach by breeding *Petunia* lines that contain low nectar volumes but resemble the rewarding species. We backcrossed an initial cross of *P. integrifolia* (low nectar volumes) and *P. axillaris* three times with *P. axillaris*, to obtain low nectar lines which can be used in behavioral assays and for fitness analysis. In each generation we selected the plants with the highest similarity to its pollen parent and the lowest nectar volumes. We have isolated a promising line with nectar volumes of 10.4 µl, displaying all other floral characteristics of *P. axillaris* except for scent emission. In behavioral assays, the foraging behavior of hawkmoths was tested on low nectar lines. The only significant behavioral difference was a reduction of drinking duration on low nectar lines. We then determined how this behavior affects one fitness parameter; seed set. Low nectar lines sired more seeds when pollinated by hands than *P. axillaris*; however this effect is neutralized in a moth pollinated set up. We therefore argue that the reduction in drinking time might act as a partner control mechanism to promote nectar production in *Petunia axillaris*.

Floral Odor Varieties of the Carnation *Dianthus sylvestris* at Different Altitudes in the Swiss Alps

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Do pollinators act as agents of disruptive selection on floral traits and contribute to the speciation process of plants? Elevational differences in flower size, calyx length and floral scent in the wild carnation *Dianthus sylvestris* suggest floral adaptation to different guilds of pollinators. Floral odor was collected by headspace adsorption and floral compounds were analyzed and identified by quantitative gas chromatography (GC) analyses and GC-mass spectrometry. We found qualitative and quantitative differences in floral scent of *D. sylvestris* at different altitudes. Furthermore, we tested for local adaptation with a reciprocal transplant experiment and compared pollinator activities. We discuss our findings in relation to adaptation of the plants to different pollinator guilds.

Making Sense of Scents: Complex Regulation of Floral Terpene Volatiles in Transgenic Tobacco

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Plant odours, a complex blend of many individual volatile components, play a fundamental role in insect-plant communication. Over 700 volatile compounds have been identified in plants, and a large proportion of these are terpenes. We have genetically modified the expression of enzymes within the terpene biosynthesis pathway of *Nicotiana tabacum* in order to modify floral volatile emissions. Analysis of headspace volatiles demonstrate that while the volatile end products of the plant terpene biosynthetic pathway can be genetically altered, the outcomes of genetic modification cannot be predicted easily due to complex regulation of the multi-compartmental biosynthetic pathway. Furthermore, genetic modification of these volatile emissions affect behaviour of the polyphagous moth, *Helicoverpa armigera*.

Speciation and Pollinator Sharing in Two Sympatrically Occurring *Ophrys* Species on Sardinia

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Orchids of the genus *Ophrys* are sexual deceptive and attract insect males for pollination. By mimicking the female species-specific sex-pheromone reproductive isolation is based on the specific attraction of males of a single pollinator species. Changes in the odour composition can lead to speciation by the attraction of a new pollinator that acts as an isolation barrier towards other sympatrically occurring *Ophrys* species¹. On Sardinia, we investigated two sympatrically occurring endemic species, *O. chestermanii* and *O. normanii*, which are both pollinated by males of the cuckoo bumblebee *Bombus vestalis*. In order to clarify the evolutionary processes underlying the origin of *O. chestermanii* and *O. normanii*, we performed bioassays with complete flower-extracts, polar and non-polar fractions of flower-extracts, electrophysiological investigations (GC-EADs), chemical analyses (GC, GC-MS) and genetic analyses (AFLPs, plastid markers). In a first series of bioassays using complete flower extracts we could exclude a supposed hybrid origin of *O. normanii*. Further bioassays using polar and non-polar fractions of pollinator attracting floral scent and chemical analyses of electrophysiologically active compounds revealed that both species attract *B. vestalis* males with the same odour bouquet of polar compounds, among them alcohols, esters and fatty-acids. However, odour analyses including compounds not involved in pollinator attraction and genetic analyses clearly showed that *O. normanii* and *O. chestermanii* evolved from different ancestors, viz. *O. normanii* from *O. tenthredinifera* and *O. chestermanii* from *O. annae*, and converged to the same pollinator². In spite of sympatry, pollinator sharing and overlapping blooming periods, there is no evidence for gene-flow between *O. chestermanii* and *O. normanii* indicating an unusual case among sexually deceptive orchids in which postmating rather than pre-mating reproductive isolation mechanisms strongly prevent interspecific gene flow.

Acknowledgments

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An Orchid Pretends to Be a Bee: Orchid Mimics Alarm Pheromone of Honeybees in Order to Attract Prey-Hunting Hornets for Pollination

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One third of the world's 30,000 orchid species are deceptive and do not reward their pollinators with nectar or pollen. Most of these deceptive orchids imitate the scent of rewarding flowers, of potential mates¹ or of prey items². In this study, we investigated the floral scent involved in pollinator attraction to the rewardless orchid *Dendrobium sinense*, an endemic species of the Chinese island Hainan, pollinated by the hornet *Vespa bicolor*. Using a combination of chemical analyses and electrophysiological methods, we demonstrate that the flowers of *D. sinense* produce Z-11-eicosen-1-ol and that the pollinator can detect this compound³. Z-11-eicosen-1-ol is also known as a component of the alarm pheromone of the Asian honeybee *Apis cerana*, as well as of the European honeybee *Apis mellifera*. Furthermore it is known as a kairomone for the European beewolf *Philanthus triangulum* to locate honeybees, its only prey. This is the first time that Z-11-eicosen-1-ol has been identified as a floral volatile. In behavioral experiments, we demonstrate that the floral scent of *D. sinense* and synthetic Z-11-eicosen-1-ol are both attractive to foraging *V. bicolor* hornets. Since hornets frequently capture honeybees to feed to their larvae, we suggest that the flowers of *D. sinense* mimic the alarm pheromone of honeybees in order to attract prey-hunting hornets for pollination. The fact that *D. sinense* produces a specific insect pheromone to attract pollinators which use the same compound as a kairomone to locate their prey insect adds an exciting novel facet to the multifarious pollination syndrome of orchids.

Acknowledgments

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Poster Session 1

Monday, 24 August

Behavioral Responses of Male Moth *Metisa plana* towards Female Pheromone Bagworm *Metisa plana* in Malaysian Oil Palm Plantations

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The bagworm *Metisa plana* Walker (Lepidoptera: Pscyhidae) has been a major pest of oil palm *Elaeis guineensis* Jaquin in Malaysia. A study on *M. plana* was conducted since January 2009 in oil palm plantations at Trolak and Bestout, state of Perak, Malaysia. In the plantation, adult male moths were observed flying towards calling female bagworm on oil palm fronds. In this study calling females glued on a long cellotape between two oil palm trees attracted flying males to approach and mate between 0400h to 0700 h. In this study, female bagworms containing different stages of *M. plana* were removed from their bags. Extracts were made from 1000 each from adult calling female, adult non calling female, female pupa, female bag and male adult after removal from bag. Adult males *M. plana* moth. were collected using light traps at 1700 h to 2300 h and from 0400 h am to 0700 h. Behavioral bioassay in T-shaped olfactometer showed male moths approaching crude extracts of: calling adult female (92%), crude adult female (88%), bag of calling female (84%) , bag of adult female (80%) between 2 sec and 3 min 45 sec. Active extracts undergo fractionation through flash column chromatography. In petri dishes, male moths approached hexane fraction of: calling female (70%), bag of calling female (65%), hexane fraction of adult female (90%), hexane fraction of bag adult female (80%). After thin layer chromatography, in petri dishes male moths were attracted towards pure hexane fraction of calling female F1 spot 1(84%), spot 2 (84%), spot 3 (55%), adult female F2 (hexane) spot 1 (84%), spot 2 (96%). In petri dishes male approached between 2 sec and 5 min 34 sec and remained arrested on the extract between 1 sec to 9 min 58 sec. In contrary, both in petri dish and T-tube, male moth gave negative results against: control, fraction F1 hexane of male bagworm after removal of bag, bag of male pupa, all ten spots of male F1. Identification of pheromone components using Gas Chromatography mass spectrometry is in progress.

Role of Human-Associated Compounds in Host-Finding by *Culex* Mosquitoes

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Culex mosquitoes are important vectors of West Nile virus and other encephalitides in North America. Mosquitoes in this genera, however, vary in their propensity for feeding on mammalian hosts and this is reflected in the difficulty in trapping some of these species using conventional traps and lures. With potential development of new lures based on human odors, an examination of responses of several *Culex* species to human-associated odors was undertaken. The role of L-lactic acid and several other human-associated compounds were evaluated for attraction of *Culex nigripalpus*, *Culex quinquefasciatus*, *Culex tarsalis* and *Aedes aegypti* (for comparison as a human-associated species) in the laboratory using a dual-port olfactometer. Previous studies indicated that lactic acid may mediate attraction of anthropophilic species to humans and conversely deter zoophilic species. Lactic acid was combined with host odors and moderately increased attraction of *Ae. aegypti* and *Cx. quinquefasciatus* and decreased attraction of *Cx. nigripalpus*, and *Cx. tarsalis*. Responses to other human-associated compounds with and without CO₂ were determined and patterns of responses of *Culex* species to human-associated odors determined.

The Level of Mate Choice in the Almond Moth, *Cadra cautella*Allison JD¹, Cardé RT²¹LSU AgCenter, Department of Entomology, 404 Life Sciences Building, Baton Rouge LA, 70803²Department of Entomology, UC-Riverside, Riverside CA, 92521; USA

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The majority of known moth pheromones are multi-component blends. The ratio of the components of these blends has been observed to influence the attraction of conspecific males and as a consequence is predicted to experience strong stabilizing selection. Because the sex pheromones of sympatric species of moths often feature the same components in different ratios, male mate choice has been hypothesized to occur at the level of species recognition. The hypothesis that mate choice occurs at the level of mate assessment in moths has received little empirical attention. For mate choice to occur at the alternate level of mate assessment, female sex pheromones should: (1) exhibit high levels of variation among individuals; (2) display relatively high levels of heritability; and (3) honestly reflect individual quality. The sex pheromone of the almond moth is a two component blend of Z9,E12-14:Ac and Z9-14:Ac. The ratio of these two components has been observed to vary considerably among individuals and to display a high narrow-sense heritability. This study tested the hypothesis that variation among individual almond moths in the ratio of Z9,E12-14:Ac to Z9-14:Ac honestly reflects female quality (condition 3 above). The ratio of Z9,E12-14:Ac to Z9-14:Ac decreases significantly as female almond moths increase in age from 1- to 5-days post-emergence. Therefore female moths were mated 1-, 2-, 3-, 4-, 5-, and 6-days post-emergence and total lifetime fitness recorded. A significant negative effect of female mating age on total lifetime fitness was observed. These data suggest that male mate choice in the almond moth, *C. cautella*, may occur, in part, at the level of mate assessment.

The Spatial Scaling of Olfactory Information

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Density-area relations of insects strongly depend on the type of sensory cues used for detecting and locating host plants or habitat patches. The area-scaling of sensory cues can be important predictors of immigration rates of insects. We examined scaling rules and attraction radius of olfactory information in a patch size gradient. We used a portable electroantennogram under field conditions and measured male moth antennal responses to female sex pheromones downwind from patches of three different size. The attraction radius of measured pheromones was linearly related to the size of the patch, scaling to patch size in a similar way as visual cues. Our result contrasts to previous suggestions that the olfactory impression of a patch should increase faster with patch size than the visual impression. The result can be used to predict immigration rates and density-area relations for the large number of insect species that uses olfactory search in long-range detection of host plants and habitat patches.

A Novel Lepidopteran Sex Pheromone Produced by Females of a Lichen Moth, *Lyclene dharma dharma* (Lithosiinae: Arctiidae)Ando T¹, Do ND¹, Adachi Y¹, Kinjo M²¹ Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan² Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, Uehara, Taketomi-cho, Okinawa 907-1541, Japan

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Lithosiinae is one of the subfamilies in Arctiidae. Larvae of the Lithosiinae species, which mainly feed on lichen in a forest, are not pest insects but important organisms contributing to the ecosystem of the forest. While many pheromones have been identified from species in the two other subfamilies, Arctiinae and Synthominae, there has been no information about the pheromones of the Lithosiinae species. The analysis of a pheromone gland extract by GC-EAD showed that the *L. d. dharma* females, which were collected in a subtropical island in Japan, produced three active components (I–III) in a ratio of 2:1:1. GC-MS analyses of the extracts before and after Wolff-Kishner reduction enabled their structures to be estimated as follows: 6-methyl-2-octadecanone (I), 14-methyl-2-octadecanone (II), and 6,14-dimethyl-2-octadecanone (III).¹ These methyl-branched ketones have not been identified as a natural product, indicating that they constitute a new chemical group of lepidopteran female sex pheromones. The planar structures of I–III were confirmed by achiral syntheses started from diols.^{1,2} Their activities were evaluated in a field, and effective male attraction was observed for the 2:1:1 mixture of I–III.² This result indicates that the females do not produce only one stereoisomer for each component or that the response of the males is not disturbed by the other stereoisomers of natural isomers produced by the females. The field test also revealed that the two-component lure of I and II captured as many males as the mixture of I–III, while lures baited with two components in other combinations and with only one component scarcely exhibited any male attraction ability².

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**Evolution of the Chemical Signature of Caste and Larval Stages of the Termite
*Reticulitermes santonensis***

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Insects recognize each other by a chemical signature, mainly based on mixtures of hydrocarbons on the surface of the cuticle (CHCs). CHCs are lipids whose primary function is to prevent water loss. This chemical profile allows individuals to identify members of their species, sex, and colony as well as caste in social insects. Termite societies have distinct castes. Nymphs and workers pass through various larval stages. Ten years ago a preliminary study¹ on several species showed that each caste had a specific blend of CHCs. This study used gas chromatography and mathematical analyses of CHC profiles in *R. santonensis* to determine, firstly, whether the process underlying the caste and development stage profiles (soldiers, undifferentiated larvae, workers and neotenics, i.e., secondary reproductives) was fixed or dynamic, and secondly, to define how profiles evolved during the differentiation of workers into pre-soldiers and then soldiers, using a juvenoid (*W-328*²) to induce the differentiation of workers into soldiers. Results showed that profiles differed between castes and stages: soldiers and neotenics always had clearly distinct profiles, and undifferentiated larvae (L1 & L2), along with first worker stages (L3 to L4), had different profiles from later worker stages (L5 to L8). A study of the differentiation of workers into soldiers showed a first cuticular profile change during the worker to pre-soldier moult and a second during the pre-soldier to soldier moult. The profile within the caste matured: during the first 4 days after moulting, pre-soldiers had a different profile from older pre-soldiers. Young and mature soldiers also had different chemical profiles. These results suggest a cycle of maturation of chemical profiles within castes and intercastes, and suggest that the mixture also change over time.

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Stereoisomeric Separation of Sex Pheromone Precursors in *Diprion pini* and *Neodiprion sertifer*

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Pine sawflies of the family Diprionidae (Hymenoptera: Symphyta), are considered to be severe defoliators of pines in the Northern hemisphere¹. Two of these species are *Diprion pini* and *Neodiprion sertifer*. *D. pini* use esters of (2*S*,3*R*,7*R*)-3,7-dimethyltridecan-2-ol as the main component of its sex pheromone², and *N. sertifer* use esters of (2*S*,3*S*,7*S*)-3,7-dimethylpentadecan-2-ol³. We present a method that separates six out of the eight stereoisomers of the precursor alcohols, by using (S)-2-acetoxypropionyl chloride⁴ to derivatise synthetic mixtures of all eight stereo-isomers of the precursor alcohols. The derivatised samples were analysed by GC-MS, using a polar HP-88 column. SIM mode was used for maximum sensitivity and selectivity. Separation of six of the eight stereoisomers of 3,7-dimethyltridecan-2-ol and 3,7-dimethylpentadecan-2-ol was obtained. A very flat temperature gradient (0.01°C/min) was needed for maximum separation. Since several insects use two or more stereoisomers in different ratios as pheromone constituents, there is also a need to separate two closely eluted peaks in very different ratios. With this method, 0.1% of (2*S*,3*R*,7*S*) in (2*S*,3*S*,7*S*)-3,7-dimethylpentadecan-2-ol, was easily separated.

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Odour Attraction of the Fly in the Wind Tunnel

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Olfactory processing of long-range cues is of high ecological relevance for many insects. Long-range active odours that induce relevant behavioural responses are suitable to study the physiological processing of olfactory neuron ligands and the sensitivity of olfactory systems. *Drosophila* has become a model organism for studying the perception and processing of odour signals but odour attraction behaviour is mainly studied in close range assays. We tested in a wind tunnel the fly's upwind flight attraction and landing on the odour source by delivering an air stream passing through vinegar. By analysing the impact of the fly's age, feeding or starvation, sex, mating state and diurnal activity we were able to induce flight and landing at the odour source in 62% of *Drosophila* during a test period of 15 min. Trapping the vinegar odour and re-vaporising it by use of a piezoelectric sprayer in the wind tunnel assay induced the same flight behaviour as to the live vinegar. We then vaporised and tested four single EAG-active vinegar compounds and their combinations. Whereas attraction to single compounds was very low, significantly more flies were attracted to some of the mixtures. The dissimilar behavioural response to odours of different quality corroborates the sensitivity and discriminative power of *Drosophila*'s olfactory system.

Feeding *Phyllotreta striolata* F. (Coleoptera: Chrysomelidae) Males Emit an Aggregation PheromoneBeran F^{1a}, Reinecke A², Srinivasan R³, Büttner C^{1b}, Ulrichs C^{1a}, Mewis I^{1a}¹ Humboldt-Universität zu Berlin, Faculty for Agriculture and Horticulture, ^aSection Urban Plant Ecophysiology, ^bSection Phytomedicine, Lentzeallee 55/57, 14195 Berlin, Germany² Max-Planck-Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Hans-Knöll-Strasse 8, 07745 Jena, Germany³ AVRDC-The World Vegetable Center, Entomology Unit, 60 Yi-Min Liao, Shanhua, Tainan, 74151 Taiwan

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The striped flea beetle, *Phyllotreta striolata*, is a serious pest of economically important crucifer crops in the tropics. This study aimed to identify volatile semiochemicals involved in host plant location in order to develop attractant-based lures for monitoring *P. striolata* in the field. Therefore, the attractiveness of volatile compounds from host plants, *P. striolata* adults, and host plants infested with *P. striolata* was analyzed. Field bioassays were conducted at AVRDC-The World Vegetable Center, Taiwan, in spring 2009. Intact seedlings, feeding-damaged seedlings with beetles removed, seedlings with 20 feeding females, and seedlings with 20 feeding males were tested for attractiveness. Only volatiles from *B. napus* seedlings with feeding *P. striolata* males attracted significantly higher numbers of both, male and female adults. However, host plant volatiles alone and volatiles from host plants with feeding females attracted only few adults. These results indicate that feeding *P. striolata* males produce an aggregation pheromone. Previously, a sesquiterpene was identified as male aggregation pheromone in the congeneric species *P. cruciferae*¹. Volatile collections from feeding males were subjected to coupled gas chromatography-mass spectrometry and coupled gas chromatography-electroantennographic detection. Several compounds elicited antennal responses from male and female *P. striolata*. The structural identification is currently under progress.

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Insect Pheromone Research in South America

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Insect pheromone research has a long and rich history built up primarily by studies carried out in the Northern hemisphere. Not surprisingly, these studies have largely targeted species relevant to these regions of the world, mainly agricultural and forest pests. Pheromone research in South American countries came a few decades behind, albeit their strong dependence in agriculture and therefore in pest management. In the last 20 years, a combination of economic, environmental and technical factors have come together to generate a small but rising number of chemical ecologists working in pheromone chemistry and biology in South America. In this contribution we summarize the results of this trend. We present a simple meta-analysis including geographical distribution, trends in collaborative or independent work, and a 20-year evolution of published articles in the field. Pheromone studies in which South American scientists have participated in collaboration with foreign scientists, mostly chemistry groups, as well as the ever-growing number of studies carried out completely within the region are summarized briefly. We have focused mainly in research involving the characterization of pheromones from native species, which involve the most important insect orders, namely Lepidoptera, Coleoptera, Heteroptera and Hymenoptera. Finally, we emphasize the importance of a coordinated effort to further promote the growth of this field in Latin America, through the endorsement of international collaborations within the region. Such goal would be facilitated by a regional academic organization, which would in turn initiate the occurrence of regular scientific meetings.

Identification of the Sex pheromone of the Citrophilus Mealybug *Pseudococcus calceolariae*

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The citrophilus mealybug *Pseudococcus calceolariae* (Maskell) is a cosmopolitan species and is thought to be native to Australia. This polyphagous species feeds on a variety of host plants including citrus, avocado, berries, sugarcane, cocoa, grape, and apple and it has spread out of its native habitat and is currently geographically distributed worldwide. In New Zealand, it is a vector of grapevine leafroll-associated virus type 3 (GLRaV-3), which causes significant declines in quantitative and qualitative parameters of vine performance. Due to quarantine restrictions imposed by several countries, the presence of *P. calceolariae* causes significant economic losses to Chilean fruit exporters. We identified the sex pheromone of *P. calceolariae* by means of aeration of cohorts of virgin females, gas chromatography-mass spectrometry, derivatization reactions, and synthesis of model compounds. The synthetic pheromone was highly attractive to males in field tests.

Female Sex Pheromone of Coconut Black Headed Caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae)

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Opisina arenosella is a serious pest of coconut palms of all ages causing decline in yield and other related problems. On account of the discrete generation cycles of *O. arenosella* and its prolonged larval period (35-56 days), success of any biological control program is dependent on timing releases of natural enemies to coincide with the preferred stages of the pest, in order to prevent population increases from developing into outbreaks. Pheromone-baited traps can play a crucial role by providing reliable, cost-effective and simple surveillance and monitoring tools for early detection of the pest. The presence of a female sex pheromone was demonstrated by Cork & Hall¹. In the present studies, GC-MS analyses of volatiles collected from virgin females confirmed the structure of the *O. arenosella* pheromone as (Z,Z,Z)-3,6,9-tricosatriene. Field trials were carried with the synthetic pheromone at different locations around Bangalore, India, to standardize the dispenser and loading using wing traps. Four pheromone loadings (100 µg, 500 µg, 1 mg & 3 mg) and 4 dispenser types (PVC vial, PVC vial with cap, black rubber septa & pink rubber septa) were evaluated. PVC vial dispensers with 100 µg pheromone loading were significantly superior to other treatments (mean 35.00 adults/ trap in 4 weeks). In confirmatory studies carried out using PVC vial and black septa dispensers with 100 µg loading, of the 521 male moths caught during the 40 day study period 390 (74.86%) were caught in traps with PVC vial dispensers, significantly more than in traps baited with the pheromone in black septa. Wing traps baited with PVC vial dispensers containing 100 µg (Z,Z,Z)-3,6,9-tricosatriene thus provide an effective monitoring tool for *O. arenosella* moths.

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Developmental Regulation of the Pheromone Biosynthesis Activating Neuropeptide Receptor (PBAN-R): Re-Evaluating the Role of Juvenile HormoneBober R^{1,2}, Rafaeli A²

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Sexual behavior of the female moth, *Helicoverpa armigera*, is dependent on the release of a unique blend of sex pheromones to attract conspecific males. The production of sex pheromone is under the control of pheromone-biosynthesis-activating neuropeptide (PBAN) which binds to its G-protein coupled receptor at the pheromone gland. The present study demonstrates the spatial and temporal differential expression levels of the PBAN receptor gene. The PBAN receptor gene transcript in the pheromone glands reaches a peak level at 5 hours post emergence. Based on our previous studies using exogenously applied juvenile hormone (JH), a possible regulatory role for JH on the PBAN receptor was implied. In order to verify the regulatory role of JH on PBAN-receptor transcript levels, decapitation studies were conducted. These studies indicate that up-regulation of the PBAN receptor transcript is not dependent on a gene regulatory factor from the head (which includes JH) since expression levels rise normally when females are decapitated before peak transcript levels are reached. In addition, sex pheromone production can be induced by PBAN in such decapitated females. On the contrary, when JH is injected *in vivo* to females at this critical period (before 5 hours post emergence, when no JH is present) the PBAN receptor gene transcript levels are inhibited. We suggest that the PBAN receptor gene transcript may be regulated early during reproductive maturation of the female pupa and conclude that the absence of JH in pharate adults, as well as its absence immediately after emergence is critical for normal up-regulation of the PBAN receptor gene expression levels.

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Identification of Sex Pheromones for the Brazilian Stinkbug Species, *Chinavia ubica* and *Chinavia impicticornis* (Hemiptera: Pentatomidae)

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Chinavia and *Nezara* spp. stinkbugs (Hemiptera: Pentatomidae) comprise more than 100 species, with high diversity in Afrotropical and Neotropical regions¹. Previous studies have shown that *Chinavia* spp. and *N. viridula* utilise trans-(Z)-bisabolene epoxide (trans-Z-BE) ((Z)-(1S,2R,4S)-4-(1',5'-dimethyl-1',4'-hexadienyl)-1,2-epoxy-1-methylcyclohexane) and the corresponding cis-isomer (cis-Z-BE) (Z)-(1R,2S,4S)-4-(1',5'-dimethyl-1',4'-hexadienyl)-1,2-epoxy-1-methylcyclohexane) as major sex pheromone components^{2,3,4}, with species specificity being guaranteed by different ratios of the two compounds. For two Brazilian *Chinavia* species, *C. ubica* and *C. impicticornis*, Y-tube olfactometer bioassays using live insects and volatile extracts collected by air entrainment showed that females were attracted by conspecific males and extracts. Gas chromatography (GC) and coupled GC-mass spectrometry (GC-MS) analysis of a male *C. impicticornis* extract suggested the presence of a single BE in high purity (>90%), which was shown by microprobe ¹H NMR to be a trans-Z-BE isomer. Similarly GC, coupled GC-MS and microprobe ¹H NMR analysis of a male *C. ubica* extract revealed the presence of cis-Z-BE and trans-Z-BE isomers in a 9:1 ratio. Coupled GC-electrophysiology (GC-EAG) recordings using the antennae of female *C. impicticornis* and *C. ubica* revealed EAG responses to the trans-Z-BE and cis-Z-BE isomers respectively. Interestingly, for the mixture of isomers present in the *C. ubica* extract, differences in the EAG responses of the two species were observed. There was no response recorded for *C. ubica* with the trans-Z-BE isomer, and no response recorded for *C. impicticornis* with the cis-Z-BE isomer. These results not only demonstrate the potential for using high field NMR to facilitate insect pheromone identification, but also show that pheromone-mediated interactions for stinkbugs involves highly specialized olfactory receptors located on the antennae.

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Phosphorylation of Native Porcine Olfactory Binding Proteins

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The identification of various isoforms of olfactory binding proteins is of major importance to elucidate their involvement in detection of pheromones and other odors. Here, we report for the first time the characterization of the phosphorylation of OBP (Odorant-Binding Protein) and Von Ebner's Gland Protein (VEG) from the pig, *Sus scrofa*. After labeling with specific antibodies raised against the three types of phosphorylation (Ser, Tyr, Thr), the phosphate-modified residues were mapped by using the β -elimination followed by Michael addition of β -thiothreitol (BEMAD) method. Eleven phosphorylation sites were unambiguously localized in the pOBP sequence and nine sites in the VEG sequence. OBPs are secreted by Bowman's gland cells and exported in the extracellular mucus lining the nasal cavity. We suggest that these proteins, after tracking the secretion pathway in the rough endoplasmic reticulum, may be phosphorylated by ectokinases that remain to be characterized. The existence of such a regulatory mechanism theoretically increases the number of OBP variants and suggests a more specific role in odorant coding than the one of odor solubilizer and transporter for OBPs. Specific binding between OBP isoforms and pig sex pheromones, measured by fluorescence spectroscopy, will be presented.

Identification and Synthesis of a Pheromone of the Wasp Spider *Argiope bruennichi*

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Spider webs and spider silk are well known vectors for chemical signals and help in the conceptual understanding of the spider behavior in communication and sexual interactions¹. Only few pheromones have so far been identified from spiders, and no successful field application has been reported till date. Extracts of volatiles from virgin and mated female *Argiope bruennichi* spiders were obtained using dynamic headspace sampling. GC-MS analysis of these extracts showed the presence of a compound closely related to the primary metabolite citric acid. Analysis of the mass spectrum and synthesis proved it to be trimethyl methylcitrate that is released only by female virgin spiders. Synthesis of this compound was performed to confirm the structure and to provide material for biotests. Since the compound has two stereogenic centers and chirality often plays a significant role in pheromone activity², the absolute configuration of trimethyl methylcitrate released by the spiders was determined by stereoselective synthesis and chiral GC. The natural trimethyl methylcitrate proved to be highly attractive for males in the field.

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Sexual Pheromones of Hybrids Males in the *Bombus terrestris* (L. 1758) Species.Coppée A¹, Rasmont P¹, Valterova I²¹ Laboratoire de Zoologie – Université de Mons-Hainaut- 20 Place du Parc B-7000 Mons, Belgium² Institute of Organic Chemistry and Biochemistry – 2, Flemingovo nam. Praha 6 , Czeck Republic

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In Bumblebees, males are known to use their cephalic labial gland secretions as sexual pheromones to attract conspecific virgin females¹. These secretions are species-specific². In the *Bombus terrestris* species, a geographic variation of these secretions has been highlighted³. This species is the most commonly used in greenhouses pollination in Europe, the most economically valuable subspecies being *B. t. dalmatinus*. This taxa is than imported in several countries assessing that few impact on local populations occurs. Sexual pheromones of *B. t. dalmatinus*, *B. t. audax* from United Kingdom and hybrids of these two taxa were compared. The main goal of this study was to show if hybrids have different sexual pheromones than their parental populations or not. The sexual pheromones (cephalic labial gland secretions, CLG secretions) were obtained by extraction of the head secretions in hexane. The solutions obtained were analysed using a GC/MS and their qualitative and relative constitution were compared. A Principal Component Analysis was applied to the data matrix. The results obtained show that CLG secretions analyses allow the identification of both *audax* and *dalmatinus*, and moreover, the differentiation of F1 hybrids from two different populations. These hybrids have intermediate CLG secretions constitution.

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Sexual Behavior and Conditions for Volatile Sample Collections in *Callisphyrus apicicornis* (Coleoptera: Cerambycidae)

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Callisphyrus apicicornis (Coleoptera: Cerambycidae) is a native and xylophagous species, affecting fruit orchards (e.g. *Malus domestica*) and ornamentals (e.g. *Betula* spp) in Chile. Since most of the developmental cycle occurs under bark, adult management using a pheromone-based control technique seems promising. We have studied *C. apicicornis* sexual behavior, characterizing calling behavior in females and searching behavior in males. Ethograms showing the behavioral sequences and respective frequencies have shown stereotyped behaviors, suggesting the existence of a long-range female-produced sex pheromone. During the spring of 2008 we attempted sample collections from virgin (but relatively old) females performing calling behavior in an all-glass aeration apparatus, trapping the volatiles on activated charcoal during 4 hours. The trap was rinsed with hexane and the extract was analyzed by GC-MS, but no signals were detected. Septa loaded with this solution did not elicit responses from males in field bioassays.

Trying to identify more precisely the conditions regulating calling behavior, a binary logistic regression was used to evaluate the effect of light intensity, temperature, wind speed, female age, and hour of the day for calling on *C. apicicornis* adults. It was found that only light intensity was statistically significant in inducing calling. Females performed calling behavior mostly between 10:00 and 12:00 AM, 15 and 25 °C, and 0.1 to 1 m/s wind. Although older females call more frequently, significantly more males responded to younger calling females. Using these data we will conduct new volatile sample collections this spring 2009 in order to obtain and identify *C. apicicornis* sex pheromone.

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Novel Desaturase Recruited for Pheromone Biosynthesis in *Operophtera brumata*

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Moths generally rely on their highly evolved sex pheromone communication for mate finding. Most of the described moth pheromones belong to the same class of chemical compounds, fatty acid derivatives that are produced *de novo* within the pheromone gland¹. The pheromone biosynthesis involves desaturation, chain shortening by beta-oxidation and final reduction, acetylation or oxidation and some of the key enzymes involved have been characterized. Desaturation of coenzyme-A esters of saturated fatty acids is thus a common feature of sex pheromone biosynthetic pathways in the Lepidoptera². The gene family of fatty acyl desaturases with different substrate-, stereo- and regio-specificities which can account for some of the pheromone variation between species, has been extensively studied. Insect desaturases are homologous to the ancestral delta9 acyl-CoA desaturases of plants, vertebrates and fungi. Several dozens of desaturases, such as delta5, delta6, delta11, delta14, from many moth species, vertebrate, and plant have been characterized. In the current study, we investigate a new member of this gene family, encoding a desaturase that inserts a double bond in the methyl end of the fatty acid molecule. We cloned a candidate gene from the winter moth (*Operophtera brumata*) and expressed it heterologously in a yeast (*Saccharomyces cerevisiae*) strain deficient in both desaturase and elongase. Using the transgenic yeast we studied the putative methyl-terminus desaturase and its ability to convert 11,14,17-eicosatrienoic acid to 11,14,17,19-eicosatetraenoic acid, a desaturation step that was postulated to be involved in pheromone biosynthesis in the winter moth based on previous unpublished *in vivo* studies.

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Keep Working under Toxic Conditions – Functional Evidence for a Pgp-like Transporter Acting as a Barrier for Cardenolides in the Lepidopteran Nervous System

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Cardiac glycosides (CGs) are highly specific inhibitors of the Na⁺K⁺-ATPase, an ubiquitous animal enzyme which is essential for many physiological processes. Insects of several orders sequester these toxic plant compounds to achieve chemical protection. In our recent research on CG-storing Lepidoptera and species with a dietary exposition to CGs we found that target site insensitivity of the Na⁺K⁺-ATPase as it is realised in the monarch butterfly (*Danaus plexippus*) and *Chrysochus* leaf beetles seems to be an exception. We could show that several other lepidopteran species exposed to cardiac glycosides possess Na⁺K⁺-ATPases highly sensitive to CGs. Immunohistochemistry with a monoclonal antibody against Na⁺K⁺-ATPase revealed a strong expression of this enzyme in the nerve cord, which possesses a neural sheath acting as a blood brain barrier. Therefore, we focussed on this interface between toxic hemolymph and susceptible nervous tissue. Using caterpillars of *Manduca sexta* as a model organism and radioactive digoxin as a tracer, we could show that the access of this CG to the nervous tissue is limited by an energy-driven barrier. We found that compounds like verapamil significantly reduce the amount of digoxin entering the *Manduca* nerve cord. Since these compounds are well known inhibitors of P-glycoprotein (Pgp), a transporter conferring multi-drug-resistance to cancer cells, we hypothesize that Pgp-like transporters are involved in the circumvention of CG-intoxication by exposed caterpillars.

HMG-CoA-Reductase and Isoprenyl Diphosphate Synthase Regulate Iridoid Biosynthesis in *Chrysomelina* Larvae

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Larvae of the leaf beetles *Phaedon cochleariae* and *Gastrophysa viridula* produce iridoids (cyclopentanoid monoterpenes) as chemical weapons to defend themselves against predators. Upon attack, the larvae release small droplets of the defensive secretion from nine dorsal pairs of reservoirs¹. Both species synthesize the iridoids *de novo* but are also able to sequester and utilize the iridoid precursor 8-hydroxygeraniol-8-O- β -D-glucoside². The precursor is assembled from isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) via the mevalonate pathway. The key regulatory enzyme of this pathway is often the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR). Real-time data indicate a high impact of HMGR in larval fat body for *de novo* production³. Enzyme assays with the recombinant protein revealed attenuated activity by addition of the aglucon 8-hydroxygeraniol, whereas no effect has been observed for the glucoside or geraniol. Homology modelling and docking experiments of the catalytic domain demonstrate binding of 8-hydroxygeraniol to the active site suggesting a competitive inhibition mechanism. Besides an enhanced HMGR activity also an elevated geranyl diphosphate synthase (GDPS) activity is detectable in larval fat body⁴. Whereas HMGR is a key enzyme controlling the early pathway, the short-chain *E*-isoprenyl diphosphate synthases act as later regulatory branch point enzymes within terpenoid biosynthesis⁵. GDPS catalyzes the single condensation of IDP and DMADP to geranyl diphosphate (GDP). In iridoid producing *Chrysomelina* larvae, 8-hydroxygeraniol-8-O- β -D-glucoside is derived from GDP, which implies participation of GDPS in *de novo* synthesis^{2, 6}. GDPS may represent the branch point between the synthesis of defensive compound and other terpenoids such as juvenile hormones or Ecdyson. Identification and characterization of the GDPS within the biosynthetic network of terpenoid biosynthesis is required to understand the regulation of iridoid production with respect to the balance of *de novo* synthesis and sequestration.

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Analytical and Behavioural Studies Directed to the Characterization of the Pheromone of *Coroebus undatus* and *C. florentinus* (Coleoptera: Buprestidae), two Insect Pests of the Cork Oak

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The oak flathead borer *Coroebus undatus* (Coleoptera: Buprestidae) invades the cork oak (*Quercus suber*) by laying eggs on bark flaps and fissures of the trunk. After hatching, the larvae penetrate into the bark and construct large galleries around the trunk, causing reduction of quality and quantity of the cork. Although the impact of *C. undatus* on the production of cork is very high, so far nothing is known about the bioecology or chemical communication of the insect and furthermore, no effective control treatments have been established. In addition, live insects are very difficult to find and so studies to the characterization of the pheromone of *C. florentinus*, a closely related species that also attacks the cork oak but much easier available, have been initiated. GC-MS analyses of abdominal and faecal extracts and of volatiles of both sexes resulted in the identification of 30 compounds, most of them linear or methyl-branched saturated hydrocarbons. In Y-tube olfactometer bioassays, males were significantly attracted to volatiles from living females, abdominal and faecal extracts of both sexes and phenol. In contrast, females only show preferences for host tree extracts and phenol. Males display higher EAG depolarization responses than females but no significant differences were observed among the different stimuli. GC-EAD analyses of volatiles revealed the presence of four active compounds that elicited significant responses in male antennae. Activity of these compounds both in the laboratory and in the field is currently being investigated.

Behavioural and Central Nervous Responses to Plant Volatiles in *Agrotis ipsilon* Males are Mating-Independent in Contrast to Sex Pheromone Responses

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We previously showed that male moths of *Agrotis ipsilon* (Lepidoptera: Noctuidae) are no longer attracted to the female sex pheromone following mating¹. This behavioural plasticity is accompanied by a change in the sensitivity of central olfactory neurons in the macroglomerular complex of the antennal lobe (AL). The loss of sensitivity after mating is restored during the next day¹. This transient neuronal plasticity serves as an energy-saving strategy by switching off the olfactory system and therefore preventing males from mating unsuccessfully. As we previously showed that the central nervous processing of plant odours (in the ordinary glomeruli of the AL) is, contrary to sex pheromone, independent of age², we investigated whether the effect of mating on the olfactory system might also be restricted to pheromone processing neurons. We analyzed the behavioural and central nervous responses of virgin and newly mated *A. ipsilon* males to the sex pheromone blend and a plant odour (linden flower extract/heptanal). By means of wind tunnel experiments, we show that, independently of the dose, the behavioural response to sex pheromone was inhibited after mating. Using intracellular recordings of AL neurons, we show that the sex pheromone starts to be detected above a specific threshold (1ng), which is much higher than in virgin males. On the contrary, both the behavioural and central nervous responses of post-mated males to the plant odour were not different from that of virgin males. By analyzing different parameters of the neuron responses³, we show that mating changes the central processing of pheromonal information, but not that of plant volatiles. This mating-dependent neuronal plasticity is therefore restricted to the sex-specific pheromonal system and does not affect the responses to general odours such as plant-volatiles.

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Keep or Losing Control: Mechanistic Aspects of a Multiproduct Sesquiterpene Synthase from *Medicago truncatula*

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All tens of thousands sesquiterpenoids known today are based on 300 hydrocarbon skeletons, formed by so called sesquiterpene cyclases, utilizing farnesyl diphosphate (FDP) as substrate. The structural diversity is created by generating an environment that binds the flexible isoprenoid substrate in a proper orientation and conformation to enforce specific trajectories for C-C-bond formation. Beside high fidelity enzymes producing only a single product also promiscuous enzymes are known that can generate up to 52 different products¹. The biosynthetic promiscuity is assumed to result from a permissive template allowing alternative conformations of the substrate and later intermediates. In order to understand to which extent the enzymes exert control over the reaction pathways, we analyzed the stereochemical course of product formation of the multiproduct sesquiterpene synthase MtTPS5 from *Medicago truncatula*². The incubation of the recombinant MtTPS5 with FDP provides 18 sesquiterpene hydrocarbons and 10 sesquiterpene alcohols with different carbon skeletons such as Germacrene- or Cubebane family. The stereochemical analysis of 17 enzyme products, using chiral gas chromatography, showed that only one enantiomer of each product was present. Additionally we could observe that products derived from a common cationic precursor share the same configuration of their stereocenters. Together with a conformational analysis of the transition states we could show that the starting conformation of FDP is under stringent control of the enzyme, predefining the configurations of the stereocenters of the early intermediates. Further labeling experiments conducted in D₂O and followed by mass spectrometric analysis, revealed information about the formation of the key intermediate (*E,Z*)-germacradienyl cation. The strong incorporation of deuterium (up to 95%) in products derived from the (*E,Z*)-germacradienyl cation show that the cationic intermediate is generated via protonation of Germacrene D. These results are consistent with an biosynthetic hypothesis of Arigoni³ and provide an alternative pathway to the 2,3-double bond isomerization of farnesyl diphosphate via nerolidyl diphosphate, essential for the formation of hydrocarbon skeletons, such as Cadinane or Bisabolane.

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Love where you Eat - the Impact of Host Plant Species on Male Mating Behavior and Cuticular Hydrocarbons in the Leaf Beetle Genus *Phaedon* (Coleoptera: Chrysomelidae)

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Specialization on new host plant species may drive diversity of herbivorous insects by reproductive isolation of herbivore populations on the ancestral and novel host plant. Do host plant species affect mate recognition in the mustard leaf beetles *Phaedon armoraciae* and *P. cochleariae*? Even though both beetle species may occur together in the same habitats, they were usually found on different plant species: *P. armoraciae* on brooklime (*Veronica beccabunga*), and *P. cochleariae* on large bittercress (*Cardamine amara*) and watercress (*Nasturtium officinale*). In the laboratory, both *Phaedon* species were kept on their natural food plants (brooklime or large bittercress), as well as on Chinese cabbage. We compared mating behavior of males with females reared on the same plant species as males with mating behavior of males with females reared on a different plant species. Male mating behavior was significantly influenced by the plant species. In intraspecific mating trials, males of *P. armoraciae* mated more often and longer with “same plant” females than with “different plant” females. In contrast, *P. cochleariae* males did not differentiate between “same plant” and “different plant” females. In interspecific mating trials, *P. armoraciae* and *P. cochleariae* showed significant premating isolation when reared on their natural host plants, but they lacked premating isolation when both were reared on Chinese cabbage. Since species and mate recognition in *Phaedon* is mediated by cuticular hydrocarbons (CHC), we performed a canonical discriminant analysis (DA) based on CHC profiles to assess whether different host plant species affect the CHC profiles. The DA clearly separated different groups according to sex, beetle species, and host plant species. Our results indicate that a host plant shift results in premating isolation induced by a change of the CHC profile that is used for species and mate recognition in mustard leaf beetles.

Mating Disruption of the Introduced Forest Pest *Megaplatypus mutatus* in Poplar and Fruit Tree Plantations in Italy: Development of Controlled Release Formulation and First Trial

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Megaplatypus mutatus (Chapuis), an ambrosia beetle native to South America, attacks standing live trees of a wide range of forest and fruit tree species, particularly damaging to commercial poplar and hazelnut plantations. In 2000, *M. mutatus* was discovered established in Italy, in the Province of Caserta, near Naples. Our lab identified pheromonal components^{1,2} and performed the first trial to monitor *M. mutatus* in Buenos Aires, Argentina, using pheromonal compounds formulated in reservoir controlled release systems and obtained good results. Taking into account that the beetle is relatively immobile, that males are monogamous, and that the pheromones are of low commercial cost, stable in field conditions and can be formulated in controlled releases systems with relatively high release rates, we evaluated the potential management by disruption of communication between the sexes through application of male pheromone in infested poplar and fruit trees plantations of Caserta Province, Italy. We developed polymeric reservoir dispensers for sulcatol, sulcatone and 3-pentanol with zero order kinetics to be deployed in the field during the females flying period. We worked in two highly infested plantations of poplars (*Populus X euramericana*) and hazelnut trees (*Corylus avellana*) in Caserta, Italy. We confirmed that initial level of attack (number of active galleries per tree) was not significantly different in selected treated and control areas. We determined the beginning of the flying period using pheromone baited traps, and then deployed pheromone devices of sulcatol, 3-pentanol and sulcatone with release rates of 79, 91 and 63 mg/day respectively. After the flying period, we re-evaluated the level of attack (number of galleries with effective mating per tree) in control and treated areas and found that in treated areas it was significantly lower than in control ones, both for poplar plantation (0.24 and 0.68 respectively) and for hazelnuts plantations (0.92 and 2.1 respectively) ($P < 0.05$).

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Emission and Localization Site of Pheromones in Ambrosia Beetle *Megaplatus mutatus* Attacking three Host Species

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Megaplatus mutatus, an Ambrosia beetle (Platypodidae) native to South America, is a main forest pest that attacks live standing trees, affecting commercial poplar and broadleaf plantations. Attack is initiated by pioneer males selecting a host tree to build a short nuptial gallery, from which they attract females using a sexual pheromone composed mainly by (+) 6-methyl-5-hepten-2-ol ((+)-sulcatol) and 6-methyl-5-hepten-2-one (sulcatone). Recently 3-pentanol was found in the chemical analyses of volatiles using an SPME coating specific for low molecular weight alcohols and ketones. Although identified in a small percentage of samples, walking behavioral bioassays with video image analysis showed that at the doses tested, 3-pentanol elicited an attractive response from females. In the present study, we determined the sites of production of the pheromones within males and the temporal pattern of pheromone emission during gallery initiation and establishment. (+) Sulcatol, sulcatone and 3-pentanol were found only in proctodeum. Sulcatol and 3-pentanol production was detected 1-2 days after gallery initiation with maximal production between 5-12 days. Sulcatone production was noted during the same period although only in trace quantities. We collected male volatile emissions during the hours of peak flight by using a specific polar microextraction phase and the extract was analyzed by GC-MS. (+) Sulcatol and sulcatone were noted in pheromone emissions from males on three different host species, between 2-12 days post gallery initiation until day 51; sulcatone always in lower amounts. The temporal patterns of sulcatol sulcatone and 3-pentanol within male *M. mutatus* proctodeum correspond to the temporal patterns of emission.

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Sex Attraction in the Potato Psyllid *Bactericera cockerelli* (Hemiptera: Psyllidae) to Male- and Female-Produced Volatiles

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The potato psyllid, *Bactericera* (= *Paratrioza*) *cockerelli* (Šulc) (Hemiptera: Psyllidae), is a major pest of potato fields in the United States, Mexico, Central America and New Zealand. Despite the economic importance of the potato psyllid as a vector of diseases, such as psyllid yellows and zebra chip, the chemical ecology of this pest has not yet been investigated. In this study, we examined the role of chemical cues in sex attraction, assessing male and female response to male- and female-produced volatiles in olfactometer assays. We also tested psyllid response to chemicals collected through whole body extracts, to determine whether this extraction method is suitable for isolating psyllid-produced chemicals. Potato psyllid males displayed attraction to odors from live females and female extracts. Males were also attracted by odors from live males and male extracts. On the other hand, females assayed in the olfactometer avoided odorant volatiles from live and extracts of both females and males. This study is the first to demonstrate sex attraction in the potato psyllid. To our knowledge, this is also the first report showing that females of any Psyllidae avoid odors associated with conspecific males and females.

Functional Fatty Acid Reductases in the Ermine Moths, *Yponomeutidae*, and their Involvement in Pheromone Biosynthesis

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The three sister species of small ermine moths (Lepidoptera: Yponomeutidae) *Yponomeuta evonymellus*, *Y. padellus* and *Y. rorellus* are distributed sympatrically throughout Europe, and they are use various alcohols and acetates as their female sex pheromone¹⁻³. This makes them a suitable group for studying the repertoire of FARs (Fatty Acyl Reductases) involved in active in the pheromone biosynthesis⁴. In pheromone biosynthesis, the FARs account for a key step by converting the fatty acyl intermediates into fatty alcohols, which may subsequently be acetylated to form pheromone component acetates. However, the molecular and biochemical properties of these enzymes have yet been investigated in detail apart from the FAR reported in the silkworm moth *Bombyx mori*⁵. We have screened the pheromone gland transcriptome of all the three species, and we isolated several FAR-like sequences. Transcriptional analysis showed that one of the transcripts, FARII, was expressed in the pheromone gland only, linking it to the pheromone biosynthesis, whereas the others were present in the rest of the body as well as in the pheromone gland. We further investigated the putative FARs by analyzing the functionality and specificity of each ORF enzyme *in vitro*, and using the same potential fatty acid intermediate pheromone precursor for each species. Our results confirm the involvement of FARII in pheromone biosynthesis. FARII was found to be a unique functional fatty acyl-CoA reductase in all three sister species, whereas the other FAR-like enzymes were found to be inactive on every substrate tested.

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Don't Talk Loudly: the Emission of Alarm Pheromone from Pea Aphids Regulated by Group-Living ConditionsHatano E¹, Kunert G², Kunert M², David A², Gershenson J², and Weisser WW¹¹ *Institute of Ecology, Friedrich-Schiller-University Jena, Dornburger Str. 159, 07745 Jena, Germany*² *Max-Planck-Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany*

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Many predators and parasitoids feed on aphids which use alarm pheromones to warn conspecifics of potential attack. The sesquiterpene (*E*)- β -farnesene (EBF), a common alarm pheromone in many aphid species, is emitted when an aphid is attacked by natural enemies¹. In addition, EBF can also mediate wing formation in parthenogenetic offspring, which allows them to leave the plant². Since some natural enemies can use the alarm signal as an allomone to locate aphids on the plant, aphids face the dilemma of alerting conspecifics and remain inconspicuous for natural enemies. Previously, the emission of EBF by aphids was studied to determine whether non-disturbed but alarmed aphids also emit this pheromone to alert further conspecifics^{3, 4}. The results indicated that alarmed aphids keep "silent" and do not release any EBF, suggesting that aphids have evolved a high affinity to EBF, perceiving very low amounts and remaining inconspicuous on the plant by not emitting excessively in the environment. Here we report that the amount of EBF released is, however, not always low and constant, and can be controlled according to interspecific interactions. The amount of EBF emitted by two aphid clones in different group conditions was assessed when attacked by predators, showing that the alarm signal of isolated aphids decreased when they are grouped. However, the biosynthesis of EBF was not affected by either condition. Furthermore, the pea aphid clones showed different dispersal and developmental strategies according to colony size and emission of alarm pheromone. The amounts of EBF detected from isolated and grouped aphids were tested in aphid colonies showing that the regulation of EBF emission according to population densities is adaptive to induce wing formation in offspring.

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Calling Behaviour and Preliminary Identification of Female Sex Pheromone of the Cocoa Pod Borer, *Carmenta foraseminis* (Eichlin, 1995) (Lepidoptera: Sesiidae)

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Carmenta foraseminis (Eichlin, 1995) (Lepidoptera: Sesiidae) is one of the serious entomological problems of cocoa (*Theobroma cacao* L.), in Venezuela. Female adult lay eggs on cocoa pods, then larvae penetrate the fruit, reaching and feeding the seeds; this causes serious damage on commercial product. At the moment, pest control is only carried out using cultural methods, so it is necessary to look for other control strategies. In the present work we studied the calling behavior and identified a putative female sex pheromone of *C. foraseminis*. The larvae and pupae were collected from damaged cocoa fruits in Choroni (Aragua state, Venezuela). Larvae and pupae were reared individually and maintaining in a room at 25°C, 63% RH and 12:12 (L:D)-h photoperiod. Daily activity of adults and female calling behavior were registered hourly during 72h. The adult was placed inside an observation chamber, which it was provided with sucrose solution sources, half cocoa pod and a young *T. cacao* plant. Also, female and male abdominal tip extracts and solid samples were prepared and analyzed by GC-MS equipped with polar and non-polar capillary columns. The calling behavior occurs the same day after emergence, at 18:00, in this case females assume the follow behavioural repertory: abdomen ventrally curved, abdominal hairs extended and gently abdominal movements. Alternatively, females walk rubbing the last abdominal segments against any surface, holding its abdomen curved. Two possible components of the *C. foraseminis* female sex pheromone were identified as (3E,13E)-octadecadien-1-ol (3E,13E-18:OH) and (3E,13E)-octadecadienyl acetate (3E,13E-18:OAc), using GC-MS libraries and retention indices (RI) reported for typically compounds of sesiid sex pheromones¹. To confirm the biological activity of these compounds, electroantennographic studies and behavioral bioassays with synthetic compounds are in progress.

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Toward Chemical Identification of Sex Pheromones Released by Male Round Gobies, Invasive Fish in the Laurentian Great Lakes

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Water in which reproductive male (RM) round goby (*Neogobius melanostomus*) have been kept attracts reproductive females (RF)¹. The present study aims to identify chemicals responsible for this attraction. We injected RM with GnRH and analyzed their tank water (16 hours after injection) using analytical chemistry, electrophysiology and behavioral responses. Estimation of steroids using ELISA revealed increased release of 11-oxo-etiocholanolone (reported to be the main biosynthetic product on incubating RM testes with androstenedione²) and its unidentified conjugate(s). Methanol extracts prepared from these waters induced stronger field potentials from the olfactory epithelium in females than those extracts prepared from saline injected RM waters. Behavioral responses, including swimming velocity and time spent near the water/odour inflow indicated that both RFs and non reproductive females (NRF) were attracted to these methanol extracts. These methanol extracts were fractionated using reversed phase HPLC and HPLC fraction pools were tested for behavioural responses in females. The fraction pool containing 11-oxo-etiocholanolone attracted NRFs, but was avoided by RFs. However, another fraction pool containing unknown conjugate(s) of 11-oxo-etiocholanolone attracted both RFs and NRFs. The fraction pool containing the unknown conjugate(s) was the only pool of fractions from the methanol extract which attracted RFs. We are currently working to identify the compound(s) present in this pool responsible for attracting RF and NRF gobies.

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Chemical Protection of Native European Eggs against Intraguild Predation by the Invasive Ladybird *Harmonia axyridis*Katsanis A¹, Hilker M², Otte T², Babendreier D¹, Kenis M¹¹ CABI Europe-Switzerland, Rue des Grillons 1, 2800 Delémont, Switzerland² Freie Universitaet Berlin, Institute of Biology, Haderslebener Str. 9, 12162, Berlin, Germany

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Harmonia axyridis is a ladybird of Asian origin that is presently invading Europe. *H. axyridis* is suspected to affect native ladybird populations through intraguild predation and competition for food. This invasive species is known to be a top intraguild predator and reported to attack the eggs of many coccinellid species. Studies have shown that while the eggs of many species were highly palatable to *H. axyridis*, some appeared to be protected against the predation of the invasive ladybird. We are currently investigating the palatability of the eggs of many native European coccinellid species to predation by neonate *H. axyridis* larvae. Results show that eggs of *Calvia quatuordecimguttata* are not eaten by *H. axyridis*, in contrast to those of most other European ladybirds. *Harmonia axyridis* eggs are also highly palatable to its own larvae. To verify whether chemical compounds that are found on the surface of the eggs are responsible for these results, we exchanged the extracts of the surface of *H. axyridis* and *C. quatuordecimguttata* eggs. When these treated eggs were provided to *H. axyridis* larvae, *C. quatuordecimguttata* eggs became more acceptable to predation while *H. axyridis* eggs became much less preferred. This finding clearly shows the importance of surface chemicals in the interactions between *H. axyridis* and native ladybirds. Chemical analysis of the eggs will be conducted to identify these surface chemicals.

The Role of Epidermal Glands in the Lacertid Lizard *Acanthodactylus boskianus*Khannoon E^{1,2}, Flachsbarth B³, El-Gendy A², Mazik K¹, Hardege J¹, Breithaupt T¹, Schulz S³¹ Department of Biological Sciences, University of Hull, Cottingham Road, Hull HU6 7RX, UK² Zoology Department, Faculty of Science, Fayoum University, El Fayoum, Egypt³ Technische Universität Braunschweig, Institut für Organische Chemie, Hagenring 30, 38106 Braunschweig, Germany

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The epidermal gland of lizards is a holocrine gland secreting semivolatile compounds which might be used by lizards for intraspecific communication. Here we show that the femoral gland secretions of one of the lacertid lizards, *Acanthodactylus boskianus*, releases semivolatile pheromones eliciting sex specific responses in conspecific lizards. We used chemical analysis and behavioural experiments. We analysed the femoral gland secretions using GC-MS and compared secretions of both sexes and different ages of males. We used the tongue flicking rate (TF) to score the interest of the lizard towards these secretions. For the first time in reptiles; monoglycerides of C11-C20 acids and glycerolmonoethers of C9-C22 alcohols were identified. We also identified steroids, alcohols, carboxylic acids, alkanes, amides, aldehydes, carboxylic acid esters, and squalene. Our study shows that there are sexual differences to the vast majority of chemicals identified. A positive correlation exists between a number of compounds and the age of the male lizards. These chemical results support the behaviour of the lizard where both males and females increased tongue flicking rate towards male secretions, with only males showing an increase in aggression towards secretions of other males. Female secretions caused responses only in males and did not elicit any aggressive behaviour. We also found significant population differences in the chemistry of these secretions, which might give rise to phylogeographic implications. Epidermal gland secretions appear to play an important role in male territorial behaviour as well as in sex recognition. This work opens opportunities to test in future the role of chemical cues in mate choice and dominance hierarchies in lizards and to use the behavioural assay to eventually purify and identify these pheromones.

Developing a Pheromone-Based Attractant for Sampling Winter MothKhrimian A¹, Lance DR², Mastro VC², Elkinton JS³¹ USDA, Agricultural Research Service, Beltsville, Maryland, USA² USDA, Animal and Plant Health Inspection Service, Otis, Massachusetts, USA³ University of Massachusetts, Amherst, Massachusetts, USA

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The winter moth, *Operophtera brumata* (Lepidoptera: Geometridae), is an early-season defoliator that attacks a wide variety of hardwoods and, in some cases, conifers. The insect is native to Europe but has become established in at least three areas of North America including southeastern New England. The species' female-produced sex attractant pheromone was identified some time ago as (3Z,6Z,9Z)-1,3,6,9-nonadecatetraene. This compound, however, also attracts a native congener, the Bruce spanworm, *Operophtera bruceata*. Dissection, or (for certainty) a DNA molecular testing is required to differentiate between males of the two species. Thus, a trapping method that is selective for winter moth would be desirable. A geometric isomer of the pheromone, (3E,6Z,9Z)-1,3,6,9-nonadecatetraene, can reportedly inhibit attraction of Bruce spanworm to traps without affecting winter moth catch, but use of the pheromone and inhibitor together has not been optimized, nor has the synthesis of the inhibitor. We present a new synthesis of (3E,6Z,9Z)-1,3,6,9-nonadecatetraene based on the intermediate (3Z,6Z)-3,6-hexadecadien-1-ol, which has also been utilized in the synthesis of the pheromone. The synthesis combines traditional acetylenic chemistry and Wittig-type olefination techniques, and benefits from a refined oxidation of the key intermediate to (3Z,6Z)-3,6-hexadecadienal. A brief summary of our field trappings in 2006-2008 will also be presented.

Off-Ratio Blends for Mating Disruption of the Citrus Leafminer, *Phyllocnistis citrella*Lapointe SL¹, Stelinski LL²¹ USDA-ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL, USA² Citrus Research & Education Center, University of Florida, Lake Alfred, FL, USA

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Pheromone studies often seek to identify an optimal response to continuously varied mixture components wherein proportionality of one component cannot be varied independently. Geometric designs were used to systematically and efficiently explore the experimental space defined by pheromone amount and proportion for the leafminer *Phyllocnistis citrella*, a pest of citrus worldwide. Deployment of single pheromone components, either (Z,Z,E)-7,11,13-hexadecatrienal or (Z,Z)-7,11-hexadecadienal in SPLAT™ (ISCA Technologies, Inc.) in citrus groves resulted in a high degree of disruption of attraction of male moths to natural blend (3:1 triene:diene) lures¹. While either individual component was effective at disrupting mating in field trials, (Z,Z,E)-7,11,13-hexadecatrienal was approximately 13 times more effective compared with (Z,Z)-7,11-hexadecadienal alone. Application in SPLAT of a third component isolated from the pheromone glands of *P. citrella*, (Z)-7-hexadecenal, did not disrupt trap catch. Our results support a non-competitive model and support sensory imbalance as the mechanism of mating disruption in this species.

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Genetics and Context Influence Hierarchical Use of Olfactory Information in *Drosophila*

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The fruit fly *Drosophila melanogaster*, like most other insects, is very selective in its use of olfactory information. This species is usually referred to as a host generalist, feeding and ovipositing on a wide range of different fruits and decaying organic material. Nevertheless, its exploitation of different food resources is likely governed by common chemosensory signals that are to a great extent shared by all these resources. We have used complex natural odours and single synthetic odours to determine response characteristics to olfactory stimuli in different contexts, ranging from attraction to oviposition. We demonstrated that truly wild type *D. melanogaster* is very selective in its attraction towards olfactory stimuli, with natural odours from optimal hosts being far more attractive than single synthetic stimuli. There is great variation among laboratory strains of *D. melanogaster*, however. Some of these strains retain a conservative wild-type phenotype whereas others are more non-selective and respond in a more generalist fashion to a wider range of stimuli almost as readily as to optimal olfactory stimuli. This broad degree of generalism in non-selective strains is limited to olfactory attraction, however. In their oviposition behaviour, both selective and non-selective strains were much more conservative than in attraction assays. Oviposition appears to be mediated by an even more narrow range of olfactory stimuli than attraction from a distance.

Phenylalanine 35 and Tyrosine 82 are Involved in the Uptake and Release of Ligand by Porcine Odorant-Binding Protein

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Structural and molecular dynamic studies have pointed out the role of aromatic residues in the uptake of ligand by porcine odorant-binding protein (pOBP). The shift of Tyr82 from its position during the opening of the binding cavity has been shown, and was supposed to participate in the entrance of the ligand. Several Phe residues in the vicinity of Tyr82 could also participate in the binding process. To clarify their involvement, we performed molecular dynamics studies to simulate the unbinding of undecanal, a ligand previously co-crystallized with pOBP. The results confirmed the key-role of Tyr82 and pointed out the participation of Phe35 in controlling the reorientation of undecanal towards the exit. To bring experimental support to both published (binding) and present simulations (unbinding), we have mutated these two residues and over expressed the wild type pOBP, the two single mutants and the double mutant in the yeast *Pichia pastoris*. As fluorescence spectroscopy implies the uptake of the fluorescent probe and release in displacement experiments, we monitored the binding ability of the four proteins for 1-aminoanthracene (1-AMA). The experimental results indicated that both residues are involved in the uptake of ligand as the three mutated proteins were unable to bind 1-AMA, contrary to the wild type recombinant pOBP that bound 1-AMA with the expected affinity.

Sex Pheromone of Wheat Armyworm *Pseudaletia sequax* (Lepidoptera: Noctuidae)Lima ER¹, Almeida AA¹, Zarbin PHG², Salvadori JR³¹ Universidade Federal de Viçosa, Entomologia, Viçosa, MG - Brasil, CEP 36570-000² UFPR, Departamento de Química, Centro Politécnico, Curitiba, PR – Brasil, CEP 81531-990³ Embrapa Trigo – Embrapa Trigo, Rodovia BR 285, km 294, Passo Fundo, RS - Brasil - CEP 99001-970

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The wheat armyworm, *Pseudaletia sequax* is distributed from Mexico to Argentina. In South America, including Brazil, Argentine and Uruguay is a serious pest in wheat, sorghum, pasture grass. Gas chromatographic and mass spectral analyses were conducted on pheromone gland extracts collected from virgin calling females of *P. sequax*. Coupled Gas Chromatograph-Electroantennographic Detection (CG-EAD) analysis of the female gland extract showed the presence of three consistent EAD-active peaks. The peaks were identified by CG-MS analyses as (Z)-11-Hexadecenal (Z11-16: Al), (Z)-11-Hexadecenyl acetate (Z-11-16: OAc) and (Z)-11-Hexadecen-1-ol (Z11-16: OH). Behavioral tests were carried out within a wind tunnel (3x1x1m). Virgin males were tested once during fifth and eighth hour of scotophase corresponding the female calling period. Analyses of flight response indicated that Z 11-16:Al and Z11-16:OAc are essential for inducing the upwind flight and landing. The minor component Z11-16:OH when added Z11-16:Al, Z11-16:OAc significantly increase flight response, but revealed to decrease the landing response of males.

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New Alkaloids from Rove Beetles of the Genus *Stenus* (Coleoptera: Staphylinidae)Lusebrink I¹, Dettner K¹, Müller T², Seifert K²¹ Department of Animal Ecology II, University of Bayreuth, 95440 Bayreuth, Germany² Department of Organic Chemistry, NW II, University of Bayreuth, 95440 Bayreuth, Germany

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Since the beginning of the last century, *Stenus* beetles have intrigued scientists due to their extraordinary method of locomotion on water surfaces. Upon falling on water they excrete a secretion from their pygidial glands that lowers the surface tension of the water and propels the beetle forward very quickly. The investigation of this secretion using the common species *S. comma* showed that the alkaloid stenusine N-ethyl-3-(2'-methylbutyl)-piperidine) is the active ingredient in the secretion responsible for this movement on water surfaces. Most previous studies on the chemical composition of the pygidial gland content in the rove beetle genus *Stenus* have been carried out using *S. comma*, but the genus *Stenus* belongs to the most species-rich genera of the animal kingdom. Up to now, 2377 taxa including 8 fossil species are known worldwide¹. We analysed the pygidial gland content of a broader range of species and discovered new alkaloids: (Z)-, (E)-3-(2'-methyl-1'-butenyl)-pyridine, 3-(1'-isobutenyl)-pyridine, and 1',3-epoxy-3-(2'-methylbutyl)- Δ^1 -piperidine representing new natural products. The chemotaxonomic significance of the occurrence of these alkaloids and stenusine in different *Stenus* species is discussed.

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Tropical Parabiotic Ants: Interspecific Nestmate Recognition in an Unusual PartnershipMenzel F¹, Schmitt T², Blüthgen N¹

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Aggression between ant colonies is ubiquitous. However, ants of the genera *Crematogaster* and *Camponotus* often tolerate each other, and certain species of these genera even live together in the same nest (parabiosis). In the tropical rainforest of Borneo, parabiotic associations between *Crematogaster modiglianii* and *Camponotus ruffifemur* are common, but the mechanisms that facilitate interspecific tolerance between associated species are largely unknown. We therefore studied interspecific nestmate recognition among the two parabiotic species. In aggression bioassays, the two species tolerated each other without involvement of any appeasement behaviour. Apparently, both species were unable to discriminate their partner colony from certain foreign colonies of the same species. This low inter-colony discrimination may be partly due to the fact that the cuticular hydrocarbons of both ant species, which usually function as nestmate recognition cues, are considerably longer than in related, non-parabiotic species. Due to their lower volatility they are harder to perceive and thus may provide fewer nestmate recognition cues than shorter hydrocarbons. Moreover, *Crematogaster* produces high amounts of several, hereto unidentified cuticular substances other than hydrocarbons, which it apparently transfers to *Camponotus*. Based on their mass spectra, these substances are chemically interrelated and probably possess several ring structures. In bioassays, these substances significantly reduced aggressiveness of *Camponotus* workers. The high interspecific tolerance thus seems to be facilitated by the low volatility of long-chain cuticular hydrocarbons, in combination with the unknown compounds that reduce aggression in *Camponotus*. *Camponotus ruffifemur* occurred in four sympatric varieties, which possess qualitatively distinct cuticular hydrocarbon profiles and different mtDNA haplotypes, and probably represent cryptic species. In contrast, *Crematogaster modiglianii* was not differentiated into genetic or chemical varieties. *Cr. modiglianii* was able to differentiate between the *Camponotus* varieties but did not discriminate between different colonies of the same variety.

***Culex* sp Oviposition Pheromone: Synthesis, Microencapsulation and Preliminary Studies for Activity over Time**Michaelakis A¹, Mihou AP², Koliopoulos G¹, Milonas P¹, Couladouros EA^{2,3}¹ Benaki Phytopathological Institute² Agricultural University of Athens³ Institute of Physical Chemistry, NCSR "Demokritos"

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The oviposition pheromone of *Culex quinquefasciatus* (6-acetoxy-5-hexadecanolide)¹ was synthesized in a racemic form with a simple (five steps), efficient, high yielding (45% total yield) and low cost way (use of relatively low cost reagents). The racemic form was tested for its oviposition stimulant in the laboratory on *Culex pipiens* biotype *molestus* which is a member of the species complex that *Culex quinquefasciatus* belongs. In the testing conditions it was found that the best oviposition stimulant achieved at 1 µg per cage². In a second step, polyurea microcapsules containing the synthetic mixture of diastereomers employed for the laboratory implementation of the attract-and-kill strategy. A dose-dependent response revealed the optimum dose of 300 mg of dried microcapsules. Activity of microencapsulate formulation over time was also studied. The microencapsulated pheromone was found to be sufficiently active to gravid female mosquitoes for a period of 40 days. We also tested the combination of the synthetic pheromone with the control agent as an attract and kill strategy. Temephos had no influence on the oviposition pattern of the females whereas mortality of larvae was very high³. Finally for the first time aging pheromone (microencapsulated) was combined with an aged infusion. *Oxalis pes-carpae* infusion was combined with the synthetic pheromone (6-Acetoxy-5-hexadecanolide) and the mixture revealed a synergistic effect only for the first day. This was a first detection for the potential use of microencapsulated synthetic pheromone with infusion and results are discussed⁴⁻⁶.

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Sex Pheromone Communication of Welsh Clearwing, *Synanthedon scoliaeformis* (Borkhausen) (Lepidoptera: Sesiidae)

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At the first glance, clearwing moths bear more resemblance to wasps than to typical moths. Moreover, clearwings are active during the light period of the day and they are not attracted by light traps at night. For detection and attraction of these peculiar moths, sex attractants and pheromones are especially valuable tools. In this work we present identification of sex pheromone for Welsh clearwing *S. scoliaeformis* moth. Gas chromatographic and mass spectrometric analyses of crude sex pheromone gland extracts revealed that virgin females produced 6 compounds structurally related to sex pheromone components of clearwing moths. Comparison of chromatographic and mass spectral data of synthetic standards with ones of natural products indicated that those compounds were *E2,Z13-18:OAc*, *E2,Z13-18:OH*, *18:OAc*, *Z3,Z13-18:OAc*, *Z13-18:OAc*, *18:OH* and occur in the ratio 92:7:1:traces:traces:traces. Trapping tests revealed that there were no formulations, including binary and three component mixtures, significantly more attractive to conspecific males compare to *E2,Z13:OAc*, tested as single compound. In conclusion, *E2,Z13:OAc* has to be considered as the sex pheromone of *S. scoliaeformis* moths. The peak of males attracted to the traps was registered within one hour after noon. Previously, *E2,Z13:OAc* was reported as the sex attractant for Welsh clearwing males¹. It is known that, *E2,Z13:OAc* functions as sex pheromone component in 9 and as sex attractant in other 17 clearwing moth species². Other compounds identified from the sex pheromone gland of *S. scoliaeformis* did not show any significant intraspecific activity, however they provide a basis to achieve specificity of a pheromone signal of *S. scoliaeformis*, thus could act in interspecific manner as attraction antagonists against other clearwing moth species. In addition, some of these compounds could be intermediates or side products in biosynthesis of a sex pheromone as well.

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The Origin of *Pieris napi* Antiaphrodisiac

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During mating *Pieris napi* L. female receive a blend of several volatile compounds from the male¹. Methyl salicylate is the main compound and acts as an antiaphrodisiac to *P. napi* males. Our earlier findings showed that the majority of methyl salicylate used by the male for the first mating was synthesized during the larval stage. By feeding the adult males by Deuterium, ¹³C and ¹⁵N labeled precursors we found that additional compounds like methyl cinnamate, benzyl cyanide, benzylalcohol and methyl benzoate can be used for continuous production of the pheromone blend and delivered by the males during a second mating.

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Natural Ligands of Porcine Olfactory Binding Proteins

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Knowledge of endogenous ligands of olfactory binding proteins is a prerequisite for studying their role in odor and pheromone transduction. Here we report the extraction, derivatization, and characterization by gas chromatography-mass spectrometry, of the natural ligands of pig, *Sus scrofa* (L.), Von Ebner's Gland protein (VEG) and odorant binding protein (OBP). We identified two isoforms (VEG1 and VEG2), which differed only by the linkage of an O-N-acetylglucosamine (O-GlcNac) group on VEG1. The natural ligands of VEG1 were characterized as two isomers of testosterone, whereas ligands of VEG2 and OBP were fatty acids or their derivatives. Our findings suggest that the binding specificity of VEG1 for steroids is governed by the presence of an O-GlcNac moiety on the protein. This specificity was confirmed by the binding of radiolabeled testosterone only by VEG1 in an in-gel binding assay. This is the first evidence for a post-translational modification in the process of odorant discrimination by olfactory binding proteins.

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Behavioral and Neurophysiological Responses of Female Oriental Fruit Moths to Changing Ratios of a Key Component in a Bioactive Mixture

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Plant derived odours used by insects for reproduction and survival are filtered out from the complex volatile blends emitted by host and non-host plants in the field. Ratios of release of plant odours, however, might vary across individual plants and across phenological stages. They might also be subjected to daily and seasonal variations in environmental conditions. Insects, therefore, face the challenge of being able to detect behaviourally active odours, regardless of changes in ratio/concentration of the components of the odour. Previous studies in our group indicated a key role of the aromatic compound benzonitrile in the perception of and response to an attractant host-plant derived volatile mixture on the oriental fruit moth (*Cydia molesta*). Surprisingly, benzonitrile is present only in minor amounts in this bioactive mixture, which consists of 3 green leaf volatiles and an additional aromatic compound. Here, we tested the behavioural and neurophysiological responses of mated female moths to changes in the concentration of benzonitrile in the mixture while keeping the ratios of the remaining components constant. The results show that the variation in the concentration of benzonitrile in the mixture affects the behavioural and neurophysiological responses to the mixture, and that these two responses mirror one another. Specifically, increases of benzonitrile up to 100 times made it progressively easier for the female moths to discriminate the mixture but further increases or decreases in benzonitrile concentration reduced progressively the magnitude of the response. Thus, the importance of relative concentrations of minor components of behavioral active blends in the olfactory recognition and behavioural discrimination of blends in a herbivore insect species is demonstrated.

Sex Pheromone Components of the Autumn Gum Moth, *Mnesampela privata*, Identification and Biological ActivityNilsson A¹, Walker P², Allen G², Davids N³, Andersson F¹, Hedenström E¹¹ Department of Natural and Environmental Sciences, Mid Sweden University, Sundsvall, Sweden² School of Agricultural Science/TIAR, University of Tasmania, Hobart Australia³ Central Science Laboratory, University of Tasmania, Hobart, Australia

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The autumn gum moth, *Mnesampela privata* (Lepidoptera: Geometridae) is an endemic Australian moth whose larvae feed upon species of *Eucalyptus*. The moths favorite host plants are *E. globulus* and *E. nitens* which are the most important species used in commercial plantations of the Australian pulpwood industry. The autumn gum moth has become one of the most significant outbreak insects of eucalyptus plantations throughout Australia. Today insecticides as pyrethroids are used for control of eucalyptus defoliators as *M. privata*. The purpose of this study is to identify the sex pheromone of the autumn gum moth and develop environmental benign pheromone based methods to monitor and control the moth population. In 2004¹ we identified 3,6,9-(3Z,6Z,9Z)-nonadecatriene as a sex pheromone component. However, in field tests the synthetic prepared C₁₉-triene did not catch as many males as expected. Now we present additional GC-MS analyses of the pheromone gland and we have identified one important missing component. We found that 3,6,9-(3Z,6Z,9Z)-heneicosatriene is present but at levels 40 times less than the C₁₉-triene. The catches of males in field tests were dramatically enhanced by a factor of ten by inclusion of a very small percentage of the C₂₁-triene in the pheromone baited traps.

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Tarsal Gustatory Response to Rotting Food Constituents in two Nymphalid Butterflies, *Vanessa indica* and *Argyreus hyperbius*

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Adult nymphalid butterflies have gustatory sense on their tarsi and respond with proboscis extension upon tarsal contact with foods. Since they use various foods from flower nectar to rotting sap and fruits, their gustatory sense might differ in conformity with the food constituents. Using *Vanessa indica* (nectar and rotting-food feeder) and *Argyreus hyperbius* (nectar feeder), we investigated behavioral and electrophysiological responses to gustatory stimulation on mid-leg tarsi. Behavioral response was estimated based on proboscis extension reflex (PER) and effective concentration at which 50% of test individuals responded (EC_{50}) was calculated for each sample. Electrophysiological response was measured from sensillum trichodeum (ST) on the tarsi by a tip-recording technique. First we examined sugar responsiveness. In PER, both species were most responsive to sucrose followed by fructose and glucose. The EC_{50} of sucrose was 100 mM for *V. indica* and 90 mM for *A. hyperbius*. Sucrose was also the most active in the ST responses and elicited remarkable spike response at the concentration of 31.3 mM in both species. It indicates that the ST is responsible for sugar reception and its sugar sensitivity is similar between the two species. Second we tested responsiveness to ethanol and acetic acid. Neither of them elicited PER and ST responses at the natural concentration^{1,2}, suggesting that these fermentation products never serve as feeding stimulants by themselves. Finally we evaluated the effect of fermentation products on sugar reception. We prepared a series of binary mixtures with a constant concentration (EC_{50}) of sucrose and a variable concentration of either ethanol or acetic acid. As increasing the concentration of ethanol, sucrose (PER and ST) responses of *V. indica* were somewhat enhanced, while those of *A. hyperbius* were gradually declined. At the natural concentration, acetic acid strongly inhibited sucrose responses of *A. hyperbius* but little affected those of *V. indica*. These results demonstrate that *V. indica* is more adaptive to rotting-food feeding than *A. hyperbius* in gustatory sense of tarsus as well as that of proboscis³.

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Experience-Dependent Plasticity in the Olfactory System of a Moth

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Male moths innately have a high sensitivity to female-produced sex pheromones. In the noctuid moth *Spodoptera littoralis*, however, behavioural responses to the pheromone can be further increased by brief pre-exposure to the sex pheromone¹. In parallel, an increase in sensitivity of neurons within the primary olfactory centre, the antennal lobe, was observed 27 h after pre-exposure with pheromone. As a first step to test if the observed effect is a form of general sensitization or rather a phenomenon of selective attention, we tested effects of pre-exposure with plant odours and gustatory stimuli on the behavioural sensitivity to sex pheromones. Brief pre-exposure to the plant-related volatile geraniol in a wind tunnel resulted in a higher response rate in subsequent tests with the sex pheromone compared to naïve male moths. Pre-exposure to another common plant volatile, linalool, however, did not change male moth responses to the sex pheromone significantly. To test gustatory stimuli, we used a pre-exposure procedure with a sucrose stimulus applied to the antenna, eliciting a proboscis extension reflex in the male moths. As a first step, sucrose-exposed moths were subsequently tested with different concentrations of sucrose solution. Sucrose-exposed males showed lower proboscis extension response thresholds to diluted sucrose solutions than naïve moths, indicating an increased sensitivity. In addition, sucrose-exposed males, tested on a locomotion compensator during stimulation with the female-produced sex pheromone, responded to lower doses of the pheromone than naïve moths. Our results indicate that pre-exposure to a variety of relevant stimuli might lead to a general sensitization of different sensory systems rather than eliciting selective attention.

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**Localization of Synergistic Aggregation Pheromone and Host Plant Odour Sources
by a Phytophagous Beetle**

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The red palm weevil, *Rhynchophorus ferrugineus*, is an important pest of date and ornamental palm trees and has become a major problem as an invasive species in the Mediterranean Basin. Due to its endophagous feeding behaviour, efficient treatments against this pest insect are still lacking. Field trapping, using aggregation pheromone and natural host plant extracts have shown a strong synergistic effect between these two types of odour sources. However, neither the nature of this synergistic effect nor the volatile compounds involved are currently known. This type of knowledge would be essential in order to optimize such traps. To better understand the specific functional roles of the aggregation pheromone and host plant stimuli and their interaction in the sensory ecology of the red palm weevil, we compared the behavioural response of males and females to these two types of odour stimuli on a locomotion compensator, which allowed us a detailed analysis of orientation behaviour.

The Flea that Came in from the Cold: Chemical Communication and Structurally Novel Metabolites in the “Snow Flea” *Ceratophysella sigillata*

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Ceratophysella sigillata, colloquially known (amongst similar species) as the “snow flea”, is a member of the order *Collembola* (springtails) whose habitat ranges from the alps to the higher regions of the Czech republic. These small, ground-dwelling insects exhibit three surface activity periods per year, in which they form large colonies that show coordinated migration behavior while feeding on algae on the ground and on tree bark. When disturbed, they secrete an alarm pheromone of unknown composition, which causes other individuals in the proximity to clear the area in a series of leaps using a tail-like appendage, the eponymous springtail (*furca*). In light of these examples of chemical communication, the composition of cuticular extracts of *C. sigillata* has recently been investigated by our work group, revealing the presence of a series of polychlorinated pentaketide bicyclic lactones. The predominant compound is “Sigillin”. A preliminary structure has been proposed, indicating the presence of a trichloromethyl- and a dichloromethylene group. This degree of halogenation is remarkable in non-aquatic species. The compound is likely derived from the polyketide biosynthetic pathway with the incorporation of three acetate and two propionate subunits. As there are no known examples of polyketide synthases in insects, it is probably produced by endosymbionts. The function of it is as yet unknown, although structurally similar lactones have shown antibacterial properties. The total synthesis of Sigillin has been attempted and proves challenging and worthwhile due to the plethora of stereocenters and the high steric demand of the trichloromethyl group.

How Do Tiny Insect Predators Search for Patchily Distributed Spider Mites? A Case Study of a Specialist Insect Predator, *Oligota kashmirica benefica*, in a Japanese Pear Orchard

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Many laboratory studies have shown that predaceous arthropods are attracted to volatiles from plants infested with prey herbivores, but not to those from intact plants. It is therefore considered that predators use volatile infochemicals from herbivores-infested plants (e.g. herbivore-induced volatiles) to search for their prey. However, it remains unanswered how tiny insect predators actually detect such volatile infochemicals from prey-infested plants mixed with many background volatiles in a natural environment. We studied foraging behavior of the specialist insect predator, *Oligota kashmirica benefica* (Coleoptera: Staphylinidae, ca. 1 mm in body length of adults), that prey on patchily distributed spider mites, *Tetranychus kanzawai*, in a Japanese pear orchard. We observed a flight foraging behavior of *O. kashmirica benefica* (31 adult individuals) toward a shoot infested by the spider mites; all of the observed predators finally landed on one of the infested leaves in a shoot. Interestingly, twenty individuals (65 %) showed zigzagging and/or hovering behavior when approaching to a prey-infested leaf on a shoot, whereas they did not show such typical flight behavior when approaching to an intact leaf on the same shoot. We also studied the olfactory response of *O. kashmirica benefica* in an Y-tube olfactometer. Adults of *O. kashmirica benefica* collected from the orchard showed a significant preference to *T. kanzawai*-infested Japanese pear leaves over clean air, while they did not discriminate between intact leaves and clean air. Both laboratory and field studies suggest that *O. kashmirica benefica* discriminated between prey-infested pear leaves and intact leaves in a shoot, at least partly by using volatile information.

(3Z,6E)- α -Farnesene as a Potential Kairomone for the Brown Spruce Longhorned Beetle *Tetropium fuscum* (F.) (Coleoptera:Cerambycidae)Silk PJ¹, Sweeney J¹, Lemay M¹, LeClair G¹, Magee D²¹ Natural Resources Canada, Canadian Forest Service-Atlantic Forestry Centre, PO Box 4000, Fredericton, NB E3B 5P7, Canada² Department of Chemistry, University of New Brunswick, Fredericton, NB E3B 6E2

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A male-produced pheromone identified as (E)-6,10-dimethyl-5,9-undecadien-2-ol (geranyl acetol) which we named "fusculol", was detected in brown spruce longhorned beetle (BSLB) *Tetropium fuscum* (F.) and *Tetropium cinnamopterum* Kirby and tested in field trapping experiments. Racemic fusculol was not significantly attractive by itself, but the combination of racemic fusculol + red spruce monoterpenes attracted significantly more male and female *T. fuscum* and *T. cinnamopterum* than did spruce monoterpenes alone¹. As a secondary alcohol, fusculol is chiral and the (**S**)-enantiomer is produced by male *T. fuscum* and *T. cinnamopterum* and, although unattractive alone, only this enantiomer is attractive in traps synergized by spruce monoterpenes and ethanol; the (**R**)-enantiomer is unattractive alone or in admixture with monoterpenes/ethanol (Silk and Sweeney, unpublished). The red spruce volatiles, all monoterpenes, were identified from our earlier work only by chemical analysis/ SPME and gas chromatography/mass spectrometry (GC/MS) techniques^{2,3} and were found to be important in trapping BSLB when synergized by ethanol and fusculol. In a more recent systematic approach, we report the analysis of red spruce volatiles by GC/EAD (electroantennography detection) analyses and GC/MS with the resultant identification of several EAD-active monoterpenes and a strongly responsive sesquiterpene, (3Z,6E)- α -farnesene. This was confirmed by synthesis and its efficacy assessed by EAD and field trapping in admixture with fusculol.

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Transport of Defensive Precursors within Leaf Beetle Larvae – Putative Transport ProteinsStrauß AS¹, Burse A¹, Heinemann SH², Boland W¹¹ Max-Planck-Institute of Chemical Ecology, Department of Bioorganic Chemistry² Center for Molecular Biomedicine, Friedrich-Schiller-University Jena

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Leaf beetle larvae of the subtribe Chrysomelina release a defensive secretion from dorsal glands to protect themselves after predatory attack. Their strategies to produce these defensive substances vary from *de novo* production to the sequestration of plant derived secondary metabolites as well as the combination of both¹. Feeding experiments with analogues of the natural sequestered defensive precursors indicated a selective transport as glucosides^{2, 3}. Hence, we mainly focus on glucose transporting proteins to identify carrier involved in the defensive system. However, in contrast to mammalian GLUTs (glucosetransporter-passive transport) and SGLTs (sodium dependent glucose transporter-active transport) which mainly transfer glucose but also glucosides, glucose transport in insects is barely understood. In search of putative glucose transporting proteins, the sequencing of cDNA libraries as well as the application of degenerated primers revealed more than ten possible GLUT-like proteins. Four of these putative GLUT sequences from different larvae species and tissues have been heterologously expressed in *Xenopus laevis* oocytes as well as in insect and mammalian cells and localized *in vivo* via GFP-fusion proteins. Although the proteins are expressed no uptake of glucose or the defensive precursors could be detected so far. In further experiments the insect expression system will be optimized to perform functional assays with the natural precursor in this expression system. Furthermore, to confine the search among transporter candidates, the application of the patch clamp technique on these native glandular cells is in progress in order to specify a possible electrogen transport system. The overall goal after finding and functional characterization of defensive transporter candidates are phylogenetic analyses to gain insights into the development of the diversity of defensive compounds and consequently, into the coevolution of leaf beetles and their host plants.

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New Sex Pheromone Compounds Identified from Females of the Plum Moth *Illiberis rotundata* Jordan (Lepidoptera: Zygaenidae: Procridinae)

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The plum moth *Illiberis rotundata* Jordan (Lepidoptera: Zygaenidae: Procridinae) is known as a pest of orchards in Japan and China. To investigate the sex pheromone of this species, extracts of glands from adult female *I. rotundata* were analyzed by coupled gas chromatography-electroantennography (GC-EAG) and coupled gas chromatography-mass spectrometry (GC-MS). Whilst GC-EAG on male moths showed an active peak which was identified as 2-butyl (7Z)-dodecenoate, GC-MS revealed the presence of small amounts of an additional compound, 2-butyl (9Z)-tetradecenoate. Electroantennographic investigations, as well as field tests, strongly suggested that the natural compounds have the (*R*)-configuration at the stereogenic center. The chemical structures of these new pheromone components show the same features as those of other procridinid species: unsaturated fatty acids esterified with a short chain chiral alcohol. Field results demonstrate 0.2 mg of a 1:1 mixture of (2*R*)-butyl (7Z)-dodecenoate and (2*R*)-butyl (9Z)-tetradecenoate to be a powerful lure that may be used in pest control measures against *I. rotundata*. This lure was used in sticky delta traps for seasonal monitoring of the pest in 2009.

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Unique Ocean-Smell Sex Pheromone of the Mushroom Fly: *Neoempheria ferruginea*

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The mating behavior of the mushroom-fly, *Neoempheria ferruginea* was observed. As a result of the wind tunnel experiment, unmated female fly and the hexane extracts of the unmated female fly attract male fly, so the existence of the sex pheromone was confirmed. As a result of the silica-gel column chromatography, the active components eluted into non-polar fraction. The active component smelled oceanic. Result of GC-EAD analysis, 3 active peaks were confirmed. These sex pheromone candidates were analyzed with GC-MS, and with 2D-NMR. As a result, it was clarified that the structure of the sex pheromones of the mushroom fly were three pentaunsaturated straight chain hydrocarbons. FTIR and GCMS suggest on these hydrocarbons that there was no conjugation and all double bonds had the *cis* configuration. The main compound identified as (Z,Z,Z,Z,Z)-3,6,9,12,15-docosapentaen.

Beyond Cuticular Hydrocarbons: Evidence of Polar Compounds Secreted by Neotenic Reproductives in Three Termite Species

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In 1959 Karlson and Lüscher introduced the new term “pheromone”, broadly used nowadays for various forms of intraspecific chemical communication. To define the term they depicted the situation in termite societies, where king and queen inhibit the reproduction of nestmates by an unknown chemical substance. Paradoxically, half a century later, neither the source nor the chemical nature of this putative pheromone is known. In this study, we report for the first time the production of polar compounds, probably of proteinaceous origin, by neotenic reproductives in 3 termites species, *Prorhinotermes simplex*, *Reticulitermes santonensis*, and *Kaloterme flavicollis*. Aqueous washes of functional neotenic reproductives contained sex-specific polar compounds *virtually* absent in non-reproducing stages. Moreover, the presence of these compounds appears to be correlated with the age of reproductives and their reproductive status. We discuss the putative function of these compounds in termite caste recognition and regulation.

Pyrokinin/PBAN Neuropeptides from the Fire Ant, *Solenopsis invicta*

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Insect neurohormones regulate various physiological and behavioral events during development and reproduction. Pyrokinin/PBAN (P_heromone B_iosynthesis A_ctivating N_europeptide) is a major neuropeptide family, characterized by a conserved 5-amino-acid C-terminal sequence, FXPRLamide. The family of peptides has been implicated in regulating various physiological functions including, pheromone biosynthesis, muscle contraction, diapause induction or termination, melanization, and puparium formation in different insect species. In this report we show the presence of pyrokinin/PBAN-like peptides in the central nervous system of the red imported fire ant, *Solenopsis invicta*, using immunocytochemical techniques. Our immunocytochemical results were confirmed by demonstrating that fire ant brain-subesophageal ganglion (Br-SG) extracts injected into decapitated females of *Helicoverpa zea* induced pheromonotropic activity. Significant pheromonotropic activity was found from all adult fire ant forms: queens, female and male alates, and workers. We have also identified four peptides produced by the fire ant pyrokinin/PBAN gene, including a new member of the PBAN family of peptides. These are the first pyrokinin/PBAN neuropeptides identified and characterized from the central nervous system of Formicidae.

Synthesis of a Component of the Male Sexual Pheromone of the African Butterfly, *Bicyclus anynana*

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Much work has been done on female sex pheromones, until now the male sex pheromones (MSP) have received little or no attention. Recently the MSP of the African butterfly, *Bicyclus anynana*, was identified. ¹Three main compounds were found to form the proposed male sex pheromone; (Z)-9-tetradecanol, hexadecenal, and 6,10,14-trimethylpentadecane-2-ol (see figure). The compounds elicited an antennal response in females using GC-EAD, and they were characterized by GC-MS. ¹Since the first two compounds are easily obtained from commercially available starting material, the focus has been on the synthesis of 6,10,14-trimethylpentadecane-2-ol. A method for the synthesis of the racemic 6,10,14-trimethylpentadecane-2-ol has been established. ¹The mixture of stereoisomers of the alcohol were derived and separated with GC-MS. By comparing the extract with synthetic mixtures of 6,10,14-trimethylpentadecane-2-ol, the most abundant stereoisomer was determined.¹

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Dual Pathways Involving $\Delta 6$ - and $\Delta 11$ -Desaturation in the Chinese Silkworm, *Antheraea pernyi* Sex Pheromone BiosynthesisWang HL^{1,2}, Liénard MA¹, Zhao CH², Wang CZ², Löfstedt C¹¹ Department of Ecology, Lund University, SE-223 62 Lund, Sweden² Institute of Zoology, Chinese Academy of Sciences, 100101 Beijing, China

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The sex pheromone of the Chinese silkworm, *Antheraea pernyi* (Lepidoptera: Saturniidae) was previously identified as a mixture of (E,Z)-6,11-hexadecadienal, (E,Z)-6,11-hexadecadienyl acetate and (E,Z)-4,9-tetradecadienyl acetate¹. We found that in addition to common saturated and unsaturated fatty acids, pheromone gland extracts contained two diunsaturated acids, (E,Z)-4,9-tetradecadienoic acid and (E,Z)-6, 11-hexadecadienoic acid, as well as several monounsaturated acids such as (E)-6-hexadecenoic acid, (Z)-6-hexadecenoic acid, (Z)-11-hexadecenoic acid, (Z)-11-octadecenoic acid and (Z)-13-octadecenoic acid. We applied deuterium labelled tetradecanoic, hexadecanoic, octadecanoic and (Z)-11-hexadecenoic acid topically to the sex pheromone gland. Labelled hexadecanoic acid was incorporated into saturated and monounsaturated aldehydes and acetates and into the diunsaturated pheromone components. Labelled (Z)-11-hexadecenoic acid was also incorporated into all three pheromone components as well as two $\Delta 11$ -monounsaturated aldehydes and acetates. Subsequently, three full-length candidate desaturase genes, namely Ape-KPSE, Ape-QPTQ and Ape-KPAE were cloned from the pheromone gland. The precise biochemical activities of both Ape-KPAE and Ape-QPTQ gene products were subsequently assessed in a desaturase and elongase deficient yeast strain. GC-MS analyses of yeast transformed with Ape-QPTQ-ORF demonstrated that this enzyme produces high amounts of (Z)-11-hexadecenoic acid and other $\Delta 11$ monounsaturated fatty acids. After supplementation with (E)-6-hexadecenoic acid, the same transformants produced high amounts of (E,Z)-6,11-hexadecadienoic acid. Yeast transformed with Ape-KPAE-ORF produced (Z)- and (E)-6-hexadecenoic acid. In addition, when supplemented with an excess of (Z)-11-hexadecenoic, the yeast also produced the (E,Z)-6, 11-hexadecadienoic acid. These findings imply that in *A. pernyi* two distinct $\Delta 11$ - and $\Delta 6$ -desaturases produce (Z)-11-hexadecenoic acid and (E)-6-hexadecenoic acid. Both monounsaturated precursors are subsequently used as substrate by the $\Delta 6$ - or $\Delta 11$ -desaturase to produce the same (E,Z)-6,11-hexadecadienoic acid, which is chain shortened to (E,Z)-4,9-tetradecadienoic acid. The acyl products are then presumably transformed to the corresponding aldehydes or acetates by a terminal alcohol oxidase or acetyltransferase, respectively.

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Effects of Sex, Age, and Mating Status on the Cuticular Hydrocarbon Components of the Asian Longhorned Beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae), and Evaluation of the Male Mating Responses to Unique Chemical Profiles

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Asian longhorned beetle, *Anoplophora glabripennis*, has been introduced from China to North America and Europe and is considered to be a serious threat to forests. A recent discovery of a large infestation in Worcester, MA (USA) in 2008 underscores the need for research into the chemical ecology of mate location and the development of semiochemical-based management tools. Cuticular hydrocarbon profiles of seven groups of beetles (separated by sex, age, and mating status) were compared using discriminant analysis. According to DA, six compounds, including the known contact sex pheromone component Z-7-heptacosene¹, discriminated female mating status. To compare groups of beetles with unique chemical profiles, bioassays compared male mating behavior response to (1) freshly killed beetles, (2) extracts applied to ceramic dummies, and (3) extracts applied to glass rods. Males clearly demonstrated a mating preference for 11-day old virgin female beetles, as percentages of males responding to freeze-killed beetles, extracts on dummies, and extracts on glass rods were highest, and time to copulation was fastest. Males had a stronger mating response to the extracts on dummies compared to extracts on glass rods in 6 of 7 beetle groups, suggesting visual cues do play a role. Newly emerged males and females also had high percentages of males responding to freeze-killed beetles, as profiles appear to be indistinguishable in the DA. Mated females had significantly different chemical profiles, as the six compounds discriminating mating status were greatly reduced, or not detectable in the profiles. Male mating responses were less intense to mated females, and the chemical compounds communicating this information may be encoded in the cuticular hydrocarbon profiles.

Acknowledgements

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Nestmate Recognition Cues in the African Termite Raiding Ant *Pachycondyla analis*

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Cuticular hydrocarbons (CHCs) are used as a means of chemical communication among nestmates in many ant species^{1,2} and they may play a role in the discrimination of nestmates and non-nestmates^{3,4}. Using a mandible opening response (MOR) bioassay, we tested the response of the African termite raiding ant *Pachycondyla analis* to CHC extracts of nestmates and non-nestmates. The ants were able to distinguish between chemical cues from control, nestmate and non-nestmate, and based on a CHC recognition threshold, aggression was demonstrated between the different ant colonies. Gas chromatography (GC) and GC-mass spectrometric analyses showed that the CHC components of the different ant colonies had chain lengths ranging from C6-C30, comprising mainly *n*-alkanes, alkenes and methyl branched alkanes, with the *n*-alkanes occurring in the same proportions among all colonies. The ants were successfully grouped according to their colonies of origin using discriminant analysis. We conclude that nestmate recognition cues in *P. analis* could be encoded in the alkenes and methyl-branched alkanes.

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Entrapping Pheromones in an Environmentally-Friendly Sol-Gel MatrixZada A¹, Falach L¹, Byers AJ²

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Development of insect pest monitoring, killing, and mating disruption systems based on semiochemicals such as pheromones are dependent on controlled release devices or dispensers. The ideal formulation should release the pheromone at a constant rate throughout the flight and mating cycle of the pest. The formulation must also protect the pheromone components from degradation by UV light and oxidation. This can be achieved by proper entrapment of the active ingredients and by the addition of antioxidants and UV blockers. The purpose of the present study was to explore the potential use of sol-gels as pheromone slow-release devices. Sol-gels are inorganic polymers of metal alkoxides, usually silicates, that exhibit glass properties and are inert materials. Due to their high porosity, the ability to vary their density in a controlled manner and their environmentally friendly decomposition products ($\text{SiO}_2 + \text{H}_2\text{O}$), sol-gel matrices are good candidates for pheromone release devices. We developed a method for the entrapment of volatile pheromones in sol-gel matrices. The pheromones that were entrapped in the gels were released at almost a linear rate. Preliminary field tests of sol-gel matrices, doped with the codling moth pheromone, showed that this new formulation can capture males in the field. Further development of sol-gel formulations for monitoring lures and mating disruption dispensers should allow optimization of release rates for efficient management of insect pests with particular pheromones in the field.

Identification and Dynamic of Production of Male-Specific Compounds Released by *Cratosomus flavofasciatus* (Coleoptera: Curculionidae)

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Cratosomus flavofasciatus (Coleoptera: Curculionidae), is a pest of economic importance for the Citrus producing areas of Brazil. The females lay their eggs inside the trunk and branches of the trees, where the larvae cause the damage, contributing to reduction in production. The control of *C. flavofasciatus* is difficult because their eggs and larvae are protected inside the host plant. Therefore, complementary strategies of pest management remain to be evaluated, including the use of semiochemicals. The objective of this study was to evaluate the volatiles released by the adults of *C. flavofasciatus* in laboratory. Volatiles from both sexes were collected by aeration at intervals of 24 h and analyzed by GC-MS. To evaluate the dynamics of volatile production, samples were collected during the photophase and scotophase. GC-MS analysis of airborne volatiles released by males and females showed the presence of three male-specific compounds, suggesting that they are responsible for the chemical communication in *C. flavofasciatus*. These compounds were identified as (1*R*, 2*S*) - Grandisol (minor), (*E*)-2-(3,3-dimethylcyclohexilideno) ethanol (intermediate) and (*Z*)-2-(3,3-dimethylcyclohexilideno) ethanol (major). The volatile release was dependent on the photoperiod, occurring mainly during the photophase, with a peak in the last hours, indicating that the activity of this insect is during the photophase. Studies are being carried out in the field to assess the biological activity of these compounds in the attractiveness of co-specific *C. flavofasciatus* and verify that these compounds act as aggregation pheromone for this species.



Poster Session 2

Tuesday, 25 August

Repellence Response of *Epilachna indica* (Coleoptera: Subfamily Epilachninae) Beetle towards Leaf Extract of *Elephantopus scaber* Linnaeus (Family: Compositae) and Identification of Repellent Chemical Compound

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The ladybird beetle pest of egg plant *Solanum melongena* was tested against methanol extracts of two medicinal plants from Malaysia *Elephantopus scaber* Linnaeus (Family: Compositae) commonly known as Elephant foot and the rose catus *Pereskia bleo* (Family: Cactaceae). Behavioral bioassay in T-tube olfactometer showed that 75% of tested beetles was repelled from *E. scaber* ($P < 0.05$). However *E. indica* was not repelled from *P. Bleo*. The repellent chemical compound from the positive fractions of *E. scaber* was identified as 2,4 bis 1,1- dimethylethyl ($C_{14}H_{22}O$).

Odor Guided Behavioral Responses of Egyptian Cotton Leaf Worm, *Spodoptera littoralis* to Plant and Floral Volatiles

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Many herbivorous insects, such as moths are known to use blends of volatile compounds emitted by green plants or flowers to locate suitable places to mate, oviposit or forage. Our main goal is to understand the odor mediation and physiological modulation of olfactory guided host-finding and foraging behavior of the polyphagous Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Using a flight tunnel bioassay, we investigated the effect of sex and mating status on the orientation behavior of *S. littoralis* towards two species of larval host-plants, cotton (*Gossypium hirsutum*) and clover (*Trifolium alexandrinum*), or flowers of lilac (*Syringa vulgaris*). Our preliminary findings showed an effect of sex and mating on the orientation behavior of *S. littoralis* towards the host-plants, however no sexual difference was found in the attraction to floral volatiles, as both males and females showed similar flight responses towards lilac flowers. Male attraction to flowers attained 46%, while 38% of males were attracted to the larval food plant cotton. In comparison, 82% of the males flew towards calling females. Up to 74% of the females were attracted to flowers for feeding, and 42% were attracted to cotton for oviposition. Both males and females showed odour-mediated anemotactic flights to all odour sources. Current work aims at the chemical identification of the attractant cues and the effect of feeding, mating and oviposition on attraction to flowers, food plants and mates. Identification of plant semiochemicals mediating host recognition and foraging behavior will help us to get better understanding of the sensory physiology and behavioral modulation in *S. littoralis*.

**Chemical Ecology of Pungency: Detoxification of Capsaicin in a Host Specialist,
*Helicoverpa assulta***

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Capsaicin (8-methyl-N-vanillyl-6-nonenamide), an alkaloid found only in *Capsicum* spp. (Solanaceae), is responsible for the pungency of hot pepper fruits. The Oriental tobacco budworm, *Helicoverpa assulta* (Lepidoptera: Noctuidae), feeds on a few solanaceous plants, including *Capsicum* spp. Larvae consume the tissue inside of the fruit, where capsaicin concentrations are highest. The objectives of this research were to study the effect of capsaicin on the development of a specialist *H. assulta* as well as other Heliothine moths, and also to investigate the mechanism of capsaicin detoxification in the specialist. Feeding experiments with capsaicin-spiked artificial diet showed that capsaicin was toxic to all herbivores tested (*H. armigera*, *H. zea*, *H. virescens*, and *H. subflexa*) except *H. assulta*. Furthermore, when capsaicin was injected into the hemocoel of fifth-instar larvae, pupal weights decreased in *H. armigera* and *H. zea*, but not in *H. assulta*, suggesting that capsaicin plays a role in the adaptation of the specialist species to its host plant. When it was fed, only a small amount of capsaicin was excreted in feces of *H. assulta* (ca. 4%) compared to *H. armigera* (ca. 20%), suggesting that the detoxification efficiency in the specialist is higher than that in the generalist. A capsaicin glucoside was identified in the feces of *H. assulta* as well as those of *H. armigera* and *H. zea*. Conjugation of toxic compounds may increase water solubility, enhancing its excretion. We are now trying to verify the detoxification mechanisms in detail by characterizing the enzymes involved in this process.

Induced Volatile Emissions from Citrus Roots Infested by the Root Weevil, *Diaprepes abbreviatus*, Recruit Entomopathogenic NematodesAli JG¹, Alborn HT², Stelinski LL²¹ University of Florida, Entomology and Nematology Department, Gainesville, FL 32611 USA² Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL 32608 USA

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It is well-documented that herbivore induced volatile emissions benefit plant hosts by recruiting natural enemies of herbivorous insects and our understanding of the dynamics of such above ground tritrophic interactions continue to expand. Recently, there has been a renewed interest in the fact that similar signals also occur belowground and that this might be an equally important aspect of the indirect defenses of plants. The larvae of the root weevil (*Diaprepes abbreviatus*) are a serious pest on citrus roots in Florida. Infestations can be controlled by the use of entomopathogenic nematodes, yet the interactions between the plant, insect and nematode are poorly understood and remain unpredictable. In bioassays using a belowground six-arm olfactometer, citrus roots ('Swingle citrumelo' rootstock) recruited significantly more entomopathogenic nematodes (*Steinernema diaprepesi*) when infested with root weevil larvae than non-infested roots, mechanically damaged roots, or larvae alone. By dynamic collection and GC/MS analysis of volatiles from the soil we determined that infested plants produced novel volatile terpenoids not found in samples from non-infested roots or in soil containing only larvae. These findings suggest Swingle citrus roots release induced volatiles as an indirect defense in response to herbivore feeding and some of these induced volatiles most likely function as attractants for entomopathogenic nematodes. Future work will focus on evaluating the use of these nematode recruitment chemicals to enhance control of *D. abbreviatus* in the field. This study may also be the first to utilize direct sampling of volatile induced compounds from an intact host-herbivore subterranean interaction.

Pre-Imaginal Experience to Host Plant Affects Male Attraction to Combined Sex Pheromone and Host Plant Odours

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There are many examples showing that pre-imaginal experience can affect host plant choice in the adult females. However, it is not known if experiences during the larval period can affect male response to plant odours. We have found that males of the moth *Spodoptera littoralis* alter their response to combinations of female sex pheromone and plant odours depending on what host plant they were reared on as larvae. In two-choice windtunnel tests males were more attracted to combinations with the host plant they had experienced as larvae.

Polymorphism of Scots Pine (*Pinus sylvestris*) and Norway Spruce (*Picea abies*) from Lithuania with regard to Monoterpene CompositionApšegaitė V¹, Karalius V¹, Danusevičius D², Būda V^{1,3}¹ Institute of Ecology of Vilnius University, Akademijos 2, LT-08412, Vilnius, Lithuania² Lithuanian Forest Research Institute, Liepų st. 1, Girionys, LT-53101 Kaunas distr., Lithuania³ Vilnius University, Faculty of Natural Sciences, M. K. Čiurlionio 21/27, LT-03101, Vilnius, Lithuania

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A study on the composition of monoterpenes in needles of 7–10-year old trees of Scots pine (*Pinus sylvestris*) and in bark of 31-years old trees of Norway spruce (*Picea abies*) from the Lithuanian population was carried out. To minimize environmental effect Norway spruce trees from experimental plantings collected in different localities of the country and planted in Vaišvydava forest, Kaunas district, were used. The composition of 7 monoterpenes (α -pinene, camphene, β -pinene, myrcene, 3-carene, limonene and phellandrene) was analysed using GC. Both in Scots pine and Norway spruce the most variable among monoterpenes were α -pinene and 3-carene. In Scots pine predominant monoterpene was α -pinene. His relative amount (of all the monoterpenes analysed) varied from 27 to 79%. Relative contents of α -pinene, 3-carene and β -pinene were found to be the most variable, and those of myrcene and camphene the least variable. The relative content of limonene varied moderately. In Norway spruce predominant monoterpenes were: α - and β -pinenes. Their relative amount varied from 22 to 37% and from 18 to 36%, respectively. Large variations were found in the relative amounts of monoterpenes, both within and among the subpopulations of Norway spruce. There was established single subpopulation of Norway spruce containing high amount of 3-carene (that originating from Dubrava).

Anti-Termite Activity of Sesquiterpene Derivatives Obtained by Autoxidation or Direct-EpisulfidationAshitani T^{1,2}, Kusumoto N², Takahashi K^{1,2}¹ Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka, Yamagata 997-8555, JAPAN² The United Graduate School of Agricultural Science, Iwate University, 18-8 Ueda 3-chome, Morioka Iwate 020-8550, Japan

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Caryophyllene is known as components of sesquiterpene part in leaf and/or heart wood extracts (eg. pine leaf, hop leaf and rauan wood oils etc). The caryophyllene is oxidized easily to give caryophyllene-6,7-epoxide which is known as a defensive compound of several plants. Recently, we have reported^{1,2} that caryophyllene-6,7-episulfide was obtained easily through a reaction between caryophyllene and elemental sulfur. However, bio-activities of the caryophyllene-6,7-episulfide have not been reported. By the way, Longifolene is also a main component in sesquiterpene parts of pine (eg. *Pinus thunbergii*) wood oil. The longifolene is easily oxidized by oxygen in the air at the room temperature to give longicamphenylone as main products. But, at the present time, investigations about bio-activities of longicamphenylone are insufficient. It is important for development of reagents against harmful insects from natural resources to investigate about bio-activities for above derivatives of the caryophyllene and longifolene, because the terpenes are occurred abundantly and the derivatives are easily obtained. In this study, bio-activities of the derivatives of caryophyllene and longifolene were investigated against subterranean termite (*Reticulitermes speratus* Kolbe) which is known as a popular harmful insect in the Japanese wood industry. Caryophyllene-6,7-epoxide and caryophyllene-6,7-episulfide were synthesized from caryophyllene by autoxidation or episulfidation. The longicamphenylone and other oxidized compounds of the longifolene were isolated from the longifolene samples stocked at the room temperature for 10 years. These compounds were impregnated into each paper disk and tested the anti-termite activities (anti-feedant and termiticidal activities). Original terpenes, the caryophyllene and longifolene, did not show any anti-termite activities. However, the anti-termite activities are observed in the some derivatives of these terpenes. Especially, caryophyllene-6,7-episulfide showed potent termiticidal activities. Thus, it is found that the caryophyllene and longifolene are changed easily by autoxidation and direct-episulfidation to bio-active compound which is useful industrially.

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The Role of Maize Volatile Sesquiterpenes in Direct Defense against HerbivoresAssefa C¹, Köllner T^{1,2}, Gershenzon J¹, Degenhardt J^{1,2}¹ Max-Planck-Institute for Chemical Ecology Jena, Germany² Martin Luther University-Institute of Pharmacy Halle/Saale, Germany

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Maize produces complex blends of sesquiterpene hydrocarbons that occur in specific plant organs and are often induced by herbivore attack. The enzyme classes responsible for the biosynthesis of these blends are the terpene synthases. Recent studies demonstrated that the products of some of these enzymes can be recruited as cues for the orchestration of indirect defenses against herbivore attack above ground^{3, 4} and below ground^{2, 4} by attracting parasitoid enemies and entomopathogenic nematodes respectively. The husk of a maize plant releases a mixture of volatiles consisting of predominantly bisabolene-, sesquithujene-, and bergamotene-type sesquiterpenes. The enzymes responsible for the production of these blends are isolated from two maize varieties B73 and Delprim and designated as TPS4 (B73) and TPS5 (Delprim)¹. Both enzymes produced the same volatile constituents but in different proportions. The current work attempts to elucidate whether these sesquiterpene blends could function as direct defense compounds against herbivore attack. To test this hypothesis, the two enzymes were overexpressed in *Arabidopsis thaliana*, a plant that does not produce much sesquiterpenes by itself at rosette stage. The performance of the larvae of the generalist herbivore, *Spodoptera littoralis*, was assessed using a series of choice and no-choice feeding bioassays conducted on wild-type and terpene expressing transgenic *Arabidopsis* plants. In the choice bioassays larvae showed feeding preference towards the wild-type Col-0 plants compared to terpene expressing genotypes. Similarly, in the no-choice feeding experiments, larvae fed on terpene expressing genotypes showed reduced mass gain, lower survivor rate, and longer development time. From these data we deduce that direct defense with TPS4 and TPS5 sesquiterpenes is possible. However, since the wild-type and terpene expressing mutants differed in the levels of JA and SA after wounding, this enhanced level of direct defense could be a concerted action with phytohormones.

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Effect of Qualitative and Quantitative Changes in Glucosinolates in Host Preference by a Crucifer Specialist Insect**Badenes-Perez FR¹, Reichelt M², Gershenzon J², Heckel D¹**¹ Department of Entomology, Max Planck Institute for Chemical Ecology 07745, Jena, Germany² Department of Biochemistry, Max Planck Institute for Chemical Ecology 07745, Jena, Germany

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Plutella xylostella (L.) (Lepidoptera: Plutellidae) is a specialist of glucosinolate-containing cruciferous plants. The ovipositional preference of *P. xylostella* was studied in plants of the family Brassicaceae and other families containing a wide variety of glucosinolates, in *Arabidopsis thaliana* mutants containing different glucosinolate or myrosinase levels, and in artificial substrates where individual glucosinolates had been added. Identity and quantification of glucosinolates was determined through LC-MS, NMR, and HPLC-UV. Oviposition was studied in non-choice and two-choice tests. Qualitative and quantitative differences in glucosinolates can play a key role in oviposition preference by *P. xylostella*, but other factors are also important.

2,3-Butane Diol, a New Attractant for the Sorghum Chafer, *Pachnoda interrupta* (Coleoptera: Scarabaeidae)

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The sorghum chafer, *Pachnoda interrupta* (Coleoptera: Scarabaeidae), is a key pest on sorghum (*Sorghum bicolor*) in Ethiopia. In search of attractants for this pest, we employed gas chromatograph-coupled electroantennographic detection (GC-EAD) and single sensillum recordings (SSR). GC-EAD on volatiles from *Sorghum bicolor* and another host plant, *Abutilon figarianum*, revealed several active compounds: (Z)-3-hexenol, methyl anthranilate, methyl salicylate, 1-octanol, 1-octen-3-ol, tetradecane, and tridecane. Field tests of these compounds, singly and in host-mimicking blends, indicated that the presence of key compounds was more important than the exact blend. The strong influence of single compounds led us to search for further attractants using SSR. Olfactory receptor neurons (ORNs) in *P. interrupta* were screened with 82 host-related compounds. We found 26 ORN classes, that in total responded to over 50 different compounds. Since it was not feasible to test all active compounds in the field, we only included compounds that were the best ligands for ORN classes, and avoided ligands that activated ORN classes that responded to previously field-tested compounds. Field tests with this selection of compounds established 2,3-butane diol as a powerful attractant for *P. interrupta*, outperforming the best previously known attractant, eugenol, by a factor of three.

Which Pattern of Oviposition-Induced Pine Odor is Relevant for Successful Host Search by an Egg Parasitoid? Electrophysiological and Behavioral Study of *Chrysonotomyia ruforum*, a Parasitoid of Pine Sawfly Eggs

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Parasitoids searching for eggs of herbivorous insects encounter odor from both egg-free and egg-laden plants; these odors may differ by the ratios of their components. Do parasitoids distinguish between such patterns by orientating towards a specific quantity of a key compound or are they able to respond to specific ratios of volatiles released by egg-laden plants? We studied these questions in the hymenopteran egg parasitoid *Chrysonotomyia ruforum* which is attacking eggs laid by the herbivorous sawfly *Diprion pini* on needles of Scots pine (*Pinus sylvestris*). Egg deposition by this sawfly is known to induce release of pine volatiles that attract the egg parasitoid. The attractive oviposition-induced pine odor differs from non-attractive pine odor especially by enhanced quantities of (*E*)- β -farnesene^{1, 2}. Olfactometer bioassays revealed that this sesquiterpene is attractive to *C. ruforum* only when offered at the background of other pine terpenes. These results suggest that the egg parasitoid is attracted when a specific ratio of (*E*)- β -farnesene and other pine volatiles is released. However, which of the numerous "other pine volatiles" are relevant for attraction of *C. ruforum*? To approach this question we recorded electroantennogram (EAG) responses by female *C. ruforum* to pine terpenes. In total, 7 of the 12 compounds tested elicited a significant EAG response, including (*E*)- β -farnesene. Those 7 terpenes were mixed in ratios corresponding to (a) attractive oviposition-induced and (b) non-attractive egg-free pine. Olfactometer bioassays showed that the parasitoid was significantly attracted only to the mixture containing terpenes in ratios as released by oviposition-induced pine. Our results show that both the presence of a key component ((*E*)- β -farnesene) and the ratio of this component to a few, *per se* non-attractive other key components are crucial for attraction of an egg parasitoid.

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Egg Deposition on a Plant Contributes to Plant Defense against Feeding Herbivores

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Feeding by herbivorous arthropods is well-known to induce plant reactions that are detrimental to the feeding stages. Furthermore, egg deposition by herbivorous insects on their host plants may elicit plant defensive responses that reduce chances of egg survival. But do the early plant responses to egg deposition also affect later responses to the feeding stages? To approach this question we tested the hypothesis that performance of herbivores starting food uptake at their hatching sites (i.e. at sites that had been laden with eggs) is lower than performance of those starting food uptake on egg-free sites. Scots pine, *Pinus sylvestris*, is known to defend against egg deposition by the sawfly *Diprion pini* by changing its quantitative pattern of terpenoid volatile emission three days after egg deposition; the altered pine odor is attractive to egg parasitoids that kill the eggs^{1,2}. In this study, we compared performance of *D. pini* raised on Scots pine that never carried eggs with performance of *D. pini* raised on pine with eggs (i.e. the sites where larvae hatched). Feeding on egg-free pine led to heavier larvae already two days after larval feeding had started, to heavier cocoons and adults, to reduced larval mortality, and to higher fecundity. Our results suggest that plants “warned” by insect egg deposition can reduce performance of herbivores. The mechanisms causing reduced performance of herbivores feeding on oviposition-induced pine are discussed with respect to priming of anti-herbivore defense.

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Plume Interaction and Odour Source Spacing of Pheromone and Non-Host Volatiles: Behavioural Effects on Bark Beetles and Moths

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The study of interactions of different odour plumes to affect the insect behaviour when the release points of different components are physically separated was done to understand the mechanism that insects use to find their host or any attractive source or avoid non-host source. We studied the behavioural response of the moth *Spodoptera littoralis* males, in a walking bioassay, to two components of the female sex pheromone blend, released from two separate dispensers at various distances (0-5 cm) from each other. The main pheromone component was tested at 1 and 10 ng in combination with the minor component 1 % of the main component. At the low dose there was higher response at 0 and 3 cm separation than other separations, whereas at high doses there was not much difference in response at 0, 2, and 3 cm separations. A Photo Ionization Detector (PID) was used to investigate plume width and overlap. The PID showed that 5 cm separation of odour sources was enough to totally separate the odour plumes from each other, whereas at 3 cm separation, plumes partially overlapped. By comparing the behavioural results with PID data it is concluded that insects are much more sensitive than the PID, since they responded at distances where the PID showed zero ppb. A field study for *S. litura* was done by spacing sex pheromone components horizontally at 0, 3, 5 and 15 cm on plastic moth pheromone traps. Males were highly attracted at 0 cm separation but spacing decreased the catch and at 15 cm spacing there was no catch. The behavioural effect of separating ph. components was further studied on the spruce bark beetle, *Ips typographus* L., in the field. The attraction of *I. typographus* to traps baited with the two aggregation pheromone components separated both vertically (0-112 cm) on extended Lindgren (19 funnel) traps and horizontally (0-80 cm) on modified windvane traps. *I. typographus* was strongly attracted when the two components were released from the same point but spacing between components decreased the trap catch. However, at 16 cm distance, in both the vertical and horizontal test, the trap catch was not much different from the positive control (0 cm separation). The effect of odour source spacing in the field on *I. typographus* was also studied with regards to repellent non-host volatiles (NHV). In this test, the aggregation pheromone were separated from a blend of repellents using the same experimental design. NHV showed a strong inhibitory effect up to 48 cm spacing, while an earlier study shown an active inhibitory range (AIR) of >1 meter. The both insect species showed a similar mechanism of odour plume tracking but moth respond at smaller scale as compared to beetle. This is, may be beetles (Scolytidae) can generate a large plume with an effective source, the size of an entire tree trunk with hundreds of odour-emitting beetles but moths may release pheromone as both a vapor and an aerosol. Beetle also showed similar range of action with respect to pheromone as well as anti-attractants effect. This study is a new interesting insight to determine the active inhibitory range of non-host volatiles to determine the scale of landscaping of non-host trees to support biodiversity hypothesis or stability hypothesis.

Understanding Sugar Sensing in Induced Plant Defences and Stress Tolerance

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Within the broader context of developing strategies of sustainable crop protection and as part of Integrated Pest and Disease Management (IPDM) framework, we are investigating how inducible plant defences to pests and diseases can be triggered by plants' ability to sense external sugars. It is already known that sugars have pleiotrophic effects on plant metabolism, growth and development and stress responses. However, the underlying regulatory networks involving multiple signalling pathways are still not properly understood. We are particularly interested in using sugar sensing to protect plants from attack by nematodes, insects and fungi, via induced multiple defences. Previous research¹ demonstrated that a naturally occurring sugar analogue, DMDP, induced systemic resistance to root feeding nematodes, implying systemic signalling pathways. We also demonstrated that externally applied sugars (via foliar sprays and soil drenches) at ultra low doses (1-10 ppm) stimulate plant growth, thus increasing the plant's potential to tolerate biotic and abiotic stresses². New multi-disciplinary collaborations are in progress to investigate the underlying mechanisms involved. This research involves efficacy trials (glasshouse and field) together with more fundamental studies on plant apoplast metabolomics, plant hormone regulation and gene expression studies. We aim to develop a more holistic understanding³ of how sugar sensing and associated signalling pathways for plant defence work for different plant genotypes growing in variable environments (G x E interactions), involving 'damaged-self recognition' as part of evolved plant defences. The ultimate aim is to develop new, more sustainable IPDM strategies utilizing both constitutive and sugar-responsive inducible resistance to pests and diseases.

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Attraction of the Stink Bug Egg Parasitoid, *Telenomus podisi*, to Defence Signals from Soybean Activated by Treatment with *cis*-Jasmone

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It has been well demonstrated that *cis*-jasmone, a volatile plant secondary metabolite, is a potent activator of plant defence^{1,2,3}. Soybean, *Glycine max*, when damaged by stink bug (Hemiptera: Pentatomidae) herbivory, has been shown to emit a blend of VOCs that attracts the stink bug egg parasitoid, *Telenomus podisi* (Hymenoptera: Scelionidae), to the plant⁴. In this study, our aim was to investigate changes in the VOC profile of soybean (L.) (var. BR16) elicited by *cis*-jasmone, and to determine the impact of these changes upon the attraction of *T. podisi* in the laboratory and in the field. *cis*-Jasmone elicited chemical defence in soybean similar to that previously reported for stink bug damage. The main components induced were camphene, myrcene, (*E*)-ocimene, methyl salicylate, and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). In Y-tube behavioural bioassays, *T. podisi* preferred *cis*-jasmone treated plants over untreated plants. Field experiments were carried out in 2 m² soybean plots, spraying *cis*-jasmone as an aqueous emulsion in the initial reproductive stage (R1) of the crop. Scelionid parasitoid populations were monitored weekly with yellow sticky traps, with the results showing that for soybean plots treated with *cis*-jasmone, the total numbers of *Telenomus* spp., along with *Trissolcus* spp. captured in traps were significantly higher than in control plots. Thus, *cis*-jasmone appears to induce defence pathways in soybean and is a promising tool for the manipulation of beneficial natural enemies in future sustainable stink bug control strategies.

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Norway Spruce (*Picea abies*) and Bark Beetle (*Ips typographus*): Search for Attractiveness Antagonist(s) of Plant Origin

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One of the possible ways to decrease the risk of plant damage by pest is selection of more resistant and less attractive genotypes for planting. The aim of our study was to determine if the Norway spruce trees differed in attractiveness for bark beetle *Ips typographus* males. Both electrophysiological and behavioral assays were conducted. Bark samples from thirty-one years old trees were extracted and analysed. The trees originated from seven different locations around the country and have been planted as collection in Vaišvydava forest, Kaunas district. The trees from two localities elicited significantly lower EAG responses compare to trees from other 5 localities. In Y-tube olfactometer the most attractive extracts were those which elicited low EAG responses. Coupled gas chromatographic-electroantennographic analysis (GC-EAD, Elite Wax column) of the Norway spruce barks revealed six compounds that consistently elicited *I. typographus* EAG responses. Analysis of the relationship between attractiveness of bark extract and relative amounts of the EAG active compounds revealed that trees containing higher amount of one compound (RI 1538) were significantly less attractive to *I. typographus* ($R = -0.86$; $p = 0.01$). MS identification of the compound is underway.

Insect Oviposition on *Arabidopsis thaliana* Results in Changes of Leaf Surface Chemistry which Affect Host Search by Egg Parasitoids

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Insect oviposition is well-known to induce plant defence. Our former studies showed that oviposition by *Pieris brassicae* on Brussels sprouts induces leaf surface changes that arrest the egg parasitoid *Trichogramma brassicae*¹. The elicitor of this plant response is benzyl cyanide, an anti-aphrodisiac transferred from *P. brassicae* males to females during mating; mated females release benzyl cyanide together with a secretion from accessory reproductive glands (ARG) for attachment of eggs on leaves². Here, we investigated whether oviposition by *P. brassicae* on *Arabidopsis thaliana* affects host search by egg parasitoids in the same way as oviposition on Brussels sprouts does. In a further step, we studied leaf surface chemistry of *A. thaliana* after oviposition, since so far the chemistry of oviposition-induced leaf surface has neither been analysed in Brussels sprouts nor in *Arabidopsis*. Our results show that the effects of *P. brassicae* oviposition on *A. thaliana* are very similar to those on Brussels sprouts: (a) oviposition on *A. thaliana* leaves had local and systemic effects that led to arrestment of parasitoids; (b) ARG secretion of mated *P. brassicae* females applied onto leaves induced the same local and systemic effects as oviposition, whereas ARG secretion of virgin females was inactive; (c) benzyl cyanide was shown to act as elicitor of *Arabidopsis* defensive response to eggs. GC-MS analyses of dichloromethane extracts from (egg-free leaf area taken from) egg-laden leaves and from egg-free leaves revealed no qualitative differences in surface chemistry. Quantitative differences were especially due to higher amounts of fatty acids on the surface of egg-free leaves. A canonical discriminant analysis significantly separated extracts from egg-laden leaves and egg-free leaves. These data suggest that the egg parasitoid *T. brassicae* is able to respond to fine-tuned quantitative changes of *Arabidopsis* leaf surface chemistry observed after host oviposition.

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Temporal Dynamics of Secondary Metabolites in *Populus nigra* L. after Herbivore AttackBoeckler A¹, Köllner TG^{1,2}, Gershenzon J¹, Unsicker SB¹¹ Max Planck institute for Chemical Ecology, Department of Biochemistry, Hans-Knöll-Str. 8, D-07745 Jena, Germany² Institute for Pharmacy, University of Halle, Germany

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Like many other plant species, *Populus nigra* L. (Salicaceae) trees respond to herbivore feeding by emitting volatile organic compounds and accumulating non-volatile secondary metabolites in their tissues. This process is referred to as induction. The volatiles emitted by *P. nigra* contain green leaf volatiles, terpenes, and monocyclic aromates. As we know from the literature, these volatiles have multiple functions e.g. in direct and indirect defense as well as for inter- and intra-plant signaling. The most abundant non-volatile secondary metabolites, condensed tannins and phenolic glycosides, primarily act as a direct defense e.g. against insect herbivores. The aim of this study was to investigate the temporal dynamics of volatile organic compound emission as well as condensed tannin, and phenolic glycoside contents in *P. nigra* after 48h of *Lymantria dispar* (Lymantriidae) caterpillar feeding. In a lab experiment, volatile emission in control and caterpillar infested *P. nigra* trees was monitored at 6 h time intervals for 4 days. In a separate experiment, the effects of herbivore feeding on condensed tannins and phenolic glycosides were measured 4, 7, and 10 days after caterpillar feeding. The volatile emission of *P. nigra* showed characteristic patterns with respect to the substance class (green leaf volatiles, monoterpenes, sesquiterpenes), light period and herbivory. Condensed tannins were significantly induced within 10 days after caterpillar feeding. During this time frame, the concentration of the three identified phenolic glycosides showed no consistent pattern of induction. Based on our results, we argue that the herbivore induced emission of volatiles in *P. nigra* is likely to cause ecological short term effects, whereas the delayed induction of condensed tannins may have long term consequences for tree-insect interactions.

Leaf-Mining Habit and Defense by Volatiles is Not Contradictory in Pseudodineurini Sawfly larvae

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Living organisms exhibit morphological, behavioral, and life history characters, tightly linked through evolution. Because insects are subject to potent and diverse selective pressures by natural enemies, their defense strategies manifest the results of these subtle interplays. It is often difficult to recognize causality in these relationships, and one adaptation may in fact seem to be in conflict with another. As one example of this, we studied here the larvae of the sawfly tribe Pseudodineurini (Hymenoptera, Tenthredinidae, Nematinae). These larvae emit, when disturbed, a clearly perceptible lemon-like odor produced by defensive ventral glands, which are a characteristic of the subfamily. Typical for the tribe is their leaf-mining lifestyle, which is assumed to protect them from some enemies, too. The glandular secretion of all analyzed species contained a well-known insect repellent, citral, which was effective against predatory ants, and which may be used when the larvae leave their mine. A potent fungicide, 8-oxocitral, was also detected, but in only some Pseudodineurini species. The discrete distribution of 8-oxocitral could not be explained by the phylogeny of the group, which suggests a switch of genes related to its biosynthesis. We discovered that the compound was present in species that typically live in humid and cold zones, but absent in species from dry and warm biotopes. It is likely that larvae crawling on and in the soil to spin a cocoon benefit from producing a fungicide when inhabiting an environment particularly conducive to fungi. Our study illustrates how multiple constraints can shape the evolution of insects. It also reinforces the pertinence of studying sawflies when addressing such eco-evolutionary questions.

Volatile Compounds Involved in the Behavior of the Woodwasp *Sirex noctilio*

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The woodwasp *Sirex noctilio* has been studied for many years and the typical volatiles emitted by its host trees are known to elicit antennal response of males and females¹. Since it is very challenging to develop a bioassay using a wild insect in the lab, we tested the attraction of *S. noctilio* to two of its host species in a trapping experiment. Scots pine (*Pinus sylvestris*) caught significantly more wasps than white pine (*P. strobus*), which could possibly be due to qualitative and quantitative differences found between the volatile profiles of the two pine species. Early reports describe swarming of males as they move from tree to tree after their emergence on warm, sunny days². We designed an experiment to collect volatiles on SuperQ filters from different numbers of males and females in a plexi-glass cage to see whether there is any volatile compound produced by the wasp mediating its behavior.

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Z-Nose Detection of Floral Volatile Emission from Rubber Septa

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Background: Volatile emission is of particular interest in a number of areas of plant-insect interactions. Septa emitting floral volatiles have been previously used to mimic floral emissions as a way to gain insight into plant-pollinator interactions, as well as in trapping of insects. 12 compounds have been identified as being present in the floral bouquets of over 50% of flowering plant species¹ and we tested these and an additional 4 compounds that have been shown to be of ecological importance in a number of studies. Knowledge of the emission rates of these compounds from septa would help improve experiments with odor manipulations through the prediction of efficacy/lifetime of volatile blends. **Methods:** We aimed to quantify the emission rate of 16 floral volatile compounds from soaked rubber septa using GC-MS and the Z-nose. Rubber septa were soaked for 2 hours in 100 mgml⁻¹ solution, air dried for 1 hour and then either sampled using tubes containing Tenax TA adsorbent or the Z-nose. The 16 compounds tested included the monoterpenes α - and β -Pinene, Limonene, E-Ocimene, Myrcene, Linalool, the sesquiterpene Caryophyllene, and the irregular terpene 6-methyl, 5-hepten-2-one. Phenolic compounds tested were: Benzaldehyde, Methyl salicylate, Benzyl alcohol, and phenylethylalcohol. In addition, we assessed the emission of two green leaf volatiles, Z-3-hexen-1-ol and Z-3-hexenyl acetate and two more phenolic compounds phenylacetaldehyde and anisaldehyde.

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Effects of Rewarding and Unrewarding Experiences on the Responses to Host-Induced Plant Odors of the Generalist Parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae)

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For numerous parasitic wasps it is known that associative learning can modify their foraging behavior¹. This behavior is in agreement with optimal foraging theory, which expects the wasps to adapt their responses to specific cues in accordance with the rewards they receive while perceiving these cues. In accordance, the generalist parasitoid *Cotesia marginiventris* shows increased attraction to a specific plant odor after this odor has been perceived during contact with hosts. This positive associative learning is common among many parasitoids, but little is known about the effects of unrewarding host searching events on the attractiveness of odors perceived during such events². To obtain such knowledge we studied the effects of unrewarding events, defined as failure to find hosts or as attacking unsuccessfully a non-host species, on the subsequent responsiveness to odors perceived during these events. Thus, preferences of female *C. marginiventris* for herbivore-induced odors of three plant species (*Zea Mays*, *Gossypium hirsutum* and *Vigna unguiculata*) were tested in a six-arm olfactometer after the wasps perceived one of the plant odors either without contacting any caterpillars or in presence of the host caterpillar *Spodoptera littoralis*, or the non-host caterpillar *P. rapae*. Our results confirm strong effects of positive associative learning, but showed no changes in innate responses to the host-induced odors after the “negative” experiences. Hence, a positive association is made during an encounter with hosts, but unsuccessful host-foraging experiences do not necessarily lead to avoidance learning in this generalist parasitoid.

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Thymol and Carvacrol Formation by Transgenic *Arabidopsis* Overexpressing a Cytochrome P450 from *Origanum vulgare*Crocoll C¹, Gershenzon J¹, Degenhardt J²¹ Department of Biochemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Strasse 8, D-07745 Jena, Germany² Institute of Pharmacy, Martin Luther University Halle, Hoher Weg 8, D-06120 Halle, Germany

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The essential oil of *Origanum vulgare* consists of complex terpene blends which are synthesized and stored in specialized structures, the glandular trichomes, on the aerial parts of the plant. The quantity and composition of this oil is highly variable but often contains the monoterpene olefins γ -terpinene and *p*-cymene as well as the monoterpene alcohols thymol and carvacrol. These two phenolic monoterpenes, thymol and carvacrol, are especially known for their antiherbivore, antimicrobial, pharmaceutical and antioxidant activities. Comparative studies of terpene biosynthesis in two *O. vulgare* cultivars suggested that the aromatic monoterpene alcohol thymol is derived from γ -terpinene by two oxidation steps that are likely to be catalyzed by a cytochrome P450 monooxygenase. A putative cytochrome P450 gene was isolated from cDNA libraries of *O. vulgare* which shares 72 % amino acid identity with a limonene-6-hydroxylase involved in carvone biosynthesis in mint. RNA hybridization analyses predicted the corresponding enzyme to be involved in thymol biosynthesis. Overexpression of the *O. vulgare* P450 gene in *S. cerevisiae* resulted in active enzyme which is currently being characterized for its substrate and product specificities. *In vitro*, the enzyme accepted several substrates including γ -terpinene, α -terpinene, (-)-*S*-limonene and (+)-*R*-limonene and appears to catalyze different types of oxygenations. To better test the role of the enzyme *in planta*, we introduced the *O. vulgare* cytochrome P450 gene into *Arabidopsis*. Feeding of monoterpene intermediates to the transgenic *Arabidopsis* plants overexpressing this *O. vulgare* cytochrome P450 resulted in the formation of monoterpene alcohols including thymol and carvacrol. Interestingly, a higher proportion of these products could only be isolated as glycoside conjugates which might be a detoxification reaction by the plant itself to prevent cell damage. The results have interesting implications for how plants produce and store such defense compounds.

Fighting Herbivores: Defense Mechanisms of Black Poplar (*Populus nigra* L.)Danner H¹, Köllner TG², Gershenzon J¹, Unsicker SB¹¹ Max-Planck Institute for Chemical Ecology, Department of Biochemistry, Jena, Germany² Institute for Pharmacy, University of Halle, Germany

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The arsenal of defense reactions of long-lived woody perennials to herbivory is an important factor for long-term survival. Direct defenses include morphological properties like trichomes and chemicals (e.g. phenolic glycosides, condensed tannins), directly influencing the attacking herbivores. Indirect plant defenses often involve the attraction of natural enemies of the herbivores either by volatile cues like terpenes and green leaf volatiles or by rewards like extra-floral nectaries. Both strategies are costly, and inducibility of the participating pathways can optimize the trade off between defense and susceptibility to herbivores and pathogens. The aim of this study was to investigate herbivore induced volatiles in a natural, old growth population of *Populus nigra* L. (Salicaceae) in northeastern Germany at both, the phenotypic, and the genetic level. We were able to detect a number of volatiles in black poplar individuals from the field that were induced by *Lymantria dispar* caterpillar feeding. Both the quality and the quantity of the emitted volatile compounds strongly depended on the tree genotype. Based on the *Populus trichocarpa* genome, we established two marker genes, (E,E)- α -farnesene, and (-)-germacrene D synthase, for quantitative analysis of gene expression levels in the natural *P. nigra* population. Those genes were heterologously expressed in *E. coli* and synthase products were confirmed by SPME headspace analysis. With these molecular tools at hand, we are able to address specific questions e.g. concerning intra-plant communication under natural conditions in the field.

A Specialist Caterpillar Seems Able to Suppress Maize Indirect Defenses

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Plants can rely on a broad range of different mechanisms to defend themselves against their herbivorous enemies. While direct defenses, like the production of toxic compounds, tend to have a direct negative effect on insect herbivores, indirect defenses help plants to attract the insects' natural enemies. These so-called tritrophic interactions are mediated by volatile organic compounds (VOCs)¹. It seems that some insect herbivores have evolved ways to manipulate or disrupt plant indirect defenses². It is hypothesized that specialist herbivores are better in manipulating the defense responses of their preferred host plant than generalists. In order to test this hypothesis, the indirect defense responses of maize plants were compared upon attack by a specialist and a generalist caterpillar species. Our results show that the specialist caterpillar *Spodoptera frugiperda* can suppress induced volatile emissions in maize, which was reflected in considerably less VOCs emitted after feeding by *S. frugiperda* than after feeding by the generalist *Spodoptera exigua*. The specialist seems to benefit from this suppression, because the plants it attacked were also much less attractive to one of its main natural enemies, the parasitoid wasp *Cotesia marginiventris*. The mechanism behind this phenomenon remains to be elucidated. We are currently studying the influence of caterpillar regurgitant composition and feeding modus.

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***Malus domestica* L. and *Cydia pomonella* (Lepidoptera, Tortricidae) Relationships and Sugar Signaling**

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A blend of 3 soluble carbohydrates and 3 sugar alcohols present on apple tree leaf surface influence host selection and egg laying of *C. pomonella*¹⁻³. Inducing resistance was investigated in orchards by triggering sugar signaling pathways. Analyses of leaf surface metabolome showed high modifications of the 3 sugar alcohol quantities and ratios within the blend. With man-made six metabolite blends, we could corroborate these changes to the reduction of egg-laying and to arrestment of neonate larvae. Analyses of leaf tissue and surface metabolomes after sugar foliar treatment, will permit to establish hypotheses to identify the signal sugar start and following signaling pathways within the plant which give *C.pomonella* resistance.

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Using Short-Lived Radiotracers to Study Short-Term Induced Changes in Resource Dynamics

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As sessile organisms, plants have evolved a wide arrange of defense mechanisms in order to reduce the impact or withstand enemy attacks. Upon damage, herbivores are confronted by the production of chemical compounds or physical structures by their host. This may reduce herbivore preference or performance through reduced tissue digestibility or/and increased tissue toxicity. However, plants can employ an alternative, not mutually exclusive, strategy in response to damage. Through tolerance mechanisms, plants can reprogram primary metabolism enabling them to fine-tune their allocation of new and existing resources to less accessible organs for the attackers. Here we investigated the effects of simulated herbivory by methyl jasmonate (MeJA), a known defense elicitor, on resource allocation in tomato and tobacco using short-lived radiotracers (¹¹CO₂ and ¹³NH₃) to measure the distribution of ¹¹C-photosynthate and ¹³N-amino acids *in planta*. Shortly after MeJA treatment (4hrs), we observed an increase in carbon and nitrogen export from treated leaves (relative to baseline responses in the same plant), as well as a significant increase in ¹³N-amino acid allocation to the roots as compared to the apex, and a trend showing an increase in carbon allocation to the roots as compared to the apex. These results suggest an increase in root sink strength in response to simulated herbivory. Furthermore, ¹¹CO₂ fixation was reduced in response to MeJA, hinting at possible trade-offs between growth and induced resistance. Overall, these results are in agreement with our hypotheses, showing a change in resource allocation after simulated herbivory reducing the chance of resources being lost to herbivores. Research was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service and by the US DOE.

Plasticity in Gall Midge Host Plant ChoiceHillbur Y¹, Boddum T¹, Hansson BS²¹ Dept of Plant Protection Biology, SLU, PO Box 102, SE-230 53 Alnarp, Sweden² Dept of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans Knöll Str 8, D-07745 Jena, Germany

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The gall midges (Diptera: Cecidomyiidae) is an insect family with more than 5000 described species and a high rate of speciation¹. Most gall midges are considered highly specialized and are commonly reported as mono- or oligophagous with reference to larval feeding². During their 1-2 day life span the adults will mate and oviposit, using pheromones and plant volatiles to find partners and host plants^{2,4}. Gall midge females are thus under strong selective pressure to find a suitable oviposition site, i.e. the right host plant. Oviposition on a non-host plant may result in a host plant shift and be a basis for speciation, given that the larvae survive on the new plant. Using two gall midge species, the swede midge *Contarinia nasturtii* – a specialist on cabbage, and the brassica pod midge *Dasineura brassicae* – specialized on oilseed rape, we studied the plasticity in host plant choice. We tested if: 1) given no choice, females will oviposit on any plant, 2) females are more likely to accept an alternative host plant that are related to and have an odor profile similar to the preferred host, 3) egg and larval survival is higher on the preferred host plant than on an alternative host. Eventually this will tell us if gall midge host specificity is based on olfaction, and if plasticity in host plant choice can be a basis for speciation.

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Enzymatic, Expression and Structural Divergences among Carboxyl O-Methyltransferases after Gene Duplication and Speciation in *Nicotiana*Hippauf F¹, Michalsky E², Barkman TJ³, Piechulla B¹

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Methyl salicylate and methyl benzoate have important roles in a variety of processes including pollinator attraction and plant defence. These compounds are synthesized by salicylic acid and benzoic acid carboxyl methyltransferases (SAMT and BSMT) which are members of the SABATH gene family¹. Both SAMT and BSMT were isolated from *Nicotiana suaveolens*, *Nicotiana glauca*, and *Nicotiana glauca* allowing us to discern levels of enzyme divergence resulting from gene duplication as opposed to species divergence. Phylogenetic analyses showed that *Nicotiana* SAMTs and BSMTs cluster in separate clades and the latter can be differentiated into the BSMT1 and the newly established BSMT2 branch. Although SAMT and BSMT orthologs showed minimal change due to species divergence, substantial evolutionary change of enzyme activity and expression patterns occurred following gene duplication. The BSMT enzymes evolved higher preference for benzoic acid (BA) than salicylic acid (SA) whereas SAMTs maintained ancestral enzymatic preference for SA over BA. Expression patterns are largely complementary in that BSMT transcripts primarily accumulate in flowers, leaves and stems whereas SAMT is expressed mostly in roots. A novel enzyme, nicotinic acid carboxyl methyltransferase (NAMT), which displays a high degree of activity with nicotinic acid was discovered to have evolved from a Solanaceae BSMT ancestral enzyme in *N. glauca*. While BSMT in flowers is clearly involved in methyl benzoate synthesis to attract pollinators, its function in other organs and tissues remains obscure.

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Guatemalan Moth Responds to Potato Odours that Signal Host QualityKarlsson MF¹, Birgersson G¹, Bengtsson M¹, Cotes Prado AM², Witzgall P¹¹ SLU, Chemical Ecology Group, Alnarp, Sweden² Corpoica, Colombian Corporation of Agricultural Research, Biol. Contr. Lab. Bogota

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Potato plants release volatile compounds that signal both phenological stage and the physiological state of the plant. Insects make use of these signals to identify suitable larval food plants and to improve survival of their progeny. This hypothesis was tested in the Guatemalan moth *Tecia solanivora*, a devastating pest of potato in Central and South America. Identification of potato volatiles in different phenological stages from sprouting to flowering showed that over 90% of the compounds emitted from potato foliage are sesquiterpenes, while potato tubers mainly emit aliphatic alcohols, aldehydes and ketones. Headspace collections were screened using electrophysiological methods and female antennae responded mainly to green leaf sesquiterpenes and floral compounds. Oviposition assays with potato plants of different phenological stages showed a strong preference for flowering plants and females attracted to floral compounds such as methyl phenylacetate. However, the females avoided laying eggs on leaves and most eggs were deposited in the soil instead¹. *T. solanivora* also avoided oviposition in the presence of damaged tubers, which correlated with an enhanced emission of sesquiterpenes. Leaves, as well as damaged potatoes produce toxic steroidal glycoalkaloids² that are involved in the resistance of potatoes to pathogens and pests³. Two of the most abundant compounds, α -solanine and α -chaconine have been found to strongly reduce the survival of Guatemalan moth larvae, but the ovipositing females cannot sense these compounds directly. Females may instead use enhanced levels of sesquiterpenes to discriminate healthy from damaged tubers, that are less suitable for larval development. While sesquiterpenes elicit avoidance, Guatemalan moth is attracted to floral compounds. Knowledge of repellent and attractant potato odours is important for plant breeding and the development of direct control techniques.

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Volatile emitted by *Trifolium pratense* L. under Different Experimental ConditionsKigathi RN^{1,2}, Unsicker SB², Reichelt M, Gershenzon J², Weisser WW¹¹ Institute of Ecology, Friedrich-Schiller-University of Jena, Dornburger Str. 159
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Plants emit a wide range of volatile organic compounds, often in response to damage by herbivores, and many of the compounds have been shown to attract the natural enemies of insect herbivores or serve for inter- and intra-plant communication. We studied the emission of volatiles from *Trifolium pratense* (L.) (Fabaceae) after herbivore feeding under two different temperature regimes (20°C and 30°C). We identified a total of 24 volatile organic compounds. After herbivory by *Spodoptera littoralis* caterpillars the emission of seven compounds significantly increased. The monoterpenes (*Z*)- β -ocimene and (*E*)- β -ocimene were the most dominant compounds in the herbivore treatment. When plants were exposed to increasingly longer feeding durations, there was a corresponding increase in the amount of herbivore induced volatiles emitted. Increase of temperature from 20°C to 30°C resulted in significantly higher emission of three compounds, limonene, nonanal, and (*E,E*)- α -farnesene. The herbivore induced compounds that we measured in *T. pratense* have also been detected in other herbaceous plant species. However, contrary to other reports the increase of temperature did not significantly increase many of the herbivore induced compounds.

Salicyl Alcohol Oxidase - an Access to Evolutionary Adaptation of *Chrysomela* ssp. to their Host Plant

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The Coleoptera represent one of the most diversified taxa on earth. Among beetles the “Phytophaga” are a megadiverse lineage, representing roughly 40% of all known species. Their enormous radiation and evolutionary success is generally agreed to be correlated with multifaceted host plant interactions. These interactions imply particular adaptations on both sides, herbivorous beetle and host plant. Understanding the adaptive mechanisms will contribute to answer the questions why there are so many beetles and how they are embedded in the plant-herbivore ecological network. Several leaf beetle species belonging to the subfamily Chrysomelinae use a chemical defense strategy to overcome predatory attack and microbial invasion. They have developed a defensive system consisting of 9 pairs of defensive glands located dorsally in the thorax and abdomen. In case of a danger, the glands are compressed and small droplets of the defensive secretion appear on top of the apocrine glands. It has been shown that larvae of the genus *Chrysomela* not only survive the general insect repellent salicin of their host plants, but also benefit from this plant secondary compound by sequestration and further modification to employ the resulting salicyl aldehyde for their own chemical defense. After transport of salicin through the gut into the defensive glandular reservoir, chemical modifications go on in two steps: deglycosylation by a glucosidase and oxidation of the aglucon salicyl alcohol to the bioactive principle salicyl aldehyde by a salicyl alcohol oxidase (SAO). We identified the SAO of two *Chrysomela* ssp. and characterized them on protein and genome level. The enzymes are highly glycosylated members of the GMC Oxidoreductase family and specifically expressed in the defensive glandular tissue. Genome investigations indicate that multiple gene duplication events led to SAO function and an increased number of GMC oxidoreductase genes in *Chrysomela* ssp. Because the SAO is a key in utilization of salicin, resulting in increased fitness of the leaf beetle larvae, discovering evolution and origin of this enzyme gives insights in adaptive mechanisms in herbivore-host plant interactions.

Development of an Assay to Measure Larval Acceptance of a New Host by Diamondback Moth (Lepidoptera: Plutellidae)

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Whether or not an herbivore can expand its host range depends on the behavior of both larvae and ovipositing females in acceptance of a new host. The diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), is a specialist on cruciferous hosts. However, during an outbreak of this insect pest in Kenya in 1999, *P. xylostella* was found infesting fields of peas (*Pisum sativum*) in addition to fields of crucifers¹. Since this time, insects from that area have been kept in colonies reared separately on both pea and cabbage (*Brassica oleracea* var. Gloria) for further study of host shift dynamics. Neonate larvae from cabbage and pea strains were examined in their response to leaf discs from those plants in a factorial design. In a 5-minute assay, recorded parameters included time to reach a leaf disc and the level of directed movement. Larvae accepting a leaf disc within 24 hours were retained for measurements of survival on the host plants. Preliminary data suggests that survival on a host is dependent on initial acceptance by neonates within the first 24 hours. Pea strain larvae accepted both cabbage and pea as a host. Some cabbage strain larvae accepted pea as a host, but pupal weights were lower and time to pupation was extended compared to the pea strain. Interestingly, larvae of both strains often prefer to remain on a leaf disc on which they have initiated feeding rather than moving to a fresh disc placed in the arena.

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Great Tits Overtake Aphids' Galls Chemical DefensesIzhaki I¹, Koplovich A¹, Lewinsohn E², Wink M³, Inbar M¹¹ Department of Evolutionary and Environmental Biology, University of Haifa, 31905 Haifa, Israel² Department of Vegetable Crops, Newe Ya'ar Research Center, Agricultural Research Organization, PO Box 1021, Ramat Yishay, Israel³ Institut für Pharmazie und Molekulare Biotechnologie (IPMB), Universität Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany.

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In the Mediterranean scrubland, the gall forming aphid *Baizongia pistaciae* induce large banana-shape (and size) galls on the deciduous tree *Pistacia palaestina*. Field observations in several sites in Israel indicate that during the autumn, Great Tits (*Parus major*) tear the tissue of 27%±13% (in 3 sites) of the galls and consume the aphids within and eventually destroy the galls. We studied the chemical defense of the galls against avian predation by (1) analyzing tannins and volatile organic compounds (VOC) in the galls, the aphids within and intact leaves; (2) testing the effect of gall tissue on feeding preference of captive tits using manipulated artificial diets. Tannins concentration in the galls (4.42%±0.45% dw, n=16) was twice as higher than in leaves (2.85%±0.32% dw, n=20) whereas no tannins were found in the aphids. Galls had similar number of compounds (33) and similar number of compounds and concentration of monoterpenes (17 and 33 µg/g, respectively) as leaves (29, 14 and 32 µg/g, respectively). The sesquiterpene concentration was almost thrice in galls (30.4µg/g) than in leaves (11.3µg/g). Although number of compounds of monoterpenes in galls was identical to that of aphids, monoterpenes concentration in aphids was twice as higher than in galls. Yet, only one sesquiterpene compound was found in the aphids (p-cymene), as compared to 16 sesquiterpenes found in the galls. Feeding trials demonstrated that tits were repelled by artificial food containing 5% galls mash (dry weight). Tits always preferred aphids-containing artificial food over gall-containing artificial food. We conclude that although *B. pistaciae* galls are rich in repellent chemical compounds, Great Tits, by tearing the protective gall tissue, can overtake gall's defenses and feed on the aphids inside.

Purification and Identification of a Lepidopteran (Caterpillar) Fatty Acid Amide Hydrolase and its Role in Nutrient Regulation

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Several fatty-acid amide compounds (FACs) in caterpillar oral secretions enhance plant defense responses during herbivory¹. The levels of these FAC elicitors within the caterpillar appear to be controlled by two enzymes, one in the gut tissue membranes that synthesizes FACs and the second in the gut lumen that hydrolyzes them²⁻³. Since FACs are persistent despite obvious fitness costs, it is believed that they are important to the performance and/or survival of the caterpillar. Recent labeling studies suggest that FACs may be a storage molecule to enhance the caterpillars' ability to take up nitrogen⁴. Therefore I hypothesize that FACs and associated enzymes enable the caterpillar to balance their nutrient absorption when consuming sub-optimal diets. I have purified the FAC hydrolase by liquid chromatography, identified the protein by Tandem MS/MS, and confirmed activity by expression in *E. coli*. In mammals, orthologous enzymes are cytosolic and follow a strikingly different role by recycling unusable amino acids from the digestion of protein. However, in caterpillars the FAC hydrolase is present in the gut lumen and hydrolyzes a specific substrate that is purposely synthesized by the caterpillar, thus supporting a novel digestive role to enhance nutrient absorption. In order to further understand the role of caterpillar FAC hydrolase, feeding studies are underway to examine the effect of increased hydrolase activity *in vivo* on the performance and nitrogen uptake of caterpillars reared on sub-optimal diets.

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Electrophysiological Responses of *Steirastoma breve* (Coleoptera: Cerambycidae) to Volatiles Isolated from Branches of its Host Plant, *Theobroma cacao*Liendo-Barandiaran C¹, Herrera B¹, Morillo F², Sánchez P², Hernández J¹

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The stem borer, *Steirastoma breve*, is one of the most important insect pests in Neotropical cocoa plantations. Larvae and adults cause severe damages in stems and branches of infested plants¹. In preliminary olfactory bioassays, we have tested the attractiveness toward branches of *Theobroma cacao* under field and laboratory conditions² and we confirm the importance of volatiles emitted by *T. cacao* in the chemical communication system of this pest. In this work, we evaluate the electroantennographic responses of *S. breve* males and females to odors released from branches of *T. cacao*. Volatile compounds were isolated from branches that were cut from healthy plants of *T. cacao* using headspace analysis combined with solid phase microextraction (HS-SPME). The optimal conditions to isolation were: combined fiber (DVB/CX/PDMS), 30g of branches, 2 hours at 40°C and 10s as desorption time. Eight peaks showing electrophysiological activity were determined by GC-DEA using male and female antennae of *S. breve* adults. Three signals were exclusive from males, only one from females and four signals for both sexes. Chromatographic-electroantennographic peaks were identified by GC-MS and retention indexes. Commercial compounds were tested by EAG and the dosis-response curves were determined, for each compound and sex. 3-octanone and limonene showed significant differences compared with the control (hexane) by both sexes. For males, we have also observed significant differences comparing benzaldehyde and styrene against the control. However, significant differences for the rest of identified compounds were not observed for both sexes. In conclusion, the active volatiles profile used by this species seems to be different depending of the sex. The kairomones emitted by branches of *T.cacao* could be constituted by these biological active compounds. From all those, styrene, benzaldehyde, 3-octanone and limonene produce response on antennae of *S. breve* males, while the two last compounds only in females.

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Monoterpene Emission from Pine Seedlings Subjected to Different Environmental Conditions and Treatments Emulating Mountain Pine Beetle AttackLusebrink I¹, Evenden ML¹ and Erbilgin N²¹ Department of Biological Sciences, University of Alberta, Edmonton, Canada² Department of Renewable Resources, University of Alberta, Edmonton, Canada

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This study is part of an interdisciplinary project (www.thetriaproject.ca) that focuses on the genomics of three interacting organisms: the mountain pine beetle (*Dendroctonus ponderosae*), its host pine trees and its fungal associates (e.g. *Grosmannia clavigera*). The chemical ecology of this interaction is examined in a functional genomics study, in which the objectives are to determine the chemical and physiological responses of lodgepole pine (*Pinus contorta*; historical host), jack pine (*Pinus banksiana*; potential host) and their hybrids to different environmental conditions (water vs. no water) and treatments emulating beetle attack. Gene expression of host chemical response to treatments will ultimately be linked to beetle fitness. The current study was conducted to test the hypothesis that the release of monoterpene volatiles from pine seedlings will vary with water regime and treatments: control, mechanical wounding and inoculation with a spore solution of *G. clavigera*. Lodgepole pine and jack pine seedlings were randomly assigned to environmental and wounding treatments. Volatiles were collected at 6 time points over 6 weeks and analyzed with gas chromatography. Further, physiological responses of seedlings to treatments, such as photosynthesis rate, stomatal conductance and soil water content were monitored. Phloem and needle samples were taken for future terpenoid and phenolic analyses. Future studies will examine the influence of water regime and wounding treatments in mature trees and their effect on upregulation of host response and subsequent beetle fitness in treated trees.

Gallsphere – the Headspace Surrounding the Gall: Characterization and Putative FunctionRostás M¹, Maag D¹, Inbar M²¹ Botany II, University of Würzburg, Germany² Evolutionary & Environmental Biology, University of Haifa, Israel

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Gall-formers manipulate and exploit the development, anatomy, morphology, physiology and chemistry of their host plants. Inside the galls, the inducing insects benefit from an improved nutrient supply and defense from natural enemies due to high levels of secondary metabolites. The insects may even control the composition of metabolites among different gall parts. Conspicuousness by color, size or shape is a common gall trait and thus galls are often easy to detect in the canopy. This conspicuousness may serve as a signal for potential natural enemies or herbivores (such as leaf chewers), indicating that the gall is well defended and not palatable. Volatiles emitted by the galls may be part of this signaling complex. We have studied the chemical components of the large, red coral-shaped galls induced by the aphids *Slavum wertheimae* on *Pistacia atlantica* trees. In a replicated field experiment, volatile organic compounds (VOC) were sampled from the headspaces of intact and artificially damaged galls, leaves and fruits, using Super Q-filters attached to portable air pumps. The emitted VOC were analyzed by GC-MS. In addition, direct defense levels were determined as total tannin concentrations. We found that 1. Compared with leaves and fruits, gall tissue contained higher concentrations of tannins and VOC. 2. Galls emitted more monoterpenes compared to fruits and especially to leaves. 3. Artificial damage increased the amounts of monoterpenes emitted to the gall headspace by two-fold. We show here for the first time that galls create a 'Gallsphere' – headspace which is particularly rich in monoterpenes that should emphasize its conspicuity in the canopy. The adaptive value of the 'gallsphere' may be explained by the aposematic gall hypothesis: galls, well-protected by secondary chemicals in the tissue, employ warning signals (shape, color and scent) to deter potential enemies.

“Lite Tobacco?” Glycoside “Sugar Substitutes” in *Nicotiana attenuata* Lead to Thinner Caterpillars

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Diterpene glycosides (DTGs) comprise a large class of compounds consisting of a twenty-carbon (diterpene) skeleton conjugated to one or more sugar groups. Some DTGs have been shown to affect sugar metabolism in rats or to affect intestinal function in human cells^{1, 2}. Many *Nicotiana* species contain high levels of hydroxygeranylinalool (HGL-) DTGs in their green tissue, on the order of milligrams per gram, and these are negatively correlated with herbivore growth. We identified ten 16-hydroxygeranylinalool diterpene glycosides (HGL-DTGs) which are highly abundant (mg/g FM) in aboveground tissues of the wild tobacco *Nicotiana attenuata*. HGL-DTGs differ in their sugar moieties and number of malonyl groups attached to sugars (zero, one or two). Concentrations of mono- and dimalonylated HGL-DTGs are highest in young and reproductive tissues but vary dynamically with plant elicitation and tissue type. We identify malonylation as the key biosynthetic step regulated by herbivore and jasmonate signaling using *N. attenuata* stable transformants impaired in jasmonate production (IR*lox3*) and perception (IR*coi1*), or production of the *N. attenuata* hydroxyproline-rich glycopeptide systemin (NappHS, IRsys). We created inverted repeat *Naggpps* stable transformants (*N. attenuata* geranylgeranyl diphosphate synthase, IR*ggpps*) to reduce precursors for the hydroxygeranylinalool DTG skeleton, resulting in reduced amounts of HGL-DTGs. Larvae of the specialist herbivore *Manduca sexta* grew up to ten times as large on IR*ggpps* as on wild-type (WT), consistent with previous results from transient silencing of *Naggpps*³. IR*ggpps* plants also received more damage from generalist herbivores in *N. attenuata*'s native habitat. However, IRsys plants which have reduced levels of malonylated HGL-DTGs are not more susceptible to herbivores⁴. The apparent defensive value of malonylated compounds may reflect a role of malonylation in transport rather than toxicity or deterrence.

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Localization of Terpenoid Accumulation in the Bark Tissues in *Larix gmelinii* var. *japonica*, as a Chemical Defense of Conifers

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The primary function of tree bark is to protect its own tree body to plant pathogens, herbivorous insects and mammals, physically and chemically. Healthy coniferous trees with well-developed resin-duct system defense chemically against these natural enemies using constitutive resins like standing armies, before epidemic outbreaks of pests occur. The major components of the resins are terpenoids, but little is known about the way of their accumulation in the bark tissues. In this study we investigated terpenoid accumulation in the different tissues within the bark of Kurile larch, *L. gmelinii* var. *japonica*. The compositions of main mono-, sesqui- and diterpenoids in secondary phloem (nearly i.e. "inner bark") and rhytidome (nearly i.e. "outer bark") of the mature branch bark of *L. gmelinii* var. *japonica* were analyzed by GC and GC-MS. We found that the terpenoid content in the rhytidome tended to be higher than those in the secondary phloem. It is conceivable that throughout the formation of scaly rhytidome of *L. gmelinii* var. *japonica*, the terpenoids, which are biosynthesized and stored in internal secretory structures within the living secondary phloem, are concentrated bit by bit in the rhytidome, as the outer part of the secondary phloem rich in resins become dead after cutted off by periderm and then transferred into the rhytidome. We will also discuss the implications of our findings for chemical defense tactics of conifers on the basis of plant anatomy, physiology, and ecology.

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Plant Root Detection by Larvae of a Stone Fruit Borer

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The larvae of the flatheaded rootborer, *Capnodis tenebrionis* (Linné) (Coleoptera: Buprestidae) develop in stone fruit (*Prunus* spp., Rosaceae) trees, feeding on the root cortex. The eggs are laid in dry soil near the root collar of the host plant. The neonates can locate a root from a distance of 60 cm. The present study aimed to study host recognition/orientation by *C. tenebrionis* neonates and to identify cues guiding its behavior. Choice bioassay was developed using sand arena. Three sources of cues which may guide the movement toward the host roots were examined: (1) humidity, (2) fresh host plant sections and (4) head space volatiles of roots, branches and leaves of plum tree (*Prunus domestica*). Ten 24-hour old neonates were allowed to choose between 2-4 baits in the sand arena. The neonates rapidly detected and oriented themselves to water, host roots and branches and preferred host upon non host branches in choice tests. Older larval stages seem to lack these capabilities. Head space collected volatiles from branches and roots were highly attractive to the neonates, while those of the leaves were less attractive. Among the branch volatile compounds that were identified, β -caryophyllene and p-cymene were the most behaviorally active. However, the attractiveness of these compounds was significantly lower than that of the crude volatile extract. Addition of water to the volatiles increases the attractiveness of the lure. We speculate that neonates maybe guided from the dry hatching spot towards the root site by humidity as a long range cue, and at close range by the host volatiles.

Do Predatory Arthropods Perceive and Respond to Individual Herbivore Induced Plant Volatiles or Do they Instead Respond to the Mixture as a Whole?

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Phytoseiulus persimilis is a predatory mite that predominately feeds on the two-spotted spider mite *Tetranychus urticae*. Plants that are infested by *T. urticae* produce herbivore induced plant volatiles (HIPV) and thereby alter the odour they emit. The blind predatory mites exploit this source of chemical information to find spider-mite-infested plants. To explain why predatory mites are attracted to the odour of spider-mite- infested plants we will explore two hypotheses that make different assumptions as to how odours are perceived by the mites. If the mites are able to detect each of the components in an odour mixture the attraction to spider-mite-infested plants could result from the attraction to specific compounds in the odour mixture produced by these plants. Under this hypothesis specific compounds convey a specific message from the plant to the mite. Alternatively, the mites may not possess the ability to detect specific compounds in odour mixtures and perceive the odour rather as a perceptual or synthetic whole. Under this hypothesis the attraction of predatory mites could result from the mite's ability to generalize from odours that have been experienced in association with spider mites to the odour of a not yet experienced spider-mite-infested plant. Hence when olfaction is synthetic, the presence of specific compounds in the headspace of a plant cannot have an a priori meaning to the predatory mites. The attraction of predatory mites rather depends on the perceptual similarity of spider-mite-infested plant odours.

Back to Beginning: Studying Jasmonate Signaling in *Pteridium aquilinum*Venkatesan R¹, Kost C¹, Bonaventure G², David A¹, Boland W¹¹ Department of Bioorganic Chemistry, Max-Planck Institute for Chemical Ecology, Jena² Department of Molecular Ecology, Max-Planck Institute for Chemical Ecology, Jena

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Plants employ volatile organic compounds (VOCs) as an indirect defence strategy to attract predators of the herbivores. In higher plants, VOCs emission is controlled via the octadecanoid pathway, in which jasmonic acid (JA) plays a key role. Although basic knowledge about regulation of VOCs is known from higher plants, fundamental questions such as those on the evolutionary origin of this trait remain unresolved. Phylogenetically ancient plants like the ferns provide the unique opportunity to focus on such questions. In the present study, we investigated the role of JA in VOCs emission of bracken (*Pteridium aquilinum* L., Dennstaediaceae) and compared our findings to a well-known higher plant *Phaseolus lunatus* (Lima bean). Bracken responded to exogenous JA application by emitting *de-novo* synthesized VOCs (mainly terpenoids) similar to Lima bean. Coronalon, a structural mimic of JA and channel-forming peptide alamethicin elicited volatile responses similar to JA in both Lima bean and bracken. Studies with three other fern species also showed similar VOCs response to JA treatment. In contrast to Lima bean, in which herbivory induces the emission of terpenoids, simple mechanical damage or feeding by herbivores on bracken elicited the emission of green-leaf volatiles and cell-wall derived compounds only. In addition, feeding by both generalist and specialist herbivores led to only marginal, but rapid increase in endogenous JA levels, suggesting the presence of a JA pool in bracken, which was further confirmed by hydrolysis experiments. In summary, although bracken responds to JA treatment similar to higher plants, wounding elicited a qualitatively different VOC blend in this fern, thereby suggesting that the signalling pathways involved in these responses differ from those found in higher plants. Experiments are in progress to test whether VOCs originated primarily as a direct defence against microbial or fungal pathogens and only later evolved into an indirect defence mechanism.

Chemical and Morphological Diversity of the Invasive Herbaceous Plant Common Tansy (*Tanacetum vulgare* L., Asteraceae)Wolf VC^{1,2}, Gassmann A², Müller C¹¹ University of Bielefeld, Universitätsstrasse 25, 33615 Bielefeld, Germany² CABI Europe – Switzerland, Rue de Grillons 1, 2800 Delemont, Switzerland

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Common tansy (*Tanacetum vulgare* L., Asteraceae) is a strongly aromatic, perennial herbaceous plant of Eurasian origin, which is invasive in Canada and Northern USA. This species is characterised by essential oils mainly consisting of mono- and sesquiterpenes such as 1,8-cineole, α - and β -thujone, camphor, borneol, germacrene D and others. The qualitative and quantitative composition of these essential oil components varies highly between individuals. Therefore, different chemotypes can be defined. To investigate the chemotype occurrence of *T. vulgare* of different origin and the relationship to morphological traits, seed samples of 13 European and 9 North American populations were collected. These seeds were grown in a common garden experiment to exclude different environmental influences. Terpene profile, trichome density, C/N ratio and dry matter content of four- to five-week old seedlings were investigated. Furthermore, several growth parameters were recorded from 3.5 month old plants. The chemotypes and morphological traits were found to be highly diverse between, as well as within different *T. vulgare* populations. Differences in chemotype and morphology and their correlation will be discussed.

Feeding Stimulant of the Pepper and Tomato Varieties against Biotype B and Q of Sweetpotato Whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae)

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Biotype B and Q of Sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) were normally developed in all tested seven tomato varieties. However, biotype B did not grow in eight red pepper varieties. Rokkusanmaru variety of tomato and Cheongpungdaegun variety of red pepper show the highest susceptibility against biotype B and Q of sweetpotato whitefly and analyzed their compounds using HPLC (ELSD Detector) to search the feeding stimulant. The result was proved these sugar such as erythritol, xylose, xylitol, fructose, glucose, mannitol, and sucrose was contained at red pepper varieties; erythritol, xylose, fructose, glucose, and mannitol was contained at tomato variety. Especially, xylitol and sucrose existing in the red pepper did not present in tomato varieties. Subsequent bioassay with these two sugars, sucrose did not show significant difference between two biotypes; however, xylitol was showed only repellent effect against biotype B. Therefore, it seems that xylitol is a play key role in the host choice of B biotype of sweetpotato whitefly.

Attraction Behavior of Pine Sawyer Beetle, *Monochamus saltuarius* Gebler (Coleoptera: Cerambycidae) to the Volatiles of *Pinus koraiensis*

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Recently, the pine sawyer beetle *Monochamus saltuarius* has been proved as a vector of pinewood nematode, *Bursaphelenchus xylophilus* in South Korea. This pest causes serious concern that widely distributed in the middle of South Korea and most likely to spread rapidly. To understand their ecological behavior, the attraction behavior of the pine sawyer beetle was tested in the volatile samples of *Pinus koraiensis* by emitting different host condition. The olfactory response of the immature (0~3 days old after emergence) adults of *M. saltuarius* preferred the volatiles from the fresh host condition (fresh twigs, artificial damaged twigs), whereas the mature (20~30 days old) adults preferred the volatiles from the stressed host condition (twigs infested by male and female, old twigs that were artificially damaged). In the GC and GC/MS analyses, contrary to the samples from the fresh host condition having monoterpene volatiles only, the samples from the stressed host condition were revealed to have more volatiles of oxygenated monoterpenes and sesquiterpenes. The attraction assay of *M. saltuarius* adults to the volatile mixtures of monoterpenes, oxygenated monoterpene, and sesquiterpene appeared to be efficient when the oxygenated monoterpene mixture was necessarily treated with monoterpene mixtures.

Oviposition Behaviour in *Spodoptera littoralis* and Plant Cues Involved

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Selection of a suitable site for oviposition is critical for the survival of offspring in phytophagous insects. Ovipositing females orient towards the host plant using volatile chemical cues. After landing, assessment of leaf surface involves also contact stimuli¹. Herbivore feeding induces chemical defence responses in plants and the release of volatiles. These compounds may attract natural enemies and function as indirect defence, but they can also affect herbivore behavior. It has been shown that cotton plants, *Gossypium hirsutum* L., either became more attractive or more repellent to ovipositing insects after herbivore damage, depending on the age of the plant². We investigated how odours from damaged and undamaged cotton plants influence the oviposition behaviour of the cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae). All experiments were performed in the green house cages. In order to observe a clear impact of odours on gravid females during the selection of suitable site for oviposition, abiotic factors (humidity, light and temperature) were kept constant in the green house chamber during all experiments. The results demonstrate the influence of volatile compounds, in addition to non-volatile compounds on *S. littoralis* oviposition behaviour.

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GC-EAD Responses of Tomato Leafminer *Liriomyza bryoniae* (Diptera, Agromyzidae) Females to Tomato Headspace VolatilesRadžiūtė¹, Apšegaitė V¹, Butkienė R¹, Būda V^{1,2}¹ Institute of Ecology of Vilnius University, Akademijos 2, LT-08412 Vilnius, Lithuania² Vilnius University, Faculty of Natural Sciences, M. K. Čiurlionio 21/27, LT-03101 Vilnius, Lithuania

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The most important pests among leafmining agromyzids are polyphagous species, and many of those belong to genus *Liriomyza*. The leafmining fly *Liriomyza bryoniae* (Kaltenbach, 1858) (Diptera, Agromyzidae) as well as many other species within the genus *Liriomyza* is economically important due to their leafmining activity, as plethora of agricultural and ornamental plants are damaged. Headspace collection from tomato plants was analyzed by GC-EAD. Seven volatile compounds in the headspace collection consistently elicited significant EAD responses in antennae of female *L. bryoniae*. Among them, there were volatiles abundant in the blend collected, but the most of the active compounds were present in relatively low amounts. Two compounds of 7 volatiles were identified as (Z)-3-hexenol and methyl salicylate. *Behavioral test under field conditions revealed that* methyl salicylate was attractive for *L. bryoniae* flies¹. When MeSa was tested in Y-tube olfactometer differences in responses among the sexes were observed. Only *L. bryoniae* females statistically significantly preferred the airflow with MeSa (the odor was 1,5 ml plastic vial supplied with 0.5 ml of tested compound and slightly plugged with cotton wool) compared to the pure airflow (control). Males did not show preference to any of the two (with MeSa or without MeSa) airflows. C₆ green leaf volatiles, *i.e.* 2-hexenal, 3-hexen-1-ol, 2-hexen-1-ol, 3-hexenyl acetate are known as attractants for closely related species *Liriomyza sativae*². If (Z)-3-hexenol possess similar biological activity towards *L. bryoniae* remains to be investigated.

Acknowledgments

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Poster Session 3

Thursday, 26 August

Analysis of Phospholipid Molecular Species in the Silkworm (*Bombyx mori*) by LCMS-IT-TOF

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Phospholipids are a major component of membrane and exhibit great diversity in the structure of the apolar and the polar moieties of the lipid molecules. They are distributed unevenly in the organ and display characteristic phospholipid compositions. Phospholipids perform various functions in the cells and tissues of the body such as natural surfactants, signaling molecules, and antioxidants. The different pattern can be regarded as an adaptation to external and internal cell environment. In this study, we analyzed phospholipid composition in various organs of silkworm, *Bombyx mori* by TLC and LCMS-IT-TOF. As a result, we found the ratio of plasmalogen phosphatidylethanolamine (PPE) to phospholipids was higher in the gut and peritrophic membrane compared to that in other organs and tissues. Plasmalogens are a unique subclass of glycerophospholipids characterized by the presence of a vinyl ether at the *sn*-1 position of the glycerol backbone, whereas usual PE has an ester bond at the same position. PPE was suggested to be synthesized by larvae since plasmalogen was not detected in the larval diet. PPE has peroxy radical scavenging property and protects unsaturated phospholipids from oxidation. Midgut tissues suffer from oxidative stress associated with phenolic oxidation in midgut lumen when caterpillars ingest phenolics, known as defense chemicals in plants. PPE may protect gut membrane against oxidative stress.

Detection and Representation of Taste Input from Tarsal Contact Chemoreceptors in Female *Spodoptera littoralis*Abou Seada M^{1,2}, Ignell R¹, Al Assuitty AN², Anderson P¹¹ Dep. Plant Protection Biology, Chemical Ecology, SLU, P.O. Box 102, SE-230 53, Alnarp, Sweden² Dep. Zoology, Faculty of science, Tanta University, Egypt

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With their contact chemoreceptors insects detect and respond to different taste stimuli, which are used when accepting, or rejecting food, mates, or oviposition sites. We have studied how gustatory sensilla on the tarsi of the moth *Spodoptera littoralis* detect different taste stimuli. We have found that on the fifth tarsomere of each of the three pairs of legs there are 13-16 pairs of gustatory sensilla. These sensilla are differentiated into two different morphological types, a and b, based on differences in the structure of their tips and basal sockets. In order to study the functional characteristics of these types of sensilla we recorded the physiological responses of these sensilla to salts (KCl, NaCl), sugars (sucrose, fructose, glucose) and a bitter substance (caffeine) using the tip recording technique. The response patterns of the gustatory receptor neurons (GRNs) in the sensilla suggest that the moth is able to discriminate between the different tastants. In most cases, stimuli elicited a tonic response in the GRNs. However, phasic tonic responses were observed in response to fructose and caffeine in some sensilla. In order to illustrate the basic morphology of the central neuropil involved in the first stages of taste processing in the thoracic ganglia we addressed 2 different questions: (1) where do chemosensory neurons from the different tarsi send their central projections, (2) how do these sensory neurons segregate in the central nervous system. We have done mass backfilling of the three pairs of legs using neurobiotin. We were able to discriminate at least 4 different glomerular neuropil in each thoracic ganglion where all afferents coming from one leg project in. These different structures may reveal a functional segregation of different GRNs categories in the central nervous system. Further work is being done to get more details about anatomical organization of the different GRNs found on the fifth tarsomere via functional single sensillum backfilling with a suitable neuromarker.

AAH from Insect Gut Bacterium *Microbacterium arborescens*: Crystal Structure, Peroxidase Properties and RegulationBüchler R¹, Zeth K², Pesek J¹, Boland W¹¹ Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena² Max Planck Institute for Developmental Biology, Spemannstr. 35, 72076 Tübingen

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In the gut of *Spodoptera exigua*, the bacterium *Microbacterium arborescens* has to resist special environmental conditions. While the bacterium needs a certain amount of iron, too much is toxic and forms reactive oxygen species (ROS) via the Fenton reaction. Further, ROS are synthesized by the NADPH oxidase enzyme dDuox in *Drosophila* epithelial cells¹. Against these effects bacteria express Dps proteins (DNA protecting proteins under starvation) which stores excessive iron in the center of its dodecameric protein sphere and detoxify ROS. Beside its typical Dps function this AAH (amino acid hydrolase) called protein of *Microbacterium arborescens* exhibits a very special ability: It hydrolyzes and synthesizes *N*-acyl glutamines². These compounds occur in the foregut and regurgitant of herbivorous larvae and are known inducers of indirect defense cascades in some plants. The protein was crystallized and structurally dissolved to a resolution of 2 Å with one dodecamer in the asymmetric unit at endogenous iron content. The overall structure of the isolated wild type enzyme shows a single iron atom positioned at the ferroxidase centers and coordinated by oxygen and water in an asymmetric conformation. Biochemically the iron content of this enzyme under wild type conditions varies between 11 and 197 per dodecameric complex. The protein shows catalase activity with a k_M of 4.8 mmol l⁻¹. Further the question about the regulation of the AAH enzyme was addressed. It was shown that there is no influence by oxidative and temperature stress but iron excess induces the production of the protein. In contrast to this, deficiency of iron inhibits the production of AAH.

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Insect Biocontrol Strategies Involving Semiochemicals: Synthesis to Product Development

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Semiochemicals are one of the most important classes of compound indigenous to plant and insects. In our laboratory we have evaluated different class of chiral chemicals utilized in insect chemical signals and their efficacy were evaluated in the field studies to attract different species of lacewings for biocontrol of aphids and other soft body insects. Our approach towards formulation of effective and long lasting semiochemical involves protected form of natural products and plant volatiles.

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Spinosad Effects on Chemically Mediated Behaviours in *Blattella germanica*.

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Spinosad are new insecticides, fermentation-derived from a soil-dwelling bacterium *Saccharopolyspora spinosa*, used to control a variety of insects. These insecticides combine the efficacy of a synthetic insecticide with the benefits of a biological pest control organism. It is known to kill susceptible insects within 1 to 2 days after ingesting, by overexciting the insect nervous system. Other physiological effects of this insecticide are not documented. We used a sub-lethal dose of spinosad to treat *Blattella germanica* adults and we observed the subsequent consequences on both the cockroach cuticular profile and their aggregative and sexual behaviours.

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Modification of Aphid Behaviour with Natural Monoterpenoids**Gabrys B¹**, Dancewicz K¹, Sznajder K¹, Rowińska E¹, Kordan B²¹ *University of Zielona Góra, Poland*² *University of Warmia and Mazury, Poland**b.gabrys@wnb.uz.zgora.pl*

Terpenes are chemicals of considerable biological activity, widely distributed in nature. The terpene molecules, of cyclic or acyclic structure, consist of joined isoprene units (monoterpene: $n = 2$). The effect of acyclic (citral, linalool), monocyclic (*p*-cymene, α -ionone, β -ionone, S-limonene, R-limonene, S-pulegone, R-pulegone, α -terpineol) and bicyclic (camphene, β -pinene) monoterpenes on the behaviour of the peach potato aphid *Myzus persicae* (Sulz.) was studied by direct observation and electronic registration of stylet penetration (EPG). Citral and linalool had repellent activity, which was manifested in the significant decrease in time spent on leaves, decrease in total and mean time of penetration, and reduced number of probes as compared to control. Citral, linalool, S-limonene, α -ionone, and camphene were feeding deterrents and reduced the total and mean probing time of aphids and their settling on the leaves. There was a difference in activity between the isomers of a given compound: α -ionone was more active than β -ionone, R-pulegone was more active than S-pulegone, and S-limonene was more active than R-limonene. A strong – lasting for 24 hours – feeding deterrent activity was exhibited by the following monoterpenes: citral, linalool, S-limonene, α -ionone, and camphene. The feeding deterrent activity of R-limonene, *p*-cymene, and β -pinene lasted less than 24 hours. β -ionone and R-pulegone were active after 1 hour. S-pulegone and α -terpineol had a very weak deterrent activity to *M. persicae*.

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Terpenoids and Flavonoids of European Larch *Larix decidua* Mill. as Defense Chemicals against Larch Woolly Adelgid *Adelges laricis* (Vallot)**Gabrys B¹, Dancewicz K¹, Rowińska E¹ Goławska S², Szumny A³**¹ *University of Zielona Góra, Poland*² *University of Podlasie, Poland*³ *University of Life Sciences in Wrocław, Poland**b.gabrys@wnb.uz.zgora.pl*

Adelgids (Hemiptera: Aphidoidea: Adelgidae) form a small group of 70 described species that occur in boreal and temperate environments of Northern Hemisphere. Adelgids are cyclically parthenogenetic and exhibit multigeneration complex life cycles. Adelgidae are highly host specific and they feed only on certain genera in the Pinaceae. Larch woolly adelgid *Adelges laricis* (Vallot) is holocyclic and alternates between spruce *Picea* spp. (primary host) and larch *Larix decidua* Mill. (secondary host). On spruce, *A. laricis* causes the development of cone-shaped galls. After overwintering, larch woolly adelgid migrate to larch trees where they feed on needles through late autumn. In our study, we compared the terpenoid and flavonoid profile of larch needles of trees infested and not infested with adelgids. The terpenoid fraction consisted of 31 different compounds. We found that adelgids avoided trees that contained considerable amount of isobornyl acetate in comparison to the trees that were accepted. In flavonoid fraction we found three compounds. There were no significant differences in absolute and relative concentration of individual compounds in infested and not infested trees.

Antimicrobial Defence of the Invasive Multicolored Ladybird *Harmonia axyridis* in Comparison to the Native *Coccinella septempunctata*

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The multicoloured ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae) is a polyphagous predatory beetle native to Central and Eastern Asia. It has been widely used as a biological control agent of aphids in America and Europe, in greenhouses as well as in field crops. Since 2007 it has established all over Central Europe and is widespread in Germany. Roy et al.¹ showed that adult *H. axyridis* were less affected by an infection with the entomopathogenic fungus *Beauveria bassiana* than the native European species *Adalia bipunctata* and *Coccinella septempunctata*. Antimicrobial defence of beetles can be exerted by either innate immune system or volatile fumigants². In order to elucidate which defence strategy is responsible for the disease resistance, we tested both the hemolymph and main components collected from headspace of *H. axyridis* for their antimicrobial activities. While some main headspace components like pinene or limonene weakly influenced the growth of microorganisms, the hemolymph of both adults and larvae of *H. axyridis* strongly inhibited the growth of the bacteria *Escherichia coli* and *Micrococcus luteus* as well as the yeast *Saccharomyces cerevisiae*, compared to *C. septempunctata* specimens. In contrast to the native species, the strong antimicrobial activity was even present without a prior contact of *H. axyridis* to microorganisms, which induces the humoral immune defence in native coccinellids.

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FT-IR and MALDI-TOF Imaging of Leaf Epicuticular Wax in *Medicago truncatula*

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Epicuticular waxes, exposed at the outermost surface of plant, are not simple physical permeability barrier, but play crucial roles in interaction with environment, protect against UV radiation, prevent invasion of pathogenes and excessive lost of water. Mechanical damage and herbivore feeding elicit defense responses in *Medicago truncatula*, including the emission of terpenoids¹. In contrast, the contribution of epicuticular waxes during this event is poorly investigated. Therefore, we present spatial distribution of cuticular wax components for the leaves of *M. truncatula* after continuous mechanical damage with *MecWorm*; a computer controlled robotic caterpillar. A combination of GC-MS, FT-IR microspectroscopy-, MALDI-TOF imaging were employed to characterize content and distribution of epicuticular waxes cryo-adhesively separated² from adaxial side of mechanically damaged leaves. An *in situ* MALDI-TOF measurements using Li⁷-DHB matrix³ was performed. For both imaging technique the rectangular aperture was set at 30 x 30 μm^2 and raster pattern in increments of 10 μm . The spectra recorded were analysed to construct both FT-IR and MALDI-TOF images, using *Opus* (Bruker) and *BioMap* (Novartis) software. The difference spectrum was subtracted from selected FT-IR image area, and thus enabled the further assignment of higher concentration of fatty acids around wounded area. Accordingly, stronger peaks at 2900 cm^{-1} , 1700 cm^{-1} and 1000 cm^{-1} had previously been recorded as characteristic stretches⁴, of very long chain alkanes and fatty acid were observed. MALDI-TOF image of the adaxial surface showed that tetradecanoic acid (m/z 228.4) and tetracosanoic acid (m/z 368.6) accumulate over the periclinal regions of the healed area compared to non-wounded *M. truncatula* leaves. Other long chain aliphatic constituents are distributed evenly across the entire adaxial surface. Thus, epicuticular waxes contribute to the mechanically-damaged wound healing. The capability of above imaging technique for the *in situ* investigation of wax distribution triggered by mechanical damage is discussed.

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EST Mining For Fragrance-Related Genes in *Silene* (Caryophyllaceae) and *Gymnadenia* (Orchidaceae)

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Our study is focused on two sister species of *Silene* (*S. latifolia* & *S. dioica*) and *Gymnadenia* (*G. odoratissima* and *G. conopsea*). These closely related sister species pairs differ in their odor composition and pollinator assemblage. Main questions of our research project are: (i) what is the molecular basis of key floral scent compounds produced in *Silene* and *Gymnadenia* species? (ii) what are the evolutionary patterns of key scent-related genes and what will be the consequences of selection of such genes? In order to obtain an understanding of the genes expressed in floral parts, we have produced a number of standard (flower specific) and suppression subtractive hybridization (flower vs. leaf; night flower vs. day flower) cDNA libraries in *S. latifolia* and *G. odoratissima*. A total of 2,659 (*G. odoratissima*) & 2,489 (*S. latifolia*) cleaned vector sequence clipped sequences were obtained and assembled into 1030 (*G. odoratissima*) & 932 (*S. latifolia*) tentatively unique genes (TUGs). Of these total TUGs, only 655 & 813 TUGs showed significant similarities with publically available non-redundant protein database (NCBI) in *G. odoratissima* and *S. latifolia* respectively. The analysis of these EST collections allowed us to identify several important genes including fourteen key candidate scent-related genes in both the species. These partial gene fragments were also further used to isolate 7 full-length cDNA sequences in our focal species. Thus, the EST resource developed here should serve as a valuable resource for floral research scientific communities. The poster will outline results from comparative EST sequence analysis, gene ontology (GO) classification and functional characterization of key scent-related genes.

Application of Wood Vinegar as a Wood Preservative against Fungi

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Korea has a lot of wooden cultural heritages, which are likely to be contaminated by microorganism, especially fungi. They damage and transfigure the wooden cultural heritages by degrading lignin and cellulose which are the main components of wood. So we should conserve from fungi and develop the preservative to keep the original shape. Recently, wood vinegar is thought of the possible agent as one of the medicine about fungi. However, there is no information on the wood vinegar effects against fungi deteriorating wooden cultural heritages. So we examined possibility of wood vinegars as wood preservative against fungi. 13 fungi tested in this study were provided from Korean Agricultural culture collection (KACC) and isolated from janggyeong Panjeon (UNESCO world heritage). And *Pinus densiflora* and *Zelkova serrata* the wooden cultural heritages in Korea take up mostly were used for antifungal activity. The used wood vinegar (oak type) was concentrated with the rotary vacuum condenser and fractionated by dichloromethane, ethylacetate, butanol. Antifungal activities of wood vinegar were examined by a disc diffusion method in PDA and ASTM method in sapwood specimens of 2 trees. As the result, dichloromethane fraction showed the highest effect at the concentration of 5.0 mg/disc. And it got inhibitory effect in sapwood of two wood specimens at 0.1g/ml concentration rate. Especially, Fungi were completely inhibited on *Pinus densiflora* but *Zelkova serrata* showed the slight growth of mold. It seemed to be more susceptible than *Pinus densiflora* against fungi. In conclusion, wood vinegar mostly showed high antifungal activity against fungi and among three fractions, the dichloromethane fraction inhibited strongly on *C. cladosporioides*, *A. versicolor*, *P. chrysogenum* (KACC42216). Thus, we think wood vinegar is a possible agent as a preservative against fungi and an effective material on growth inhibition of even wood specimens treated by fungi.

Bee Moth *Aphomia Sociella* and Scent of Host Bumblebee NestJiros P^{1,2}, Kalinova B¹, Kindl J¹, Zacek P¹, and Valterova I¹¹ UOCHB AV CR vvi, Flemingovo nam. 2, Praha 6, 166 10, Czech Republic² Institute of Chemical Technology Prague, Technicka 5, 166 28, Czech Republic

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Aphomia sociella (Lepidoptera, Pyralidae, Galleriinae) is one of the most important parasites of bumblebees. The larvae attack the nests, destroy the brood combs, eat food storages and host offspring. Contrary to majority of lepidopteran species, *A. sociella* premating behavior is initiated by males that emit sex pheromone from wing glands¹. We identified the composition of *A. sociella* sex pheromone recently², but place of mating behavior and the way of nest infestation are not known. We observed that it takes just a few days for the moths to find a bumblebee nest. Thus, the nest scent may be a chemical cue. Here we report data on the nature of attracting chemical signals guiding *A. sociella* moths to the host nest. Volatiles from *B. terrestris* nests were collected in laboratory using dynamic head space technique. Samples were analyzed using GC-EAD and GCxGC-MS. Analyses revealed 7 antennally active compounds in the collected samples.

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Insecticidal Activity of Wood Vinegar against *Lasioderma serricorne*, Fabricius

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The wood vinegar (*Quercus*) traditionally known as effective insecticide was screened for the insecticidal activity against *Lasioderma serricorne*, Fabricius deteriorating the wooden cultural heritage widely in Korea. The insecticidal activity against *L. serricorne* F. adults was examined by the fumigation method in the filter paper (70 mm) applied to 250 μ l of the crude wood vinegar and the fractions divided into the dichloromethane, ethyl acetate and n-butanol respectively with different concentration rate. Experiment was carried out and kept in 28°C, 70% condition. Morality and knockdown of cigarette insects were counted for 72hours after treatment. As the result of this study, cigarette insects treated by the dichloromethane fraction compared with other fractions got highest morality. And they showed slowest activity in spite of the survival condition. By contrast, n-butanol fraction showed the lowest morality and even nearly same condition as the control treatment. All fractions except the control treatment exhibited the sharp morality in 3hours and fumigant treatment in more than 2days showed no effect by fumigant treatment. These results showed that dichloromethane fraction of wood vinegar presented possibilities as preservative for conservation of wooden cultural heritage against biological deterioration by insects, especially *Lasioderma serricorne*, Fabricius. Thus, we have plan to develop the preservative made from the wood vinegar, especially dichloromethane fraction and investigate the fumigant effect widely on other insects. Throughout the result, we think that the treated wooden cultural heritages would get fast effect for short time if we apply to fumigant treatment in wooden cultural heritages for conservation against the cigarette insects.

Volatiles Emitted by *Serratia odorifera* 4Rx13

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Some bacteria produce rich volatile – spectra, e.g. *Streptomyces* spp., *Bacillus* spp., *Myxococcus* spp.. Previous studies demonstrated that *Serratia odorifera* is also able to release an opulent range of volatiles^{1,2}. The role of such volatile emissions is still not well understood. The volatiles of *S. odorifera* were shown to suppress the growth of the plants *Arabidopsis thaliana* and *Physcomitrella patens* as well as fungi including phytopathogenic strains (*Rhizoctonia solani*, *Sclerotinia sclerotiorum*). The volatile emission profile of *S. odorifera* was now investigated in more detail. Altogether 73 compounds could be detected; a maximum of emission of both, the quality and quantity of compounds was reached in the early stationary phase. The distribution of volatiles in the different growth intervals was very similar. The typical alkyipyrazines released as main components of a specific *S. odorifera* strain² was not present in the volatile profile of *S. odorifera* isolate 4Rx13. The latter, however emits dimethylpyrazine, and furthermore dimethyldisulfide (DMDS), dimethyltrisulfide, 2-phenylethanol at higher levels and “Sodorifen” (a newly identified compound) is the main compound and contributes with ca. 45% to the total amount of volatiles. In assays DMDS exhibits antagonistic effects to *A. thaliana* and to some fungi (IC50: 20 µmol). Additionally, inorganic volatiles, e.g. NH₃ and CO₂ were also released from *S. odorifera* 4Rx13.

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Fat Body Studies of *Bombus terrestris* Queens in Various Life PhasesKofronová E^{1,2}, Šobotník J¹, Jiroš P^{1,2}, Komzáková O¹, Valterová I¹¹ Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo nám. 2, Prague 166 10, Czech Republic² Institute of Chemical Technology, Technická 5, Prague 166 28, Czech Republic

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Bumblebees are important pollinators. Their queens are characterized by their longevity and ability to hibernate¹. In insects, the major tissue for intermediary metabolism and storage of nutrients is the fat body. As to bumblebee's males, triacylglycerols (TAGs) could be precursors of aliphatic compounds in the marking pheromone². After eclosion, the fat body of bumblebees is developed and reserves of fat and glycogen are accumulated³. As our recent research shows, the amount of TAGs and their profiles are caste-specific. Moreover, some inter-caste qualitative differences were observed as well, but some individual differences exist. Therefore, we focused on analyzing bumblebee queens' fat bodies of various life phases. We did parallel assays of glycogen and neutral lipids of *Bombus terrestris* queens after eclosion, before hibernation, and after hibernation with 20 samples within each category. Then we extracted TAGs from 5 individuals from each category and we measured their TAG profiles by LC-MS. The ultrastructure of the fat body was studied in all three life phases. The results show that the queens after eclosion have minimum of glycogen, namely 0.1 mg per individual, and in average 11 mg of neutral lipids. The queens before hibernation have about 2.29 mg of glycogen and 35 mg of neutral lipids per individual. The queens after hibernation have about 2.33 mg of glycogen and 6 mg of neutral lipids per individual. These results correspond with the image analysis of the TEM photographs.

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Modifications of the Chemical profile of Hosts after Parasitism Allow Parasitoid Females to Assess the Time Elapsed since the First Attack

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In solitary parasitoids, only one adult can emerge from a given host. When several eggs are laid on the same host, supernumerary individuals are eliminated by lethal larval fights. In the solitary parasitoid *Anisopteromalus calandrae*, the probability a second larva win the fight strongly depends on the time elapsed since the first oviposition¹. The most the first egg is old at the moment at the second egg is laid, the less the second egg have chance to win the competition. As a consequence, females of this species lay preferentially their eggs on recently parasitized hosts rather than on hosts parasitized by an egg about to hatch¹. *A. calandrae* females parasite bruchid larvae located into cowpea seeds. In a series of choice test experiments using an artificial seed system, we demonstrated that the cue perceived by parasitoid females allowing them to discriminate hosts parasitized for different times is not brought by the seed nor by the egg previously laid but by the host. Moreover, pentane extracts of bruchid larvae elicited the same response than hosts themselves. This cue is perceived at short range distance, indicating that chemicals involved in this discrimination are probably partly volatile. Chemical analyses of pentane extracts show differences in the cuticular profiles of hosts after parasitism which evolve with time. These differences could thus be at the origin of the discrimination by parasitoid females.

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A Chemical Signal of Offspring Quality Affects Maternal Care in a Social InsectMas F¹, Haynes KF², Kölliker M¹¹ *University of Basel, Zoological Institute, Evolutionary Biology, Vesalgasse 1, 4051 Basel, Switzerland*² *University of Kentucky, Department of Entomology, Lexington, KY 40546-0091, USA**flore.mas@stud.unibas.ch*

Begging signals of offspring are condition-dependent cues that are usually predicted to display information about the short-term need (i.e., hunger) to which parents respond by allocating more food. However, recent models and experiments revealed that parents, depending on the species and context, may respond to signals of quality (i.e., offspring reproductive value) rather than need. Despite the critical importance of this distinction for life-history and conflict resolution theory, there is still limited knowledge of alternative functions of offspring signals. In this study, we investigated the communication between offspring and caring females of the common earwig, *Forficula auricularia*, hypothesizing that offspring chemical cues display information about nutritional condition to which females respond in terms of maternal food provisioning. Consistent with the prediction for a signal of quality we found that mothers exposed to chemical cues from well-fed nymphs foraged significantly more and allocated food to more nymphs compared to females exposed to solvent (control) or chemical cues from poorly fed nymphs. Chemical analysis revealed significant differences in the relative quantities of specific cuticular hydrocarbon compounds between treatments. To our knowledge, this study demonstrates for the first time that an offspring chemical signal reflects nutritional quality and influences maternal care.

The Role of Phenolics and other Mechanisms of Resistance of Poinsettia to the Silverleaf Whitefly

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The silverleaf whitefly (SLWF), *Bemisia tabaci* biotype B, is one of the most prevalent insect pests found attacking poinsettias (*Euphorbia pulcherrima*, Euphorbiaceae), USA's top selling potted ornamental. This project is the first one to evaluate the impact of physical and chemical resistance traits in economically relevant poinsettia cultivars and how these traits affect SLWF. Preliminary data showed that dark green leaf cultivars appeared to be more resistant and less preferred by the SLWF than light green leaf cultivars. Based on this evidence, two cultivars, one resistant (dark green leaf), and one susceptible (light green leaf), were chosen to evaluate and compare particular physical and chemical traits. The purpose of this part of the study is to elucidate the role of phenolic compounds, as a possible chemical trait associated with resistance. Research in a similar system, cassava (*Manihot esculenta*, Euphorbiaceae) with mealybugs (*Phenacoccus manihoti*), has shown a correlation between phenolics present in the phloem sap and antibiotic resistance¹. Phenolic compounds have been detected in phloem sap of poinsettias², but characterization and concentration of such compounds has not been reported. To evaluate plant resistance effects on SLWF we will measure survivorship and fecundity of F1 SLWF between the selected varieties. Additionally, physical mechanisms of resistance were investigated. From our studies we have shown that visual cues are strongly associated with preferential adult SLWF settling and oviposition, significantly contributing to the higher susceptibility of light green leaf cultivars. Among the physical traits we have found associated with this visual preference are chlorophyll content and leaf thickness, since the susceptible cultivar has significantly less chlorophyll content and thinner leaves compared to the resistant poinsettia cultivar. Trichome density was evaluated but no significant differences were observed. The applications for integrated pest management arising from this study will be discussed.

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Repellent Activity and EAG Response of Caraway and Clove Bud Oil against Bean Bug *Riptortus clavatus* (Thunberg) (Hemiptera: Alydidae)

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Thirteen plant essential oils were tested for their repellent activity against the bean bug *Riptortus clavatus*. Among the tested oils, caraway (100%) and clove bud oil (92%) significantly repelled the bean bugs at a dose of 0.142 $\mu\text{l}/\text{cm}^2$ by using a Y-tube olfactometer. GC and GC-MS analyses revealed that the active components responsible for the effective repellency of caraway and clove bud oil were carvone (75%) and limonene (76.9%); eugenol (100%), isoeugenol (54.3%) and β -caryophyllene (60.0%), respectively. Of the different active fractions, eugenol was the most significant one than the other components with reference to repellent activity against the bean bugs. In the GC-EAD, the antenna of *Riptortus clavatus* was responded to the volatile of limonene and carvone of caraway oil and eugenol and β -caryophyllene of clove bud.

Feeding Stimulant against Adult of Pine Sawyer beetle, *Monochamus saltuarius* Gebler (Coleoptera: Cerambycidae)

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Pine sawyer beetle, *Monochamus saltuarius* preferred the current twig than one-year and two-year twig during the feeding. It means that the current twig is appropriate to lengthen the lifespan and to increase the reproduction of this insect. In a bioassay after fractionated the hexane layer from the water layer, the hexane layer did not show feeding response; however, the water layer was preferred in order of current twig > one-year twig > two-year twig. From the analysis of sugar by HPLC, we identified three kinds of sugar, namely, fructose, sucrose, glucose. The quantity of fructose was the highest in current twig and next order was one-year twig > two-year twig. In the preference test using each standard sample, the order was fructose > sucrose > glucose at 10, 30 ug/filter paper, and fructose > sucrose > glucose at 50ug/filter paper. From the above result, fructose plays an important role as a feeding stimulant against pine sawyer adult.

Diurnal and Nocturnal Herbivory on Maize Leaves Elicit Different Response of the Fall Armyworm Parasitoid, *Campoletis flavicincta*

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Plants induced by herbivory produce specific volatile substances that attract natural enemies of herbivores. This response occurs a while after the time of induction, as a result of chemical reactions that change the plant physiology. Fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) can feed on maize all periods of the day. It has been shown that plant needs light to synthesize *de novo* herbivore-induced volatiles. Therefore, volatile emission may be changed depending on which time of the day the plant is induced which could interfere in natural enemy foraging. In this sense, the current study aimed to investigate the differential attractiveness of maize elicited by fall armyworm regurgitant under light and dark conditions to its larval parasitoid *Campoletis flavicincta* (Ashmead) (Hymenoptera: Ichneumonidae). All bioassays were conducted in Y-tube olfactometer during photophase to assess the response of the parasitoid to odors from undamaged maize, mechanical damage and simulated herbivory at time intervals 0-1, 5-6 and 24-25h. For the time 5-6h it was evaluated the attractiveness of plants induced in light or dark by inducing at 03:00pm and 08:00am and measuring the parasitoid choice at 08:00-09:00am and 01:00-02:00pm. Light in the room were turned on at 6:00am and turned off at 8:00pm. The results showed that naïve *C. flavicincta* were attracted to volatiles emitted by maize plants at 5-6h after the treatment with regurgitate in scotophase. Interestingly, they were not attracted to volatiles released at the same time interval elicited in the photophase. None of the other treatments were significantly attractive to wasps. These results suggest that the differential attractiveness of diurnal and nocturnal induction is due to a physiological wound response of the plant depending on the light conditions at the moment of induction or to an intrinsic response of parasitoid behavior, what it will be elucidated in future studies.

(1R,2S,5S)-Iridodial, an Aposematic Signal of *Chloridolum lochooanum*Nakashima T¹, Ohmura W¹, Makihara H¹, Nakamura K²¹ Forestry and Forest Products Research Institute² Faculty of Horticultural Science, Chiba University

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Freely flying adults of *Chloridolum lochooanum* were observed to secrete a fragrant odor in the daytime at clear-cur field, and not observed to be attacked by predators¹. The metasternal gland excretion of the disturbed beetles was identified to be (1R,2S,5S)-iridodial and assigned as a defensive chemical². The identity of a fragrant odor and a defensive chemical, (1R,2S,5S)-iridodial suggested that defensive weapon of the flying beetles may act rather as an aposematic volatile signal to deter predators from chasing them than as a physiologically harmful compound to counterattack the predator.

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Identification, Biosynthesis, and Function of Bacterial VolatilesNawrath T¹, Thiel V¹, Dickschat J¹, Sasse F², Kunze B², Müller R³, Schulz S¹¹ *Institut für Organische Chemie, TU Braunschweig, Hagenring 30, 38106 Braunschweig, Germany*² *Helmholtz-Zentrum für Infektionsforschung, Inhoffenstraße 7, 38124 Braunschweig, Germany*³ *Institut für Pharm. Biotechnologie, Universität des Saarlandes, Geb. C2.3, 66123 Saarbrücken, Germany*

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Many bacteria produce and emit volatile compounds. We have investigated many of these volatiles, using headspace techniques like Closed Loop Stripping Analysis, GC-MS-analyses, and following synthesis of the molecule to proof the structure proposal. During our investigations we have found five major classes of compounds, belonging to different biosynthetic groups, that have been produced by various strains of different bacterial classes. Most compounds are either fatty acid derivatives, aromatic compounds, pyrazines, terpenoids, or sulfur compounds. The ecological significance of these volatiles is mostly unknown to date, but aspects like chemical defense or communication come immediately to mind. After proving the structural proposal by synthesis the biological activities of the corresponding compounds were evaluated in different bioassays. Interestingly, some of the volatiles were strongly active in the inhibition growth of other microorganisms in diffusion assays, while selected ones showed inhibitory influence on fruiting body formation in myxobacteria. Activities on a cancer cell line were generally weak, but individual components showed a high activity given to the small size of the molecule. Furthermore the biosyntheses of different prominent volatiles were investigated in feeding experiments using labeled precursors and mutant strains.

Nicotine in Nectar: Consumption and Perception by Honey Bees and its Effect on their SurvivalAfik O^{2,3}, Inbar M², Ne'eman G¹, Shafir S³, Izhaki I²

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The role of secondary compounds in deterring herbivores from vegetative parts of plants is well established, whereas their role in plant reproductive organs remains unclear. The present study tested various effects of nicotine on honey bees as a model system to understand the possible roles of secondary compounds in floral nectar. Eight honey bee colonies were tested for their preference among six feeders containing all the combinations between two sugar concentrations (20% or 50% w/w) and three nicotine concentrations (0, 5 or 50 ppm). Individual bees from these colonies were tested for the effect of nicotine on learning performance using the proboscis extension response paradigm. Afterwards, half of the tested colonies were fed for three weeks with pure sucrose solution and the rest were fed with sucrose solution enriched with nicotine. Then the bees were tested again for their preference and learning. In addition, harnessed bees were tested for their daily food consumption and survival while being fed with sucrose solution with various nicotine concentrations. We found that nicotine repels bees, but repellency can be partially overcome by increased sugar concentration and that early exposure to nicotine may decrease its repellency. Nicotine added to the reward did not affect learning performance and survival, but at high concentration it decreased food consumption. We conclude that nicotine concentration, in the range that naturally occurs in nectar (up to 2.5 ppm), repels bees. Higher concentrations are more repellent and reduce consumption. Interestingly, prolonged exposure to nicotine resulted in habituation to nicotine (though a follow up experiment failed to confirm this effect). Thus, nicotine in floral nectar may serve as a repellent for occasional visitors, but less so to regular visitors, which would be more efficient pollinators.

Comparative Electrophysiological Responses of a Specialist (*Microplitis croceipes*) and Generalist (*Cotesia marginiventris*) Parasitoid to Cotton Volatiles Induced by two Caterpillar Species

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Herbivore-induced plant volatiles are used by parasitoids for host location. Plants emit volatile blends that may be quantitatively and qualitatively different in response to different herbivore species. These differences may convey herbivore-specific information to parasitoids and thus shape their foraging strategies. One current theory regarding the evolution of parasitoid host location strategies is that the degree of specificity of the signals needed by a parasitoid to successfully locate its host correlates with its level of host specificity. This hypothesis was tested using as models two parasitoids of cotton caterpillars with different degrees of host specificity: *Microplitis croceipes* (Hymenoptera: Braconidae), a specialist parasitoid of *Heliothis* spp. and *Cotesia marginiventris* (Hymenoptera: Braconidae), a generalist parasitoid of several caterpillar genera including *Heliothis* spp. and *Spodoptera* spp. We compared GC-EAD (coupled gas chromatography electroantennogram detection) responses of both parasitoid species to headspace volatiles of cotton plants damaged by *H. virescens* (a host species for both parasitoids) versus *S. exigua* (a host species for *C. marginiventris* but not for *M. croceipes*). Qualitative and quantitative differences were recorded in the composition of the volatiles emitted by cotton plants in response to feeding by the two caterpillar species. Importantly, notable differences were recorded in the GC-EAD responses of both parasitoid species to the volatiles. The generalist (*C. marginiventris*) showed comparatively greater GC-EAD response than the specialist (*M. croceipes*) to the green leaf volatile components of cotton headspace, whereas the inducible components elicited relatively greater response in the specialist. These results suggest that herbivore-induced plant volatiles may play an important role in mediating host specificity in specialist parasitoids.

Antennal Response of Codling Moth (*Cydia pomonella*) to Dilute Concentrations of Apple Volatile Chemicals

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The codling moth (*Cydia pomonella*) is an important pest in apple and pear orchards throughout most of the world. The objective of this study was to identify volatile compounds from immature apples that elicit antennal responses at lower concentrations. It is thought that these lower concentrations may be more relevant to our search for compounds that are attractive at some distance from the apple. Volatile compounds were collected from immature apples and analyzed by gas chromatography coupled with electroantennographic detection (GC-EAD). The apple volatile samples were analyzed at increasingly diluted concentrations to 1) determine whether lower concentrations triggered fewer antennal responses, and 2) identify the compounds that elicited antennal responses at the lower concentrations. At the lower concentrations, four compounds were identified which consistently elicited antennal responses in gravid female codling moths.

Investigating the Interaction Between Maize and the Leafhopper, *Cicadulina storeyi* China (Homoptera: Cicadellidae), a Major Vector of Maize Streak Virus

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The chemical ecology of the leafhopper, *Cicadulia storeyi* China (Homoptera: Cicadellidae), a major vector of Maize Streak Virus (MSV), the most important virus affecting maize, *Zea mays* L. (Poaceae), throughout sub-Saharan Africa¹, has been studied with a view to understanding the potential for exploiting induced and constitutive defence pathways in novel leafhopper control strategies. Volatile organic compounds (VOCs) were collected from 10-12 day-old uninfested and *C. storeyi* - infested maize seedlings (cv: Delprim). Analysis of the VOCs by gas chromatography (GC) and coupled GC-mass spectrometry (GC-MS) confirmed the release of previously reported VOCs from un-infested seedlings, i.e. myrcene, linalool, (*E*)-2-decen-1-ol and decanal, and from insect-infested maize seedlings, i.e. (*Z*)-3-hexenyl acetate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), benzyl acetate, indole, geranyl acetate, (*E*)-caryophellene, α -bergamotene, (*E*)- β -farnesene, β -sesquiphellandrene and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene². Methyl salicylate, which is known to be produced as a herbivore and pathogen-induced plant VOC^{3,4}, was also identified in the blend of *C. storeyi* – infested plants. Y-Tube olfactometer studies showed that VOCs from un-infested maize seedlings were more attractive to *C. storeyi* than the control ($P = 0.003$), whereas VOCs from *C. storeyi*-infested maize seedlings were less attractive than the control ($P = 0.021$). *C. storeyi* also preferred VOCs from un-infested maize seedlings compared to VOCs from *C. storeyi*-infested seedlings ($P < 0.001$). When methyl salicylate was added to the blend of VOCs from uninfested maize seedlings, *C. storeyi* preferred the control arm ($P = 0.016$). Finally, *C. storeyi* preferred the control arm over methyl salicylate when tested alone ($P = 0.002$). These results show that induced plant defence in maize has the potential to be exploited in the control of viruliferous leafhoppers in sub-Saharan Africa.

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Selective Iridoid Glycoside Sequestration by two Sawfly Species of the Genus *Athalia*Opitz SEW¹, Jensen SR², Müller C¹¹ Department of Chemical Ecology, University of Bielefeld, D-33615 Bielefeld, Germany² Department of Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark

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Iridoid glycosides (IG) are characteristic secondary plant metabolites of Plantaginaceae. Larvae of the sawfly species *Athalia circularis* and *A. cordata* (Hymenoptera: Tenthredinidae) are specialized on species of this plant family. Therefore, the ability and specificity of IG sequestration in the sawfly species was investigated. The main IG fraction of the host plant *Veronica beccabunga* is comprised of four catalpol esters, whereas the concentration of catalpol itself is very low. Additionally aucubin and some other IG were identified in this plant. In both sawfly species high amounts of catalpol were concentrated in the larval hemolymph, followed by relatively low amounts of aucubin. No catalpol esters were detectable in larval hemolymph as well as in the frass of this species. Instead the esterified protocatechuic acid of the catalpol ester verproside could be identified in the frass of *A. cordata*. This indicates that the esters are hydrolyzed in the insect and catalpol is sequestered selectively. Other IG of the host plant (e.g. epiloganic acid) were excreted by the sawfly larvae unchanged. To test for an ecological relevance of selective catalpol sequestration the feeding deterrence of catalpol ester versus catalpol against the ant *Myrmica rubra* was investigated.

Electrophysiological and Behavioral Responses of *Leiodes cinnamomea* to Volatiles Isolated from Conspecifics and its Host FungusOrtiz A¹, Hidalgo F¹, Pérez-Andueza G²¹ *Inorganic and Organic chemistry Dept. EPS Linares. University of Jaen. 23700 Linares, Spain*² *Agroforestry and Environmental Dept. Universidad Católica de Ávila, 05005 Ávila Spain*

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The truffle beetle, *Leiodes cinnamomea*, (Coleoptera: Staphylinoidea, Leiodidae) (Panzer, 1793) is an important pest of black truffle (*Tuber melanosporum*). Through their feeding and oviposition habits, both the adults and the feeding larvae, contribute to economic losses by the destruction of truffles, but little is known¹ about the chemical communication between truffle beetles. Electrophysiological and behavioral responses of the truffle beetle to host volatiles from the black truffle and from conspecific insects were investigated to identify semiochemicals involved in insect attraction. Volatile compounds released by adult insects and ripe truffle airborne volatiles were collected by dynamic headspace adsorptions on Tenax-TA or static adsorptions by using solid-phase microextraction (SPME). Coupled gas chromatography-electroantennography (GC-EAG) and gas chromatography-mass spectrometry (GC-MS) resulted in the identification of at least five EAD active compounds from airborne volatiles release for male and female adults, including: 6-Methyl-5-hepten-2-one, camphor and 1-octen-3-ol. From selected compounds from ripe truffle odours, showed that only Dimethylsulfide (DMS) significantly elicited electroantennogram response and some did not evoke EAG responses at all. The overall EAG response profiles of the male and females to the tested fungus odours were similar. The EAG-active compounds were subsequently investigated for behavioral activity in an arena bioassay. The results of this behavioral assay showed that, at the doses tested, camphor and DMS were significantly better than the six tested compounds at stimulating male and females to land and walking locomotors reactions on target.

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Specialist Egg Parasitoid *Telenomus remus* Uses Herbivore-induced Volatiles or Green Leaf Volatiles from Maize as Cues to Find its Host, *Spodoptera frugiperda*

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Egg parasitoids face many challenges in host search because cues derived from the eggs themselves are hard to detect. Oviposition-induced volatiles are known to act in the host search by egg parasitoids¹, however, this plant response was only reported in three tritrophic systems so far. In contrast, insect herbivory commonly induces volatile emission by the plant attracting natural enemies. Herbivore-induced volatiles do not represent direct cues of eggs, although they may indicate indirectly the presence of eggs in the case of multivoltine hosts. In this sense, the current study aimed to investigate the olfactory response of the specialist egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Scelionidae) to volatiles released by fresh mechanical damage in maize (mainly green leaf volatiles-GLVs) and herbivore-induced volatiles elicited by *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) regurgitant in different time intervals. All bioassays were carried out in a Y-tube olfactometer during photophase to assess the response of naïve and experienced wasps to odors from undamaged maize, fresh mechanical damage, or simulated herbivory at time intervals 6-7 and 12-13h. Experienced wasps were attracted to fresh mechanical damage and 6-7h herbivore-induced volatiles, whereas naïve wasps didn't respond to any of the treatments. These results indicate that *T. remus* is able to learn to respond to volatiles induced by fall armyworm herbivory as well as to GLVs. Searching for host community using herbivore-induced volatiles seems to be an adaptive behavior of *T. remus*. However, learning GLVs is not expected to be a successful strategy as they are common to several plant species and are not specific of host damage.

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SuperScent - a Database of Flavors and Scents

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Volatiles are efficient mediators of chemical communication acting universally as attractant, repellent, or warning signal in all kingdoms of life. Beside this broad impact volatiles have in nature, scents are also widely used in pharmaceutical, food, and cosmetic industries, so the identification of new scents is of great industrial interest. Despite this importance as well as the vast number and diversity of volatile compounds, there is currently no comprehensive public database providing information on structure and chemical classification of volatiles. Therefore, the database 'SuperScent' was established to supply users with detailed information on the variety of odor components. The version of the database presented here comprises the 2D/3D structures of approximately 2,100 volatiles and around 9,200 synonyms as well as physicochemical properties, commercial availability, and references. The volatiles are classified according to their origin, functionality, and odorant groups. The information was extracted from the literature and web resources. 'SuperScent' offers several search options, e. g. name, Pubchem ID number, species, functional groups, or molecular weight.

'SuperScent' is available online at: <http://bioinformatics.charite.de/superscent>.

Chemical Camouflage in the Codling Moth, *Cydia pomonella*, Feeding on Different Host Plant SpeciesPiskorski R¹, Trematerra P², Dorn S¹¹ ETH Zurich, Institute of Plant Sciences, Applied Entomology, 8092 Zürich, Switzerland² University of Molise, Department of Animal, Plant and Environmental Science, 86100 Campobasso, Italy

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Cuticular hydrocarbons (CHCs) of fruit endophytic larvae of the codling moth, *Cydia pomonella* L. (Lepidoptera, Tortricidae) were reported to resemble those of its main host, the apple fruit. To evaluate the role of diet in potential chemical camouflage, we have analyzed the CHCs of larvae that developed on: (I - ii) two unrelated host plant species, the apple (*Malus domestica* Borkh., Rosaceae) and the common walnut (*Juglans regia* L., Juglandaceae) from the same region (Molise, Italy); and (iii) artificial diet. In parallel, the hydrocarbons of host fruits and artificial diet were analyzed. Our results show quantitative differences between CHC profiles (consisting of *n*-alkanes, C23-C31). Quantities in the CHCs from the walnut-collected larvae are shifted towards higher alkanes, in comparison to the apple-collected larvae, and a similar shift is observed between the walnut and apple fruits. Similarity of the CHC profiles is higher between larvae from apple and apple fruits than between larvae from walnut and walnut fruits, suggesting a higher level of camouflage in apple populations, and indicating that the apple population might be the progenitor of the walnut population. Finally, the comparison of the CHCs of larvae reared on artificial diet with the diet itself proves that the larval hydrocarbons are biosynthesized, at least at their larger share, by the moth itself.

Inducible Root Defenses in Maize

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Plants are confronted with a great number of pathogenic microorganisms and herbivores in their environment. In response to these attacks, plants have evolved a broad range of defense mechanisms. Such induced plant responses can occur aboveground, in the leaves, and also belowground, in the roots. Moreover, despite being separated in space, belowground organisms can induce defense responses aboveground and *vice versa*¹. That indicates the important role of above- and belowground systems for the whole plant resistance. However, aboveground interactions have been more intensively studied than belowground interactions. The aim of our project consists in a better understanding of the mechanisms that lead to root defense induction in maize. Therefore, we will investigate the effects of bacteria, pathogenic fungi and oomycetes and herbivores on root resistance.

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Bootstrap Estimation and Inference on an Index of Phylogenetic CorrelationPrice WJ¹, Hinz HL², Shafii B¹, Schwarzlaender M³, Rapo CB^{2,3}, Eigenbrode SD³¹ University of Idaho, Statistical Programs, Moscow, Idaho, USA² CABI Europe-Switzerland, Rue des Grillons 1, 2800 Delémont, Switzerland³ University of Idaho, Department of Plant, Soil, and Entomological Sciences, Moscow, Idaho, USA

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A common objective of bioinformatic analyses is to assess the similarity of species or genotypic variations. Such measures provide a means to evaluate evolutionary models and history as well as having potential application to ecological systems such as host preference selection. Phylogenetic correlation¹ is one index of similarity typically employed. Given a phylogeny, the correlation, λ , measures the deviation or adjustment relative to a dependent Brownian evolutionary model. Values for λ are found through maximum likelihood estimation of a generalized linear model assuming a variance-covariance structure, where off diagonal elements are scaled by λ . A value of λ equal to 1.0 is indicative of the Brownian model, while $\lambda = 0.0$ indicates an independent random process. Statistical inference on λ has traditionally been assessed using a likelihood ratio test comparing the estimated value to the theoretical null values, $\lambda=1.0$ and $\lambda=0.0$. These tests, however, rely on the assumption of a Normal likelihood within the phylogeny. In addition, statistical comparison of estimated λ values has not been addressed. An alternative procedure is proposed here which relies instead on the resampling methodology of the bootstrap. Here, λ is estimated with an underlying bootstrap distribution which provides both confidence limits, as well as, hypothesis testing capabilities without distributional assumptions. The method will be demonstrated using phylogenetic and metabolomic data related to the host specificity of *Ceutorhynchus cardariae* Korotyaev on Brassicaceae species.

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Egg Parasitoids Behavior towards the plant Chemicals

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Plants are the highly valuable resources even for the herbivore community. Plants develop different mechanisms to reduce insect attacks of which production of induced volatile chemicals is of major concern. They provide important information for insect natural enemies during their long-range host searching. Parasitoid's attraction to these induced chemicals is now well established in several plant-pest-parasite systems. We have studied the behavioral responses of the egg parasitoid, *Trichogramma chilonis* ishii towards the leaf surface chemicals emitted from castor bean plant, *Ricinus communis* (L) due to the herbivory and their ovipositional stimulant activity to the parasitoids. The feeding activity by the capsule borer, *Dichocrocis punctiferalis*, Guenée (Lepidoptera: Pyralidae) on the *R. communis* plants resulted with the production and emission of leaf surface chemicals that arrested the parasitoids. *T. chilonis* responded to the pest induced volatiles and also there was a significant induction of oviposition stimulation that lead to increased parasitization of the host eggs. In laboratory bioassays, the parasitoids discriminated the odors of intact and *D. punctiferalis* damaged castor plants and oriented towards the patch consisting the infested plant chemicals. The uninfested (intact) leaves of the capsule borer infested *R. communis* plants were extracted in dichromethane, purified by column chromatography and identified by analytical techniques through bioguided fractionation. A GC and GC-MS study revealed quantitative and qualitative differences between the emissions of pest damaged and un damaged plants. Delta -3-carene, 1-8-cineole, para- cymen-8-ol, (-)- α -terpineol, Piperidin-2-one, Hexadecyl oxirane were found in the borer infested leaf chemicals apart from several long chain hydrocarbons..

No Root Priming in Maize?

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When plant leaves are attacked by phytophagous insects, they emit several volatile chemical compounds, which are known to be attractive to natural enemies of insect herbivores. These volatiles are also detected by neighboring plants, which, in response, can enhance or accelerate defense responses, a phenomenon called priming. Plant roots also release volatiles in response to herbivory and it has been shown that they attract entomopathogenic nematodes, but their potential role in priming of nearby plants is still unknown. In a first series of experiments we investigated root priming and found no evidence that induced root volatiles primed neighboring roots, neither within plants nor between plants. However, more experiments are needed to fully exclude the occurrence of root priming by neighboring roots.

Show Me the Way to the Oil: How *Macropis* Bees Find its Oil-HostSchäffler I¹, Milchreit K, Dötterl S¹ University of Bayreuth, Department of Plantsystematics, 95440 Bayreuth, Germany

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Meeting the right host plant is essential for survival of many insect species, and volatile organic compounds are important mediators in many plant-insect interactions. The European solitary ground nester bee *Macropis fulvipes* is highly specialized on some species of the genus *Lysimachia* (180 spp., Myrsinaceae). In this plant genus about 40 % species produce oil instead of nectar, and this oil is used by *Macropis* to impregnate their cells, and to feed (together with pollen) their larvae¹. Here, we describe the flower scent of oil- and non-oil producing *Lysimachia* species, and determine the attractiveness of visual and olfactory *Lysimachia* cues on flower naive as well as experienced *Macropis* bees. The floral scent analyses revealed that scent emitted by *Lysimachia* differs qualitatively as well as quantitatively among species, and this is also true for the scent of different oil-producing species. From the bioassays we learned that scent plays an important role for naive bees to find their host plants, and that experienced bees also use visual plant cues to find their host. Bees also responded to whole flower, pollen and oil extracts (in pentane), respectively². Experiments are currently under way to identify the compounds being responsible for host-plant finding of *Macropis*. We also test the hypothesis that *Lysimachia* oil plants have an oil-marker in common.

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Sweet Stuff for the Good Guys: Do Polysaccharide Plant Strengtheners Enhance Plant Attractiveness to Parasitic Wasps?

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The ability of parasitic arthropods to locate and attack hosts is a result of successive behavioural steps regulated by physical, chemical and biochemical factors. In the host location and selection process, cues associated with the host such as volatile compounds emitted by plants as a consequence of herbivore activities play a role in attracting parasitoids to patches infested with hosts¹. Many plants release volatile compounds as a response to feeding or egg-laying by insects². Parasitoids often are highly attracted to these plants since the emitted compounds may serve as long-range cues enabling the location of hosts. Therefore, plants under attack can benefit from attracting parasitoids³ and this herbivore-induced volatile emission is considered to be an indirect induced plant defense². In maize, a number of abiotic and biotic factors can modulate the quality and quantity of the herbivore-induced volatile blend, which in certain cases may have consequences for parasitoid attraction⁴. Recently, Rostas and Turlings (2008)⁵ found that BTH (benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid *S*-methyl ester), which is known to induce the plant's defense against pathogens, also enhances the plant's indirect defense, *i.e.* its attractiveness to parasitoids. Furthermore, a variety of molecules, including oligo- and polysaccharides, peptides, proteins, and lipids can act as elicitors for pathogen defense. Laminarans induce the formation of antifungal compounds in alfalfa cotyledons⁶. Nevertheless, the mechanism of BTH in the plant is already well known, while the mechanism of polysaccharides in the plant is not fully understood⁷. Objectives: to perform series of olfactometer experiments using the tri-trophic system that comprises *Zea mays*, *Spodoptera littoralis*, and several of their larval endoparasitoids⁸ to test the effect of both BTH and laminarin on the attraction of the endoparasitoids; *Microplitis rufiventris*, *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). The results indicate that both Laminarin and BTH have a significant effect on the attraction of the studied parasitoids to maize plants infested with *S. littoralis* when compared to the control treatment.

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Biosynthetic studies: *In Vitro* Incubation of the Labial Gland and Fat Body of the Bumblebee Males (*Bombus lucorum* and *Bombus terrestris*) with [1,2-¹⁴C]acetate uptakeŽáček P^{1,2}, Tykva R, Kindl J¹, Valterová I¹¹ Academy of Sciences of the Czech Republic, Institute of Organic Chemistry and Biochemistry
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Biosynthesis of the marking pheromone of the bumblebees males of species *Bombus lucorum* and *Bombus terrestris* were studied. Pheromonal components of the bumblebee males are produced and stored in the cephalic part of the labial gland¹. Both species differ in their labial gland secretions. *B. lucorum* males produces aliphatic compounds with long carbon chain. The most abundant is ethyl (*Z*)-tetradec-9-enoate (53%)². Labial gland of *B. terrestris* males contains mixture of terpenic and aliphatic compounds³ where 2,3-dihydrofarnesol and ethyl dodecanoate are the most abundant. It was proposed by Luxova et al.⁴ that fatty acids stored in the fat body are precursors of aliphatic components of the labial gland. Aim of this study was to confirm this hypothesis. Labial glands and fat bodies of the species were incubated *in vitro* with radioactive [1,2-¹⁴C]acetate. After certain time of incubation, the gland extracts were analysed by means of thin layer chromatography with using non-destructive detection of ¹⁴C-distribution by position sensitive detector. Analysis showed that [1,2-¹⁴C]acetate was incorporated into various kind of compounds such as fatty acids, triacylglycerols, esters and hydrocarbons. Differences were found in radioactivity distribution between labial gland and fat body in both studied species. The authors thank to Dr. Josef Holík from the institute of Experimental Botany, CAS, for making possible the radioanalysis and Czech Science Foundation (203/09/1446) for financial support.

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