ISCE 21st ANNUAL MEETING

WASHINGTON, DC JULY 23-27, 2005

OMNI Shoreham Hotel and Conference Center
2500 Calvert Street
Washington, DC 20008

I. Mosquitoes II. Semiochemistry III. Marine Chemical Ecology IV. Insect-Plant Interactions

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Conference at a Glance

Saturday, July 23

1pm – 3	ISCE Council Meeting – Empire Ballroom
3pm – 6:00	Registration – Blue Prefunction
4pm – 5:30	Session Sponsor set up – Blue Prefunction
6:30pm – 8	Opening Reception - Blue Prefunction
8pm – 10	Poster Mounting – Blue Prefunction

Sunday, July 24

7am – 8:30	Poster Mounting – Blue Prefunction
8am – 6pm	Registration – Blue Prefunction
8am – 6pm	All Workshops – Blue Room
10:15am – 10:30	BREAK – Blue Prefunction
12pm – 1:30	LUNCH ON OWN
3:40pm – 3:55	BREAK – Blue Prefunction
6pm – 7:30	Poster Session/Cash Bar – Blue Prefunction

Monday, July 25

8am – 6pm	Poster Room – Blue Prefunction
8am – 6pm	Registration Desk – Blue Prefunction
8am – 6pm	All Workshops – Blue Room
10:10am – 10:25	BREAK – Blue Prefunction
11:55am – 1:30	LUNCH ON OWN
3:15pm – 3:30	BREAK – Blue Prefunction

Tuesday, July 26

8am – 6pm	Registration Desk – Blue Prefunction
8am– 6pm	All workshops – Blue Room
10:05am – 10:25	BREAK – Blue Prefunction
12pm – 1:30	LUNCH ON OWN
12pm – 1:30	Journal of Chem. Ecol. Luncheon (officers only) – Hampton Ballroom
Arrange	"Duck Tour" of Washington D.C – Hotel Lobby
Arrange	Tour of Beltsville Agricultural Res. Center – Hotel Lobby
6:30pm – 10	Banquet – Diplomat Ballroom

Wednesday, July 27

8am – 5pm	Registration Desk – Blue Prefunction
8am – 6pm	All workshops – Blue Room
10:20am – 10:35	BREAK – Blue Prefunction
3:00pm – 3:15	BREAK – Blue Prefunction

Hotel Map

Program

Saturday, 23rd afternoon

3:00-6:00 **Registration**

1:00-3:00 **ISCE Council Meeting (Officers only)**

6:30-8:00 **Opening reception**

Sunday, 24th morning

8:30-8:35 ***Hanna Mustaparta:*** **Welcome from ISCE President**

8:30-8:45 ***Jeff Aldrich:*** **Announcements and Introductions**

8:45-8:55 ***Phyllis Johnson*** **(Director, Beltsville Agricultural Research Center): Welcome & Introduction**

Invited Lecture

8:55-9:35 ***Robert Davis*** **(Molecular Plant Pathology Laboratory, Beltsville, Maryland): "*Spiroplasma kunkelii* – Helical, motile, wall-less, plant pathogenic bacterium: A model of obligate parasitism in plants and insects"**

Symposium on Mosquitoes / *Sponsored by Avon*

9:35-9:40 ***Walter Leal*** **(Organizer): Introduction**

9:40-9:45 ***Michele Duggan*** **/ Avon**

9:45-10:15 ***Walter S. Leal:*** **"Reverse chemical ecology: Prospecting for oviposition attractants for *Culex* mosquitoes"**

10:15-10:30 **Coffee Break**

10:30-11:00 ***Linda Field:*** **"Molecular basis of olfaction in *Anopheles gambiae*"**

11:00-11:30 ***Coby Schal:*** **"Trapping gravid *Aedes mosquitoes* with bacteria-derived semiochemicals"**

11:30-12:00 ***Ring Cardé:*** **"Behavioral responses of *Culex* mosquitoes to oviposition attractants"**

12:00-1:30 **Lunch**

Sunday, 24th afternoon

1:30-1:45 ***Jerome Klun:*** **"Novel behavioral assay for mosquito deterrents"**

1:45-2:00 ***Irving Wainer:*** **"Immobilized odorant binding protein liquid chromatographic stationary phases: Going with the flow in chemical ecology"**

Contributed Papers (mosquito session) / *Sponsored by BASF*

2:00-2:05 ***Jerome Klun:*** **Introduction**

2:05-2:10 ***Robert Farlow*** **/ BASF**

2:10-2:22 ***Michael Birkett:*** **"Differential attractiveness of vertebrate hosts: The search for natural repellents against haematophagous insect pests"**

2:22-2:34 ***Kamlesh Chauhan:*** **"Development of new piperidine analogs as mosquito deterrents"**

2:34-2:46 ***Junwei Zhu:*** **"Development of natural products as mosquito repellents and larvicides"**

Student Competition Contributed Papers (*Ken Haynes:* Moderator)

2:46-2:51 ***Ken Haynes:*** **Introduction**

2:51-3:03 ***Marco D'Alessandro:*** **"How soil microorganisms and microbial volatile organic compounds affect a tritrophic signaling network"**

3:03-3:15 ***Michelle Marko:*** **"Chemical attraction of a specialist insect herbivore to the freshwater macrophyte *Myriophyllum spicatum*"**

3:15-3:27 ***Maaike Bruinsma:*** **"Effects of phenotypic manipulation of herbivore-induced plant defense on the insect community associated with cabbage"**

3:27-3:39 ***Yasuyuki Choh:*** **"Induced production of extrafloral nectar in intact lima bean plants in response to volatiles from spider mite-infested conspecific plants as a possible indirect defense against spider mites"**

3:39-3:55 **Coffee Break**

Silverstein-Simeone Award Lecture

3:55-4:00 ***Walter Leal:*** **Introduction**

4:00-4:40 ***John Carlson*** **(Yale University, New Haven, Connecticut): "Chemosensory reception and coding in *Drosophila*"**

Student Competition Contributed Papers (cont.)

4:40-4:52 ***Seong-Gyu Lee:*** **"Morphological pathways of olfactory neurons in the pheromone-responsive sensilla of male *Heliothis subflexa*"**

4:52-5:04 ***Nina Fatouros:*** **"Sex, spies and pheromones: phoretic egg parasitoids exploiting a butterfly anti-aphrodisiac"**

5:04-5:16 ***Panagiotis Vamvatsikos:*** **"Host-habitat selection by female aphid parasitoids: to what extent do innate and learned factors determine behaviour?"**

5:16-5:28 ***Sven Steiner:*** **"Female sex pheromone in immature insect males – a case of pre-emergence chemical mimicry?"**

5:28-5:40 ***Jared Fine:*** **"The sea lamprey migratory pheromone is a synergistic blend of three novel sulfated steroids"**

Sunday, 24th evening

6:00-7:30 **Poster Session / Cash Bar**

Monday, 25th morning

Symposium on Semiochemistry Honoring Professor Kyung Saeng Boo
Sponsored by ISCA Technologies Inc.

8:30-8:35 ***Tom Baker*** **(Co-organizer): Introduction**

8:35-8:40 ***Agenor Mafra-Neto*** **/ ISCA Technologies Inc.**

8:40-9:10 ***Kye Chung Park:*** **"Overview of semiochemicals research and application success in Korea"**

9:10-9:40 ***Man-Yeon Choi:*** **"PBAN and PBAN receptors"**

9:40-10:10 ***Allard Cossé:*** **"Chrysomelid semiochemistry"**

10:10-10:25 Coffee Break

10:25-10:55 ***Tom Baker:*** **"Heliothine moth olfaction"**

10:55-11:25 ***Jeffrey Aldrich:*** **"Catnip, aphids and lacewing predators: Tritrophic coincidence or confusion?"**

11:25-11:55 ***Jocelyn Millar:*** **"Sex pheromones of the navel orangeworm and *Pyralis farinalis*: the missing pieces to the puzzle"**

11:55-1:30 Lunch

Monday, 25th afternoon

Contributed Papers
(*Jocelyn Millar & Allard Cossé:* Moderators)

1:18-1:30 ***Baldwyn Torto:*** **"Kairomone-based trapping of the small hive beetle *Aethina tumida* in a Florida apiary"**

1:30-1:42 ***John Byers:*** **"A cost of alarm pheromone production in cotton aphids, *Aphis gossypii*"**

1:42-1:54 ***Jeremy McNeil:*** **"Pheromone-mediated mating in the aphid parasitoid, *Aphidius ervi*"**

1:54-2:06 ***Imene Saïd:*** **"Chemical compounds inducing aggregation in *Periplaneta americana* (L.) (Insecta: Dictyoptera)"**

2:06-2:18 ***David Hall:*** **"Sex pheromones of apple leaf curling midge, *Dasineura mali*, and raspberry cane midge, *Resseliella theobaldi*: a new class of pheromone structures"**

2:18-2:30 ***Aijun Zhang:*** **"Female sex pheromone of the dogwood borer (DWB) *Synanthedon scitula*"**

2:30-2:42 ***Bente Gunnveig Berg:*** "**Characterisation of olfactory pathways involved in pheromone and interspecific signal information in three heliothine species"**

2:42-2:54 ***Michael Domingue:*** **"Olfactory receptor neuron responses of Asian and European corn borer hybrid males to parental pheromone components"**

2:54-3:06 ***Angel Guerrero:*** **"Antagonistic activity of pheromone responses in Lepidopteran males by esterase inhibitors"**

3:06-3:18 ***Claude Wicker-Thomas:*** **"Elongases in *Drosophila*: role in fatty acid and sex pheromones metabolism"**

3:18-3:33 **Coffee Break**

3:33-3:45 ***Stephan Schulz:*** **"The chemistry of collembolan"**

3:45-3:57 ***Antje Burse:*** "**Characterization of early stages of the iridoid biosynthesis in larvae of the leaf beetle *Phaedon cochleariae*"**

3:57-4:09 ***Gary Blomquist:*** "**An update on the juvenile hormone regulation of pheromone production in bark beetles"**

4:09-4:21 ***Gregory Wheeler:*** **"Plant chemistry-based host specificity of *Oxyops vitiosa*, a weevil introduced for weed biological control of *Melaleuca quinquenervia*"**

4:21-4:33 ***Anna-Karin Borg-Karlson:*** **"Antifeedants in the faeces of the pine weevil *Hylobius abietis*: identification and biological activity"**

4:33-4:45 ***Rikard Unelius:*** **"Structure-activity relationships of benzoic acid derivatives as antifeedants for the pine weevil *Hylobius abietis*"**

4:45-4:57 ***B.G. Zhao:*** **"Nematicidal activity of quinolizidine alkaloids and field tests to control pine wilt disease with aloperine"**

4:57-5:09 ***Nancy Gillette:*** **"Control of western pine beetle, *Dendroctonus brevicomis*, populations using aerially applied verbenone flakes"**

5:09-5:21 ***Brian Sullivan:*** **"Reexamination of the pheromone system of the southern pine beetle, *Dendroctonus frontalis*"**

5:21-5:33 ***Peter Gregg:*** **"Area-wide effects of a plant volatile-based attract and kill formulation against *Helicoverpa armigera* and *H. punctigera* in Australia"**

5:33-5:45 ***Bruce Schulte:*** **"Pheromones and chemosensory investigative behavior by African elephants"**

5:45- 5:57 ***Anne-Geneviève Bagnères:*** **"Does chemical signature reflect genetic relatedness between population and colonies in termites?"**

5:57-6:09 ***Manfred Ayasse:*** **"Identification of queen sex pheromone components of the bumblebee *Bombus terrestris*"**

6:09-6:21 ***Aivlé Cecilia Cabrera:*** **"Chemical cues for nestmate recognition in *Acromyrmex landolti*"**

6:21-6:33 ***Michael Greene:*** **"Structural complexity of chemical recognition cues affects perception of group membership in ants"**

Tuesday, 26th morning

Symposium on Marine Chemical Ecology

8:30-8:35 ***Nancy Targett*** **(Organizer): Introduction**

8:35-9:20 ***Robert Thacker:*** **"Sponge-microbe symbioses: model systems for integrating molecular and chemical ecology"**

9:20-10:05 ***Thomas Arnold:*** **"Seagrass-pathogen interactions: attack by the wasting disease pathogen, *Labyrinthula* spp., causes the 'pseudo-induction' of phenolics"**

10:05-10:25 Coffee Break

10:25-11:10 ***Nicole Lopanik:*** **"The tale of an ordinary bryozoan, its symbiont, and an anticancer compound: the chemical ecology and biosynthesis of the bryostatins"**

11:10-11:55 ***Marc Weissburg:*** **"Sensory biology and ecology of chemosensory foraging: a tale of two predators (and their prey)"**

Tuesday, 26th morning-afternoon

12:00-1:30 Journal of Chemical Ecology luncheon meeting (officers only)

Arrange **"Duck" tour of Washington D.C.**

Arrange **Tour of Beltsville Agricultural Research Center**

Tuesday, 26th evening

Banquet / *Sponsored by Sterling International Inc.*

6:30-7:00 **Cash bar**

7:00-7:05 ***Jeff Aldrich:*** **Introductions**

7:05-7:10 ***Rod Schneidmiller:*** **Sterling International Inc.**

7:10-7:20 ***Hanna Mustaparta:*** **Comments of outgoing ISCE President**

7:20-8:30 **Dinner**

8:30-9:15 ***Jacques M. Pasteels*** **(Universite de Libre Bruxelles, Belgium) – Social Lecture: "Wonderful meetings and unique opportunities"**

9:15-9:45 ***Hanna Mustaparta:*** **Presentations of Student Awards**

Wednesday, 27th morning

Silver Medal Award Lecture

8:30-8:35 ***Tom Baker:*** **Introduction**

8:35-9:05 ***James Tumlinson*** **(Pennsylvania State University, University Park): "Chemical ecology of plant defenses against insects and pathogens"**

Symposium on Insect-Plant Interactions / *Sponsored by Trécé Inc.*

9:05-9:10 ***Jeff Aldrich:*** **Introduction**

9:10-9:15 ***Bill Lingren*** **/ Trécé, Inc.**

9:15-9:20 ***Wilhelm Boland*** **(Co-organizer)*:* Introduction**

9:20-9:50 ***Phillip Reymond:*** **"Plant-insect interactions in *Arabidopsis*: From transcript profiling to induced resistance"**

9:50-10:20 ***Rayko Halitschke:*** **"Genetically silenced defense responses: Consequences for the herbivore community composition on *Nicotiana attenuate*"**

10:20-10:35 **Coffee Break**

10:35-11:05 ***Martin Heil:*** **"Physiological and molecular adaptations stabilizing symbiotic ant-plant mutualisms"**

11:05-11:35 ***Jürgen Engelberth:*** **"Signals, effects, and specificity of volatile-induced plant defense responses"**

11:35-1:30 **Lunch**

Wednesday, 27th afternoon

Symposium on Insect-Plant Interactions (cont.)

1:30-2:00 ***Jo Handelsman:*** **"Chemical signalling in the microbial community of the lepidopteran gut"**

2:00-2:30 ***May Berenbaum:*** **"Xenobiotic metabolism by caterpillars: inductions and deductions"**

2:30-3:00 ***Axel Mithöfer:*** **"MecWorm, a novel tool to study plant-herbivore interactions"**

3:00-3:15 **Coffee Break**

Contributed Papers (*Ted Turlings:* Moderator)

3:15-3:27 ***Joachim Ruther:*** **"Emission of herbivore-induced volatiles in absence of a herbivore – response of *Zea mays* to green leaf volatiles, terpenoids, and ethylene"**

3:27-3:39 ***Ted Turlings:*** **"Above and below ground tritrophic signaling in maize"**

3:39-3:51 ***David Puthoff:*** **"Elucidation of resistance and disease mechanisms in roots using sugar beet and sugar beet root maggot as a model system"**

3:51-4:03 ***John Tooker:*** **"Jasmonate in lepidopteran eggs and neonates"**

4:03-4:15 ***Brice McPherson:*** **"The influence of the introduced pathogen *Phytophthora ramorum* on saprotrophic beetle (Coleoptera: Scolytidae) host selection behavior"**

4:15-4:27 ***Teunis Dekker:*** **"Peripheral and central shift in the olfactory circuitry mediate preference for toxic fruit in *D. melanogaster* sibling *D. sechellia*"**

4:27-4:39 ***Kotaro Konno:*** **"Plant latex and its diverse ingredients protect plants from insect herbivory: tales of cysteine proteases in papaya and fig latex and sugar-mimicking alkaloids in mulberry latex"**

4:39-4:51 ***Nadir Erbilgin:*** **"Geographic variation in response of *Dendroctonus valens* to host volatiles of *Pinus* spp.: a holarctic perspective"**

Final Business Meeting

5:00-5:45 ***Hanna Mustaparta:*** **Introduction of new offices and Society business**

Lecture Abstracts

Spiroplasma kunkelii – Helical, Motile, Wall-less, Plant Pathogenic Bacterium: A Model of Obligate Parasitism in Plants and Insects

Robert E. Davis, Molecular Plant Pathology Laboratory, USDA-Agricultural Research Service, Beltsville, MD 20705, USA

Prior to the late 1960s and early 1970s, hundreds of plant diseases known as yellows diseases were presumed to be caused by viruses. Yet, in spite of decades of intensive research, no virus could be isolated from, or visualized in, infected plants. The conviction, that viruses were the causal agents, influenced the direction of research worldwide, until the surprising discovery in disease plants of microbes resembling mycoplasmas previously known in animal diseases. The causal agent of corn stunt disease was among those pathogens termed mycoplasmalike organisms (MLOs, now phytoplasmas). This new conviction was held until the unexpected discovery in 1972 that diseased corn plants and insect vectors contained a previously unknown, unique helical and motile, wall-less prokaryote, termed spiroplasma. Since then, other plant pathogenic spiroplasmas have been found, and non-plant pathogenic spiroplasmas have been reported in numerous insect species, in ticks, and in a crab, and have been implicated in human disease. Plant pathogenic spiroplasmas reside in the enucleate sieve cells of phloem tissue in infected plants and are transmitted by insects, primarily leafhoppers that feed in the phloem and that also serve as hosts in which these pathogens multiply. In their evolutionary descent from low G + C, Gram positive bacteria in the *Clostridium*/*Bacillus* group, spiroplasmas underwent massive reductions in genome size, progressing toward minimal sets of genes required for trans-kingdom parasitism and pathogenicity in insects and plants.

The startling discovery of the corn stunt spiroplasma, *Spiroplasma kunkelii*, opened the search for spiroplasmas in other diseases and created new opportunities to understand an important plant disease and unique pathogen-host interactions. Progress in genome sequencing is now providing new means to address fundamental issues concerning *S. kunkelii* and spiroplasmas in general. After decades during which the corn stunt disease agent was incorrectly pursued as a virus, later believed to be a phytoplasma, and finally unveiled by the revelation of its identity as a spiroplasma, to be followed by another quarter century during which much about spiroplasmas remained mysterious, genome sequencing is now making it possible to begin answering questions that have been of intense interest since the belief in viral etiology faded. Understanding how the genome evolved to gain new functions for plant pathogenicity, insect transmission, and adaptation to an obligately parasitic life style, should aid the identification of molecular targets for the design of measures to control corn stunt and other spiroplasmal diseases.

Reverse chemical ecology: Prospecting of oviposition attractants for *Culex* mosquitoes

W.S. Leal[1], A.M. Chen[1], T.I. Morgan[1], Y. Ishida[1], and D.J. Pesak[2]. [1]Honorary Maeda-Duffey Laboratory, Department of Entomology, University of California-Davis, Davis, CA 95616 USA, [2]Bedoukian Research Incorporated, 21 Finance Drive, Danbury, CT 06810, USA

Female mosquitoes use airborne semiochemicals integrated with other sensory modalities to find and determine the suitability of host for a blood meal, oviposition sites, etc. Reception of these semiochemicals by specialized apparatus in the periphery, such as antennae and maxillary palpi, is a sine qua non step prior to integration with other stimulus modalities in the brain and subsequent translation into behavior. Odorants reach the aqueous sensillar lymph (in the antennal and maxillary sensilla) through pore tubules (Kaissling 1987), but solubility prevents the intact odorant from reaching the membrane-bound odorant receptors (ORs). Largely, odorants (pheromones and other semiochemicals) are hydrophobic molecules and the sensillar lymph is an aqueous barrier. As demonstrated in moths (Leal 2005), odorant-binding proteins selectively bind physiologically relevant chemicals (odorants) and solubilize them by trapping the odorants in a large hydrophobic binding cavity (Sandler et al. 2000). Odorants encapsulated by OBPs are ferried to the ORs as an OBP-odorant complex, which also protects the odorant from odorant-degrading enzymes. At the end of the journey, odorants are delivered to the ORs by a pH-dependent conformational change (Wojtasek and Leal 1999; Damberger et al. 2000; Horst et al. 2001). Previously, we have isolated, identified, and cloned the first female antennae specific odorant–binding protein from mosquito species, *Culex quinquefasciatus* (CquiOBP1) (Ishida et al. 2002). We have now expressed CquiOBP and demonstrated that this mosquito olfactory protein undergoes a pH-dependent conformational change similar to that observed in the pheromone-binding protein from the silkworm moth, *Bombyx mori* (Wojtasek and Leal 1999). Using both cold binding assay (Leal et al. 2005) and a fluorescence reporter (Ban et al. 2002), we showed that CquiOBP binds to a mosquito oviposition pheromone (Laurence and Pickett 1982) at the sensillar lymph pH, but not at low pH. These findings validate CquiOBP as a molecular target for the screening of potential oviposition attractants.

Ban L, Zhang L, Yan Y, Pelosi P. (2002). Binding properties of a locust's chemosensory protein. Biochem Biophys Res Commun 293:50-54.

Damberger F, Nikonova L, Horst R, Peng GH, Leal WS, Wuthrich K. (2000). NMR characterization of a pH-dependent equilibrium between two folded solution conformations of the pheromone-binding protein from *Bombyx mori*. Protein Science 9:1038-1041.

Horst R, Damberger F, Luginbuhl P, Guntert P, Peng G, Nikonova L, Leal WS, Wuthrich K. (2001). NMR structure reveals intramolecular regulation mechanism for pheromone binding and release. Proc Natl Acad Sci USA 98:14374-14379.

Ishida Y, Cornel AJ, Leal WS. (2002). Identification and cloning of a female antenna-specific odorant-binding protein in the mosquito *Culex quinquefasciatus*. J Chem Ecol 28:867-871.

Kaissling K-E. (1987). R. H. Wright lectures on insect olfaction. Simon Fraser University.Burnaby, British Columbia

Laurence BR, Pickett JA. (1982). *erythro*-6-Acetoxy-5-hexadecanolide, the major component of a mosquito oviposition attractant pheromone. J Chem Soc Chem Commun 1982.

Leal WS. (2005). Pheromone reception. Top Curr Chem 240:1-36.

Leal WS, Chen AM, Ishida Y, Chiang VP, Erickson ML, Morgan TI, Tsuruda JM. (2005). Kinetics and molecular properties of pheromone binding and release. Proc Natl Acad Sci USA 102:5386-5391.

Sandler BH, Nikonova L, Leal WS, Clardy J. (2000). Sexual attraction in the silkworm moth: structure of the pheromone-binding-protein-bombykol complex. Chem Biol 7:143-151.

Wojtasek H, Leal WS. (1999). Conformational change in the pheromone-binding protein from *Bombyx mori* induced by pH and by interaction with membranes. J Biol Chem 274:30950-30956.

Supported by NIAID-NIH (1U01AI058267-01).

Molecular basis of olfaction in *Anopheles gambiae*

Linda M Field, Rothamsted Research, Harpenden, Herts. AL5 2JQ, UK.

The detection of semiochemicals plays a major role in host-seeking behavior by insects. The proteins involved in insect olfaction include the odorant receptors (ORs) and the odorant-binding proteins (OBPs), the latter functioning as carriers within the antennae for transporting odours to the ORs. There is also increasing evidence that OBPs play a more active role in the molecular discrimination of the semiochemicals. In mosquitoes olfaction is involved in the detection of animal hosts by females prior to blood feeding and it is likely that this involves a subset of the OBPs present in the females. We have used an algorithm, MotifSearch, to identify genes in the *Anopheles gambiae* database which encode putative OBPs, revealing more than 70 genes. Semi-quantitative RT-PCR has shown that some of these genes are expressed in female heads and therefore could be involved in host location, indeed a subset of these show specific expression in female heads. We have also identified homologues of each of the *A. gambiae* genes in a closely related species *Anopheles arabiensis*, which unlike *A. gambiae* prefers non-human animals. Some of the OBP genes show differential expression between the two species and therefore could be involved in their different host preference. The expression profiles of the genes in the two members of the *Anopheles* complex provides the first step towards further molecular analysis of the mosquito olfactory apparatus.

Trapping gravid *Aedes* mosquitoes with bacteria derived semiochemicals

Coby Schal, Ning Xu, Loganathan Ponnusamy, Charles S. Apperson, Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695, USA

Emerging viral diseases, such as dengue hemorrhagic fever and West Nile viral encephalitis, pose a significant threat to public health. Our long-term research goal seeks to develop an area-wide management strategy for mosquito vectors that is based on their biology and behavior. Egg-laying females of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) choose container habitats for their progeny. Physical, chemical and biological factors influence the female as she searches for and selects an oviposition site. Our working premise is that in the aqueous mixture of an oviposition site, microbial degradation of organic material produces volatile attractants or repellents, as well as nonvolatile arrestants and oviposition stimulants or deterrents. Gravid mosquitoes use these volatile metabolites as semiochemical cues to locate oviposition sites that provide favorable conditions for production of offspring. Our research seeks to identify the most significant environmental cues, the chemical structure of the active/attractive bacterial metabolites, and to demonstrate that the semiochemicals can be used as lures to enhance devices used for surveillance and control of these container inhabiting mosquitoes. We are currently characterizing the cues that attract gravid female mosquitoes to containers for oviposition and the bacterial communities associated with productive and unproductive containers in nature. Ultimately, attractants will be used in combination with insecticides and biological control agents in "lethal ovitraps", devices that should suppress populations of *Ae. aegypti* and *Ae. albopictus* mosquitoes in urban habitats.

Support: NIH-NIAID 1 U01 AI058303-01.

Behavioral responses of *Culex* mosquitoes to oviposition attractants

R.T. Cardé and Marieta Braks, Department of Entomology, University of California, Riverside, CA 92521, USA

Habitat-related odors and a pheromone associated with the egg rafts can influence the propensity of gravid female *Culex* mosquitoes to oviposit and they may influence the flight and landing maneuvers that precede oviposition. Collectively such semiochemicals have been termed "attractants," although there is little information available on what kind of orientation behaviors might be involved. Such information is essential for the improvement of gravid traps commonly used in arboviral surveillance and control programs. The chemical ecology studies for identifying these semiochemicals have largely depended on bioassays measuring end-point behaviors, that is, did a given odor increase or decrease the number of eggs deposited?. To establish how such odors contribute to egg laying we have used "still air" bioassays that measure oviposition and wind tunnel assays that record flight tracks. Our model system has been *Culex pipiens quinquefasciatus* and the odors tested have been mainly natural complex blends, including hay infusion and egg rafts.

Oviposition "still air" bioassays, including egg raft counts and sticky screen assays, were performed in small (0.3 by 0.3 by 0.3 m) and medium (0.7 wide, 1.5m high) indoor cages and large (4.0 by 4.0 by 2.0 m) outdoor net cages to compare the effect of cage size on source finding. Additionally, a novel wind tunnel bioassay was developed, and a 3-D video recording and analysis system, initially created for studying the host-seeking behavior of mosquitoes, was adapted for studying their oviposition-related behavior. Individual gravid and non-gravid mosquitoes were released downwind of a turbulent odor (hay infusion or its control) plume and its flight track was recorded, digitized, and analyzed. Here, we report only the data of gravid females. Video analysis showed that a higher proportion of gravid female mosquitoes found and returned to hay infusion than to humid air. After obtaining the 3-D flight coordinates of a standardized section of the initial upwind flight track, multiple flight parameters were calculated and compared between the two stimuli. With this bioassay and recording system, events and flight patterns to different olfactory stimuli can be studied. By digitizing a standardized, relatively short segment of each flight track, orientation behavior to different odor stimuli can be distinguished.

Novel behavioral assay for mosquito deterrents

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A test module, previously developed for quantitative measurement of the efficacy of mosquito biting deterrents on human volunteers, was adapted for *in vitro* evaluation of chemical deterrency by coupling the module with a membrane-blood reservoir. Performance of standard repellents Deet, Bayrepel® and SS-220 in the new *in vitro* system was compared with their performance on humans against mosquitoes using our standard *in vivo* system. For each compound, *in vitro* dose-response assays were conducted with compounds applied to cloth positioned over blood reservoirs covered with Baudruche membrane against *Aedes aegypti* (L.). The compounds were also tested *in vitro* against *Anopheles stephensi* Liston and *Ae. aegypti* at a fixed dose of 24 nmol compound/cm^2 cloth over the Baudruche and Edicol collagen membranes. Concurrently, the compounds were tested at the fixed dose using the module on human volunteers. The observed proportions of both mosquito species deterred from biting in the fixed doses in the *in vitro* assays were similar to those obtained using humans. Dose-response relationships of the *in vitro* and *in vivo* systems were also very similar. This new *in vitro* assay system can be used in high through-put screening to identify new chemicals having potential for use as topical mosquito biting deterrents on humans.

Immobilized protein-based liquid chromatographic stationary phases containing pheromone binding proteins or nicotinic receptors: Going with the flow in chemical ecology

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Liquid chromatography columns have been developed for use in the screening of biologically active compounds. One column was developed by covalently bonding the pheromone binding protein from the silkworm moth, *Bombyx mori* (BmorPBP). The resulting column was evaluated using radiolabeled bombykol and the results demonstrated that the immobilized protein retained its ability to bind this ligand . The data also demonstrate that the BmorPBP column was able to distinguish between four compounds, and rank them in their relative order of affinity for the protein from highest to lowest: bombykol > bombykal >1-hexadecanol > (Z,E)-5,7-dodecadien-1-ol, and that the immobilized BmorPBP retained its pH-dependent conformational mobility. The column was stable over a 10 month period. A second series of columns was developed through the immobilization of membranes from cell lines expressing neuronal nicotinic acetylcholine receptors (nAChRs). The resulting columns have been characterized and can be used to identify and study nAChR agonists, competitive antagonists and non-competitive inhibitors. A series of neonicotinoids have been studied on the immobilized nAChR columns and the results will be presented. The data from these studies demonstrate that the concept of immobilizing pheromone binding proteins, odorant binding proteins or receptors in order to create affinity chromatographic columns is viable approach to the development of online screens for new insect repellants and insecticides.

Differential Attractiveness of Vertebrate Hosts: The Search for Natural Repellents against Haematophagous Insect Pests

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Blood-sucking and biting insects are differentially attracted to vertebrates. This is likely to be caused by differences in the semiochemical profiles of individual hosts. Despite substantial research into insect-human and other insect-animal interactions, the responsible semiochemicals have not yet been identified. Unattractive individuals either lack attractant chemical compounds or may produce compounds that "mask" the activity of attractants. This latter hypothesis has been tested in two different scenarios, one involving cattle and their associated fly pests, and the other involving human beings and the yellow fever mosquito, *Aedes aegypti*.

For cattle flies, initial counting studies confirmed the differential attractiveness of Holstein-Friesian heifers to the horn fly, *Haematobia irritans*. Volatiles were collected from individual heifers and analysed by gas chromatography (GC), coupled GC-electroantennography (GC-EAG) and coupled GC-mass spectrometry (GC-MS). Using the antennae of *Ha. irritans*, and another pest, the face fly, *Musca autumnalis*, 23 compounds were located and identified by GC-EAG and GC-MS. In field trials involving small herds of heifers ranked according to fly-load, one of the identified compounds, 6-methyl-5-hepten-2-one, reduced fly-loads on high fly-loading individual heifers.

For *Ae. aegypti*, behavioural studies confirmed the differential attractiveness of human volunteers. A novel air entrainment technique was developed and used to collect volatile chemicals from volunteers, and the extracts analysed by GC, coupled GC-EAG and GC-MS. For the first time, electrophysiological responses of *Ae. aegypti* females to volatiles were recorded, and EAG active compounds identified. A quantitative and qualitative analysis of compounds within extracts from individuals known to be differentially attractive revealed significant differences in chemical profiles. Chemicals found in greater amounts in unattractive extracts showed a masking effect when presented together with attractive odours.

These studies demonstrate, for the first time, the role of volatile semiochemicals in conferring the differential attractiveness of individuals within a single host species. The identification of naturally-occurring semiochemicals that reduce fly attractancy opens up the way for the development of new and improved control technologies, whereby masking compounds could be incorporated into new, safe and natural repellents against biting insect pests.

Development of new piperidine analogs as mosquito deterrents.

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Piperidine analogs applied to skin are known to have significant deterrent effects against blood sucking arthropods. We identified ligand responsible for topical insect repellency, and designed piperidine amide ligands to mimic this compound. Evaluation of all piperidine analogs resembling this core ligand showed either equal or better mosquito repellency compared to N,N-diethyl-3-methyl–toluamide (DEET) and chiral 1S-(cyclohex-3-ene-ylcarbonyl),2S'-methyl piperidine (S,S-220) in three different *in vitro* bioassays. If all or some of these compounds lack undesirable toxic effects and plasticizer properties, the novel piperidine analogs will be commercially viable candidates in the mosquito repellent/deterrent market worldwide.

Development of natural product as mosquito repellents and larvicides

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The present study reports our most recent findings on the development of natural products as mosquito adult repellents and larvicides. The behavioral responses of mosquitoes to natural product repellents are also investigated in the wind tunnel. Finally, the potential use of these natural products in preventing mosquito bites will be discussed.

How soil microorganisms and microbial volatile organic compounds affect a tritrophic signaling network

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Plants are known to respond to herbivory with a wide range of defense mechanisms, which may act either directly against the herbivores or indirectly by attracting natural enemies of the herbivores. The indirect defense strategy involves volatile organic compounds (VOCs) emitted by the herbivore-infested plants that serve as cues for the natural enemies. We investigated how soil microorganisms might modify such blends of herbivore-induced VOCs. Volatile blends released by caterpillar-damaged maize seedlings grown in autoclaved soil were compared with volatile blends from similar treated plants to which we added an extract of a non-autoclaved soil. It was found that the latter plants released substantial amounts of a racemic mixture of 2,3-butanediol in addition to the known herbivore-induced VOCs. In olfactometer bioassays these additional compounds affected the attraction of the parasitoid *Cotesia marginiventris* to caterpillar-infested maize seedlings. Females of the parasitoid preferred a blend with 2,3-butanediol after they had learned to associate such a blend with the presence of the hosts. We are currently confirming our hypothesis that 2,3-butanediol is produced by microorganisms in the maize seeds, and will test if this compound also affects the feeding behavior of the caterpillars and promotes plant growth, as has previously been reported for *Arabidopsis* plants.

Chemical attraction of a specialist insect herbivore to the freshwater macrophyte *Myriophyllum spicatum*

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Many insects locate host plants by the perception of semiochemicals emitted by their hosts. In aquatic systems, the variety of compounds a specialist insect is exposed to may be quite different from its terrestrial relatives. The milfoil weevil, *Euhrychiopsis lecontei*, is a fully aquatic specialist insect that feeds, oviposits, and mates on the freshwater macrophyte *Myriophyllum spicatum*. Bioassay-driven fractionation was used to isolate the chemicals released by *M. spicatum* that attracted *E. lecontei*. Mass and NMR spectroscopy were used to identify the attractive compounds released into the water by *M. spicatum*. Dose-response curves of *M. spicatum*-released exudates and the isolated chemicals, glycerol and uracil indicate that weevils preference increased as sample concentration increased. Weevils were attracted to glycerol from 0.018 to 1.8 mM, a concentration similar to which terrestrial insects are attracted to sugar alcohols; and to uracil from 0.015 to 15 μM, a level more indicative of a plant-specific attractant than a nutrient. Uracil concentration was significantly greater in the exudates of *M. spicatum* than of other *Myriophyllum* spp. The weevils are likely responding to metabolites that are released as a result of the rapid growth of *M. spicatum*. This is the first characterization of a water-borne insect attractant in a freshwater system. Analysis of the watermilfoil-weevil interactions will provide further understanding as to how insects locate their host plants in an aquatic system.

Effects of phenotypic manipulation of herbivore-induced plant defense on the insect community associated with cabbage

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Plants have evolved many ways to defend themselves against herbivory. When under attack defense compounds are induced to deter herbivores and to attract natural enemies. We are using phenotypic manipulation as a tool to improve our understanding of induced defense and its effect on the insect community associated with the plant. Phenotypic manipulation can be achieved by inducing or inhibiting different steps in the biochemical pathways that produce defense compounds. In this study we used the phytohormone jasmonic acid (JA) that is known to be involved in many plant processes including direct and indirect defence against herbivores. JA induces a volatile blend that is similar to that induced by herbivore damage. After JA application to Brussels sprouts (*Brassica oleracea* var *gemmifera*) the behavior of herbivores and their natural enemies was studied. When herbivore-induced plant defense compounds are produced, they might affect all species associated with the plant. Insects from different trophic levels are expected to use this information in different ways. While for herbivores it might be a cue signaling reduced hostplant quality, for parasitoids and predators it could signal the presence of a host or prey on the plant. In wind tunnel experiments the effect of JA-induced plant volatile production on attraction of several species of parasitoid wasps was tested. Oviposition preference of the herbivore *Pieris rapae* for JA-induced and control plants was assessed in dual-choice assays. In addition to this induction treatment with JA, we tested an inhibitor, phenidone. Phenidone is an inhibitor of lipoxygenase, a key enzyme catalyzing an early step of the octadecanoid pathway. The effect of the phenidone treatment on host location behavior was investigated for the parasitoid wasp *Cotesia glomerata*.

Induced production of extrafloral nectar in intact lima bean plants in response to volatiles from spider mite-infested conspecific plants as a possible indirect defense against spider mites

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Plants emit a specific blend of volatile compounds in response to herbivory. The volatiles are called herbivore-induced plant volatiles (HIPV). One of the ecological functions of HIPV is to attract the carnivorous natural enemies of herbivores. In some cases, HIPV are known to mediate interactions between infested plants and intact plants. We studied such plant-plant interactions in a system consisting of lima bean plants (*Phaseolus lunatus*), herbivorous mites (*Tetranychus urticae*), and predatory mites (*Phytoseiulus persimilis*). In this system, it has already been reported that HIPV-exposed intact plants attracted the predatory mites.

Firstly we confirmed the increased attractiveness of HIPV-exposed intact plants to *P. persimilis* in our experimental setups. However, there are no preys on the intact plant, and thus the attracted predators will eventually move to the neighboring infested plants. For the exposed plants, therefore, it would be important to arrest predators on the plants in addition to the attraction. We tested whether the exposed plants could arrest *P. persimilis* or not. Here, we focused on the extrafloral nectar (EFN). We found that lima bean plants increased the amounts of EFN not only after infestation by *T. urticae*, but also after exposure to HIPV. EFN secreted by lima bean plants contained fructose, glucose, and sucrose. *P. persimilis* dispersed more slowly from an exposed intact plant than from control plant (plant exposed to intact conspecific volatiles). The predators also dispersed more slowly from control plants to which we added EFN using glass capillary than from untreated control plants. We further showed that EFN was a potential alternative food source for *P. persimilis*. From these results, we concluded that increased EFN was involved in the slowed dispersal of *P. persimilis* from the plants exposed to herbivore-induced plant volatiles. Our data suggest that the increase of EFN in a HIPV-exposed intact plant could be one of the induced indirect defense strategies against spider mites.

Chemosensory reception and coding in *Drosophila*

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We used a genomics approach to identify odor and taste receptors from the *Drosophila* genome database. We isolated two families of 60 genes each, the *Or* genes and the *Gr* genes, which encode odor and gustatory receptors.

We developed an *in vivo* functional expression system to identify the ligand specificities of individual odor receptors. A mutation was used to delete two adjacent *Or* genes, thereby eliminating the response of a particular neuron to all tested odors. We call this mutant neuron the "empty" neuron and use it as a "decoder": we introduce into it another receptor gene and determine the odor specificity conferred by the transgenic receptor. This approach allowed systematic investigation of the odor response spectra of the antennal repertoire of odor receptors. We found that some receptors responded strongly to many of the tested odors, whereas others responded strongly to only one or none. Likewise, some odors strongly activated many receptors, and some odors activated only one. We also found that some individual receptors are able to respond in different ways to different odorants: they are activated by some odorants and inhibited by others. Finally, the work allowed us to deduce which receptors are expressed in which types of neuron in the antenna, thereby establishing a receptor-to-neuron map of the olfactory system.

We have now extended this approach to express odor receptors from the malaria vector mosquito *Anopheles gambiae* in *Drosophila.* We found that the female-specific receptor AgOr1 responds to 4-methylphenol, a component of human sweat. These results suggest the possibility that AgOr1 may play a role in the human host-seeking behavior of the female mosquito, and they demonstrate the utility of *Drosophila* for the study of odor receptors from other insect species.

Morphological pathways of olfactory neurons in the pheromone-responsive sensilla of male *Heliothis subflexa*

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Male *Heliothis subflexa* have three types of electrophysiologically characterized, pheromone-related long trichoid sensilla on their antennae. The olfactory receptor neurons (ORNs) in each type of sensillum respond to one component of the female-emitted sex pheromone. The A-type sensillar ORNs respond only to *(Z)*-11-hexadecenal (Z11-16:Ald), the B-type sensillar ORNs only respond to *(Z)*-9-hexadecenal (Z9-16:Ald) and those in the C-type sensilla respond only to *(Z)*-11-hexadenenol (Z11-16:OH). In addition to the pheromone-component-responsive ORNs, some of the A-type- and all of the B-type-sensilla house functionally unidentified neurons that are not responsive to any of our test compounds; these we have named "silent" ORNs.

We used the cobalt staining technique to demonstrate the neuronal pathways to the antennal lobe, of individual ORNs housed in each type of sensillum after physiological characterization by means of single-sensillum-recording. Neuronal projection patterns to the glomeruli were highly consistent for the ORNs from each type of sensillum. Three different glomeruli in the male-specific macroglomerular complex (MGC) were the primary targets of the three different types of sensillar ORNs. The glomerular destinations of the co-compartmentalized silent neurons in A- and B-type sensilla, respectively, were revealed to each project reliably to different glomeruli comprising a distinctive glomerular complex situated posterior to the MGC. This complex shows morphological conformity to a homologous structure in the antennal lobe of *Helicoverpa zea* that we previously described and called the Posterior Complex (PCx). Surprisingly, a few of the examined sensilla also housed ORNs that projected to ordinary glomeruli, in addition to the pheromone-component-sensitive ORNs housed in these same sensilla that projected to the MGC or to the PCx. Furthermore, the ordinary glomerular destinations of these ORNs were not arbitrary, but were restricted to only a few glomeruli.

Sex, spies and pheromones: phoretic egg parasitoids exploiting a butterfly anti-aphrodisiac

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Infochemicals are important stimuli for parasitoids during the process of host location. Eavesdropping on the host's sexual communication system is a known strategy found in several egg parasitoid species[1,2]. Sex pheromones emitted to attract mates can allow egg parasitoids to find adult hosts. Recently we have discovered a novel type of chemical espionage by the tiny wasp *Trichogramma brassicae* exploiting the anti-aphrodisiac of its host, the large cabbage white *Pieris brassicae*[3]. Anti-aphrodisiacs are species-specific pheromones, passed from male to female during mating, to render them less attractive to conspecific males[4,5]. Males of *P. brassicae* are known to cloak females with their own male odour benzyl cyanide during mating[6]. *Trichogramma brassicae* wasps can detect this scent and subsequently employ phoresy (defined as the transport of certain insects on the bodies of others for purposes other than direct parasitism[7]). A wasp first mounts a mated female butterfly labelled with the anti-aphrodisiac benzyl cyanide, and thereafter hitch-hikes with her to a host plant. When the butterfly starts to lay eggs, the wasp descends onto the leaf and parasitises the females' freshly laid eggs. To our knowledge, these results are the first to show chemical espionage of an anti-sex pheromone by parasitic wasps, which can lead to a dramatic reduction of host offspring survival. This fascinating strategy may have evolved frequently in nature and adds a new dimension to our understanding of host-parasitoid associations.

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Host-habitat selection by female aphid parasitoids: to what extent do innate and learned factors determine behaviour?

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This study investigates the olfactory responses in the generalist parasitic wasp *Aphidius colemani* in order to provide some insight to the origin of behavioural preferences. Bioassays were conducted in an olfactometer and insects were offered choices between leaves from different plant varieties. After the parasitoids' emergence from peach potato aphids (*Myzus persicae)* that had been reared entirely on Brussels sprout (*Brassica oleracea* L. var. *gemmifera*) variety Bedford-Winter Harvest, they preferred the leaf odour of that sprout variety over that of others. When the parasitoids were reared on aphid hosts from a continuous culture on a chemically defined artificial diet, the emerging females preferred several plant-leaf odours to clean air but did not distinguish between different sprout varieties. However, when parasitoids emerging from diet-reared hosts were allowed to contact sprout-reared aphid mummies for 24 h, they showed preference for the sprout variety on which those mummies had been reared. The parasitoids appeared to be influenced by post-emergence experience of the chemical cues contacted on the cuticle of the mummies. There also appears to be a maternal effect on preference in that, if the parasitoid's mother was reared on Winter Harvest, a single mummy from that same variety provided a large enough stimulus to evoke preference for Winter Harvest odour over that of a second sprout variety, Red Delicious. However, in order to switch preference to another sprout variety, Red Delicious, experience of a single mummy was not enough, five were needed. There seems to be a hierarchy of preference determination in the adult parasitoid from innate, through maternal influence to post-emergence experience. Thus parasitoids emerging from completely diet-reared hosts show preference for plant odours over clean air but not between plant varieties, but only a small exposure to a mummy is needed to elicit an odour preference if the mother was cultured on the same plant variety as the mummy stimulus. However, a stronger post-emergence experience of mummies from a different variety can switch the parasitoid's preference to that variety.

Female sex pheromone in immature insect males – a case of pre-emergence chemical mimicry?

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Sexual selection by competition for mates is a formidable force that has led to extraordinary adaptations in males. Here we present results suggesting a novel case of pheromone mimicry in males of *Lariophagus distinguendus*, a parasitic wasp of beetle larvae that develop in stored grain. Females of *L. distinguendus* produce a pheromone already before they emerge from a grain. Males are attracted to the parasitised grain and wait for females to emerge. Males emerging later than others are under enormous selection pressure since females mate only once. We show evidence that developing males fool their earlier emerging competitors by mimicking the female pheromone. Males exposed to pupae of either sex exhibit typical courtship behaviour. Searching males are not only arrested by grains containing developing females, but spend as much time on grains containing developing males. Hence, by distracting their competitors away from receptive females late males may increase their own chance to mate with these females. After emergence, males decompose the active compounds within 32 h probably to decrease molestation during their own search for mates. Chemical analyses of active pheromone extracts and bioassays using fractions demonstrate that the active compounds are among the cuticular hydrocarbons.

The sea lamprey migratory pheromone is a synergistic blend of three novel sulfated steroids

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The sea lamprey (*Petromyzon marinus*) begins life in freshwater streams which it then leaves to parasitize lake or oceanic fishes before eventually re-entering streams to spawn. Our previous studies have demonstrated that adult lampreys locate spawning streams using a migratory pheromone released by stream-resident larval conspecifics. Last year we reported the isolation of three behaviorally-active compounds with pheromonal activity from larval holding water. One of these we identified as the lamprey-specific bile acid, petromyzonol sulfate (PS) while the other two we found to be novel disulfated steroids which we have tentatively named '590' and '704' based on their molecular weights. Electroolfactogram recording from adult lampreys found 590 and 704 to have detection limits of 10^{-12} Molar (M) and 10^{-13} M, respectively. More recently, we have asked whether and how these compounds might function as a mixture. EOG cross-adaptation studies have found that all three are detected by independent receptor sites suggesting that lamprey may be capable of discerning them. Further, when tested in a two-choice preference maze, we have found that a mixture of all three is much more active than 704 alone, even when the latter was tested at elevated concentrations. A mixture of 704 and 590 was also more attractive than 704 alone. We conclude that migratory sea lamprey discern all components and that the migratory pheromone functions as a blend. Experiments are presently underway to determine the importance of specific ratios of the components needed to effect full activity so that the complete cue can be developed to control sea lamprey in the Great Lakes where this species is an invasive. Funded by the Great Lakes Fishery Commission.

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	Chemical Compound	Compound Common Name		Chemical Compound	Compound Common Name
1	1,4 Diaminobutane	Putrescine	72	Z,Z & Z,E-7,11- Hexadecadien-1-yl Acetate	Gossyplure
2	*i*-butyl acetate		73	Z,Z & Z,E-7,11- Hexadecadien-1-yl Acetate OH free	Gossyplure
3	*i*-butyl alcohol		74	Z-8- 14-Methyl Hexadecenal	
4	E-2- Hexenal		75	E,Z-9,11- Hexadecadienal	
5	E-2- Hexenol		76	Z,Z-11,13- Hexadecadienal (NOW Technical pheromone)	
6	1- Hexanol		77	Z,Z-7,11- Hexadecadien-1-yl Acetate	
7	Z-3 Hexenol		78	Z,E-7,11- Hexadecadien-1-yl Acetate	
8	2,4- Hexadienyl Acetate		79	Z-13- Octadecenal	
9	2,4- Hexadienyl Butyrate		80	Z-13- Octadecen-1-yl Acetate	
10	2,4- Hexadienyl Isobutyrate		81	E,Z-3,13- Octadecadien-1-yl Acetate	
11	2,4- Hexadienyl Propionate		82	Z,Z-3,13- Octadecadien-1-yl Acetate	
12	Hexanal		83	Z-9- Tricosene	
13	Hexanoic Acid		84	Z,Z,Z-3,6,9- Tricosatriene	
14	Z-3- Hexen-1-ol		85		(+) Disparlure
15	Z-3- Hexenyl Acetate		86		*Alfa*-bisabol
16	Methyl Cyclohexene		87		*beta*-Farnesene
17	Methyl Cyclohexadiene		88		Caryophylene(trans)
18	Trimethyl Cyclohexene		89		Caryophyllene oxide
19	Heptyl butyrate		90		Ceralure
20	E-2- Heptenal		91		Ceralure B1
21	6-methyl-4-hydroxi-E-2-heptene	Rhyncophorol	92		Cirtonellol Prime
22	Octyl butyrate		93		Citronellal
23	E,2- Octen-1-ol		94		Citronellol 96 FCC
24	1- Octen-3-ol		95		Citronellol AJ FCC
25	4,6-Dimethyl-7-hydroxy nonanone	Serricornin	96		Citronellyl Acetate
26	4-methyl-5-nonanol		97		Citronellyl Anthranilate
27	Ethyl-2,4-Decadienoate		98		Citronellyl nitrile
28	Z&E-5- Decen-1-yl Acetate		99		Creosol
29	1,7-Dioxaspiro[5.5]Undecane		100		Cuelure
30	E-3- Dodecen-1-yl Acetate		101		Dichloro phenol (2,6)
31	Z-5- Dodecen-1-yl Acetate		102		Dipentene tech
32	Z-7- Dodecen-1-yl Acetate		103		Disparlure (Racemic)
33	E-8- Dodecen-1-yl Acetate		104		Eugenol
34	Z-9- Dodecen-1-yl Acetate		105		Farnesene
35	Z-8- Dodecen-1-yl Acetate		106		Grandlure complete
36	E-9- Dodecen-1-yl Acetate		107		Grandlure I
37	E-10- Dodecen-1-yl Acetate		108		Grandlure II
38	E,E-8,10- Dodecadien-1-yl Acetate		109		Grandlure III and IV
39	E,Z-3,5- Dodecadien-1-yl Acetate		110		Guaiacol
40	Z-8- Dodecen-1-ol		111		Guava Oil
41	Z-7- Dodecen-1-ol		112		Invert sugar
42	E,E-8,10- Dodecadien-1-ol		113		Latic Acid 85%
43	Z,E-7,9,11- Dodecatrien-1-yl formate		114		Latic Acid Solution
44	Tridecanal		115		Linalool
45	E&Z-4- Tridecen-1-yl Acetate (TPW Technical pheromone)		116		Lycolure
46	E,Z-4,7- Tridecadien-1-yl Acetate		117		Methyl amine HCl
47	E,Z,Z-4,7,10 Tridecatrien-1-yl Acetate		118		Methyl Eugenol
48	E&Z-11- Tetradecenal		119		Methyl indole (3)
49	Z-7- Tetradecen-1-yl Acetate		120		Mosquito Oviposition Pheromone
50	Z,E-9,12- Tetradecadien-1-ol		121		Myrcene
51	Z-9- Tetradecen-1-ol		122		Oleic acid
52	Z-7- Tetradecen-2-one		123		Orange Oil
53	Z-8- Tetradecen-1-yl Acetate		124		Oriental fruit moth (OFM Technical pheromone)
54	Z-9- Tetradecen-1-yl Acetate		125		Peach tree borer (PTB Technical pheromone)
55	Z-11- Tetradecen-1-yl Acetate		126		Pearlate
56	E-11- Tetradecen-1-yl Acetate		127		Phenethyl alcool
57	3,8- Tetradecen-1-yl Acetate		128		Prenyl Acetate
58	E,Z-3,8- Tetradecadien-1-yl Acetate		129		Prenyl Benzoate
59	Z,E-9,11- Tetradecadien-1-yl Acetate		130		Prenyl Caproate
60	E,E-9,11- Tetradecadien-1-yl Acetate		131		Prenyl Formate
61	E,Z,Z-3,8,11- Tetradecatrien-1-yl Acetate		132		Prenyl Isobutyrate
62	Tetradec-1-yl Acetate		133		Sorhamentin
63	E-11- Tetradecen1-yl Acetate		134		Styrene
64	Z-11- Hexadecen-1-ol		135		Sugar distillated
65	E-11- Hexadecen-1-ol		136		Terpinyl Acetate
66	Z-9- Hexadecenal		137		Terpinyl formate
67	Z-11- Hexadecenal		138		Trimedlure
68	Z-9- Hexadecen-1-yl Acetate		139		Vanillin
69	Z-7- Hexadecen-1-yl Acetate		140		Vannila extract
70	E-11- Hexadecen-1-yl-Acetate		141		Verbenone
71	Z-11- Hexadecen-1-yl Acetate				

Overview of semiochemicals research and application success in Korea

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A rapid progress has been made in research on insect pheromones and other semiochemicals in Korea during the last two decades. Sex pheromone compositions have been identified in several major orchard and other horticultural moth pest species and some other species in Korea, such as *Helicoverpa assulta*, *Grapholita molesta*, *Carposina sasakii*, *Adoxophyes orana*, *Lyonetia prunifoliella*, *Phyllonorycter ringoniella*, *Dichocrocis punctiferalis*, *Ostrinia furnacalis* and *Peridroma saucia*. Some of these sex pheromones identified have already been successfully incorporated into IPM system, now being used as direct and indirect pest control means in Korea. Korean populations of many of these species showed significant differences in pheromone compositions from those reported in her neighbor countries of Japan and China, suggesting that such interpopulational pheromone polymorphism is more common across insect world than it was thought. Sex pheromone compositions were also identified in some aphid species in Korea such as *Aphis spiraecola* and *Tuberocephalus momonis*, and it was soon found that the aphid sex pheromone components were highly attractive to their predator lacewings. Presence of kairomones responsible for attracting egg parasitoids was shown in a pheromone component, hot pepper odor and male scale factor for *H. assulta*, and in male accessory gland for *Lymantria dispar*. Studies on the regulation of pheromone biosynthesis and release were accompanied in some species in Korea, resulting in the characterization of PBAN (pheromone biosynthesis activating neuropeptide) in *H. assulta* and *Adoxophyes* sp. Semiochemical research system has been firmly established in Korea through these studies. In some areas, successful monitoring and mating disruption technology has been successfully transferred from researchers to the end users in Korea, which will be soon expanded to wider areas. Continuous attempts to identify and use semiochemicals as well as wider applications of semiochemicals in pest control are expected in Korea in the coming years.

Insect PBAN and PBAN receptor

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Many female moths produce and release sex pheromones to attract and successfully mate with a conspecific male in field. Sex pheromone production in lepidopteran moths is known to be under the regulation of a pheromone biosynthesis activating neuropeptide (PBAN). PBAN is produced in the suboesophageal ganglion (SEG) and released into the hemolymph via the corpora cardiaca. All PBANs identified are consisted with 31-35 amino acids from moths, and have the common five amino acid sequence in C-termini. The conserved pentapeptide, FXPRL amide, is the minimum amino acid sequence required for physiological activity. This conserved sequence is found 1) stimulating pheromone biosynthesis in moths, 2) the embryonic diapause hormone (DH) in the silkworm, 3) inducing melanization and reddish coloration hormone (MRCH) in larval moths, 4) myotropins or pyrokinins to stimulate muscle contraction in cockroach and locust, 5) accelerating puparium formation in flies and 6) regulation of pupal development and diapause in heliothine moths. The group of these peptides with FXPRL amide in C-terminal sequence is named with the PBAN/Pyrokinin family.

PBAN acts directly on pheromone gland cells by using calcium and cAMP as second messengers. PBAN receptors have been identified a gene encoding a G protein-coupled receptor (GPCR) from pheromone glands of the female moths, *H. zea*, *H. armigera* and *B. mori* with functional expression and Ca^{2+} binding assay with various FXPRL peptides *in vitro* insect cells. PABN receptor consisted with 7-transmembrane domain and tree extracellular loops, is related to several receptors from insects (*Drosophila* and *Anopheles*) and to neuromedin U and ghrelin receptors from vertebrates. This study will provides to find physiological role and mode of action of similar neuropeptides, and to develop a novel biological pesticide as non-toxic peptide mimic compound for insect pest management.

Leaf beetles of the genus *Diorhabda* (Coleoptera: Chrysomelidae): studying highly volatile blends of semiochemicals

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The leaf beetle *Diorhabda elongata* Brullé (Coleoptera: Chrysomelidae) is a newly released biological control agent for saltcedars, *Tamarix* sp., an exotic, invasive weedy tree in the Western US. The recently identified male-produced pheromone components, (2*E*,4*Z*)-2,4-heptadienal and (2*E*,4*Z*)-2,4-heptadien-1-ol, and the synergistic saltcedar "green leaf" volatiles are highly attractive to males and females in the field. These semiochemicals could be useful for studying various key population attributes of newly released *Diorhabda* populations/species.

Different day-length adapted *Diorhabda* populations, imported from Asia and Europe, show differences in pheromone blend ratios and are deployed in habitats with various *Tamarix* species.

In the absence of a sensitive behavioral bioassay, an electrophysiological dose-response study has been developed based on released pheromone and plant materials, to predict possible attractive field blends, and the results are used to study the behavioral significance of these highly volatile and variable blends of semiochemicals.

Heliothine moth olfaction

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Much has been learned over the past few decades about insect olfaction through comparative neuroethological and neuroanatomical studies of the sex pheromone olfactory pathways in heliothine male moths. The efforts of many groups around the world have resulted in new insights about how these moths perform pheromone blend quality discrimination, and how this ability might have evolved from species to species. Professor Kyung Saeng Boo's research on *Helicoverpa assulta* and his collaborative research on this species with Professors Hanna Mustaparta and Bente Berg has provided some particularly informative results with regard to olfaction-related variation across species. There are several consistent aspects concerning the sex pheromone olfactory pathways of heliothine moths. One of these is how the tuning profiles of olfactory receptor neurons (ORNs) vary across species in response to the same pheromone-compound-related ligands. Another is how the axons of these ORNs target particular glomeruli in the macroglomerular complex (MGC); tuning profiles of projection interneurons that arborize in these same target glomeruli match those of the ORNs, creating linear, odorant-specific pathways for transmission of pheromone-compound-related information. Recent work involving hybrids between *Heliothis virescens* and *H. subflexa* has shed light on the different ways in which ORN tuning profiles might shift and affect behavior. In our studies, the most noteworthy shift in ORN responsiveness in hybrid males was an overall increase in sensitivity to Z9-14:Ald exhibited by ORNs responsive to Z9-16:Ald in B-type sensilla. Heightened cross-responsiveness to Z9-14:Ald by these hybrid ORNs correlates well with the behavioral cross-responsiveness observed in hybrids in which Z9-14:Ald was found to be able to substitute for Z9-16:Ald in the pheromone blend, a behavior not observed in parental types. The greater sensitivity to Z9-14:Ald in hybrid ORNs also correlates well with hybrid male antennal lobe projection interneurons that also exhibited a shift toward greater cross-responsiveness to Z9-14:Ald and Z9-16:Ald. We propose that the differences between parental *H. virescens*, *H. subflexa*, and hybrid males' levels of ORN cross-responsiveness to Z9-16:Ald and Z9-14:Ald are due to differential enhancement or repression of gene expression for two different receptors that are co-localized on the same ORN in B-type sensilla. We also propose that a flexibility in the level of expression of the receptor for Z9-14:Ald that is co-expressed with receptors for either Z11-16:Ac or Z11-16:OH on ORNs in the C-type sensilla of these two species would explain differences in other ORN tuning profiles related to upwind flight behavior. We have recently characterized the existence of a previously undescribed structure, the Posterior Complex (PCx), in the antennal lobe of males of *H. zea* and *H. subflexa* that is closely associated with the MGC. One particular PCx glomerulus receives input from the type of 'silent' ORN that is co-compartmentalized with Z11-16:Ald-sensitive ORNs in the A-type sensilla of both species. Axons from another type of silent ORN that is co-compartmentalized with Z9-16:Ald-sensitive ORNs in B-type sensilla of *H. subflexa* also project to the PCx, but to a different PCx glomerulus than do the silent ORNs from A-type sensilla. It will be interesting to see whether or not these ORNs are truly unresponsive to all odorants; such silence might be the result of the repression of expression of a gene for a receptor that would have otherwise imparted sensitivity to some unknown pheromone-related compound.

Catnip, aphids and lacewing predators: Tritrophic coincidence or confusion?

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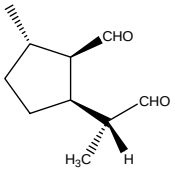
Lacewings, especially green lacewings (Neuroptera: Chrysopidae), are among the most common predators of aphids and other soft-bodied insects. Some lacewings are attracted to certain isomers of nepetalactone and nepetalactol, which are found in the catnip plant (Lamiaceae: *Nepeta cataria*) and are also components of aphid pheromones, leading to speculation that these lacewings use aphid pheromones to find prey. However, we discovered that males of the goldeneyed lacewing, *Chrysopa oculata*, produce 1*R*,2*S*,5*R*,8*R*-iridodial (plus nonanal, nonanol and nonanoic acid) from elliptical epidermal glands on the 3rd-8th abdominal sternites, and that this iridodial isomer occurs as an impurity in 1*R*,4a*S*,7*S*,7a*R*-nepetalactol. Initial experiments with sticky traps baited with 1*R*,2*S*,5*R*,8*R*-iridodial caught many goldeneyed lacewing males (but no females), whereas males were only weakly attracted nepetalactol (Chauhan et al. 2004, Zhang et al. 2004). GC-EAD experiments showed that *C. oculata* males and females were up to 10000 times more sensitive to 1*R*,2*S*,5*R*,8*R*-iridodial (threshold between 0.1 and 1 pg) than to other compounds tested. We also found that the herbivore-induced plant volatile, methyl salicylate, significantly synergized attraction of males to 1*R*,2*S*,5*R*,8*R*-iridodial.

In 2004, additional field-testing of 1*R*,2*S*,5*R*,8*R*-iridodial was conducted in the vicinity of Beltsville, Maryland, and Spokane, Washington. In Maryland, we demonstrated that female goldeneyed lacewings are attracted to and lay eggs nearby pheromone-impregnated lures, although they do not enter pheromone-baited traps. In Washington State, traps baited with iridodial were powerfully attractive to goldeneyed lacewing males, as well as males of another species tentatively identified as *Chrysopa nigricornis* (Zhang, unpubl.). However, iridodial occurred only in thoracic extracts of *C. nigricornis* males; no iridodial was detected in abdominal extracts. Sweep netting and observations in the vicinity of traps indicated that *Chrysopa* females of both species were attracted to pheromone-treated areas.



1*R*,2*S*,5*R*,8*R*-Iridodial

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Sex pheromones of the navel orangeworm and *Pyralis farinalis*: the missing pieces to the puzzle.

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The major component of the sex pheromone of the navel orangeworm (NOW, Lepidoptera: Pyralidae) was identified in the late 1970's as (11Z,13Z)-hexadecadienal (Coffelt et al., 1979). However, this compound as a single component has worked neither consistently nor well as an attractant in traps, suggesting that there might be one or more missing components to the pheromone blend. Sporadic efforts by a number of research groups over the past two decades, including ours, failed to identify the missing components. Consequently, we took an indirect approach to identification of possible missing components by taking advantage of the known cross attraction between NOW and another pyralid moth *Pyralis farinalis* (Landolt and Curtis, 1982). This approach proved successful: in fall 2003, careful analyses of pheromone gland extracts of *P. farinalis* by GC-EAD revealed a minor component with an unexpectedly long retention time that elicited strong and consistent responses from male antennae. This compound was subsequently identified as (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-tricosapentaene [(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-23:H]. Subsequent analyses of NOW pheromone gland extracts in early spring of 2004 identified the same compound, as well as the homologous (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene [(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-25:H]. Interestingly, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-23:H elicited much weaker and less consistent responses from antennae of male NOW than it did from antennae of *P. farinalis*. This weak response, in combination with its unexpectedly long retention time in comparison to the major component of the pheromone, partially explains why it was missed by previous researchers. However, laboratory and field bioassays have demonstrated the critical importance of the pentaenes to the attractiveness of the pheromone blend, both for NOW, and also for another pyralid species, *Dioryctria abietivorella* (Millar et al., 2005). Overall, these blends have several remarkable features. First, their dissimilar components almost certainly arise from two independent pheromone biosynthesis pathways, with the shorter chain components probably being synthesized in the pheromone gland, and the longer chain hydrocarbons possibly being synthesized in oenocyte cells and transported to the gland through the hemolymph. Second, it is unclear how calling female moths successfully emit blends of compounds with such substantial differences in size and vapor pressure. Third, it is surprising, but not unprecedented, that the polyunsaturated hydrocarbon components elicit relatively weak responses from antennae of male NOW moths, despite their being crucial to eliciting behavioral responses.

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Kairomone-based trapping of the small hive beetle *Aethina tumida* in a Florida apiary

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The small hive beetle, *Aethina tumida*, originating from Africa, is an invasive destructive parasitic pest of European honey bees in the USA. The adult beetle is strongly attracted to adult worker honey bee volatiles. In GC-EAD analysis of Super Q-trapped volatiles of worker honey bees, several components elicited antennal responses by both males and females of the beetle. When bioassayed in a wind tunnel, eight of the EAD-active components detected dominated the volatile profile released into the tunnel by both living worker honey bees and their Super Q-trapped volatiles. GC-MS analysis identified these components as isopentyl acetate (worker honey bee alarm pheromone), 2-heptanone, octanal, hexyl acetate, nonanal, 2-nonanone, methyl benzoate and decanal. In an Apiary in Florida, PVC pipe traps baited with natural media that released these same compounds as part of a blend of volatiles caught significantly more beetles than unbaited traps. Significantly more beetles were caught in baited traps placed in the shaded woody areas than in baited traps placed in the sunny open field surrounding hives. These results demonstrate the kairomonal response of adult beetles to honey bee volatiles.

A cost of alarm pheromone production in cotton aphids, *Aphis gossypii*

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The sesquiterpene, (*E*)-β-farnesene, is used by many aphid species as an alarm pheromone to warn related individuals of predation. Disturbed cotton aphids, *Aphis gossypii* Glover, released (*E*)-β-farnesene into the air as detected by SPME (solid phase microextraction) and gas chromatography mass spectrometry (GC-MS). Solvent extracts of cotton aphids of various life stages and weights also were analyzed by GC-MS for sums of ions 69 and 93, which discriminated (*E*)-β-farnesene from coeluting compounds. Aphids of all life stages and sizes reared on cotton plants in both an environmental chamber and glasshouse contained (*E*)-β-farnesene in amounts ranging from 0.1 to 1.5 ng per individual. The quantities of (*E*)-β-farnesene in aphids increased in relation to increasing body weight, and variation in individual weights explained about 82% of the individual variation in alarm pheromone. However, the concentrations (ng/mg fresh weight) declined exponentially with increasing body weight. These findings indicate that aphid nymphs try to compensate by producing relatively more pheromone per weight than adults but still cannot approach an evolutionary optimal load, as assumed in adults with the greatest total amounts. This suggests that young aphids need to balance costs of growth and maturation with costs of producing the alarm pheromone.

Pheromone-mediated mating in the aphid parasitoid, *Aphidius ervi*

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Aphidius ervi was introduced as a biological control agent of the pea aphid nearly 50 years ago and is now well established in North America. More recently it has been introduced into South America and Australia. However, despite its wide use very little is known about the reproductive biology of this species. We undertook a study to examine the mating behaviour of this species under both laboratory and field conditions, looking at the effects of age, time of day and certain abiotic conditions. The findings will be compared with data from a previous study on an indigenous potato aphid parasitoid, *Aphidius nigripes.*

Chemical compounds inducing aggregation in *Periplaneta americana* (L.) (Insecta: Dictyoptera)

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The cockroach *Periplaneta americana* (L.) is a gregarious species as male and female adults and larvae of all developmental stages gather together in common shelters during their diurnal rest phase [1]. Chemical communication among cockroaches is necessary to induce the aggregation process and to maintain the cohesion of aggregates. Behavioural tests proposing a choice between two filter papers used as resting sites, estimated the efficiency of chemical compounds to induce aggregation. Cockroaches selected and aggregated on papers conditioned by direct contact of conspecifics [2]. Our aim was to identify the chemical compounds that mediate the formation of aggregates. High concentrations of dichloromethane extracts of cuticular hydrocarbons induced aggregation in binary choice tests [3]. Volatile compounds emitted by *P. americana* were sampled without contact with the insects. These compounds were attractive in a Y-olfactometer and induced aggregation in binary choice tests. GC-MS analyses of volatile extracts revealed ten fatty acids. The role of each fatty acid in inducing aggregation was investigated and only three of these compounds were efficient in inducing aggregation. Aggregation on papers conditioned by the three fatty acids was enhanced when low concentrations of cuticular hydrocarbons were added. Currently, experiments aim to specify whether this chemical blend is the aggregation pheromone of *Periplaneta americana*.

This study was supported by the European program LEURRE (IST-2001-35506).
Key words: *Periplaneta americana*, cuticular hydrocarbons, volatile fatty acids, aggregation.

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Sex pheromones of apple leaf curling midge, *Dasineura mali*, and raspberry cane midge, *Resseliella theobaldi*: a new class of pheromone structures

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Components of the female sex pheromones of the apple leaf curling midge, *Dasineura mali* Kieffer, and the raspberry cane midge, *Resseliella theobaldi* Barnes (Diptera: Cecidomyiidae) have been identified and synthesised and the synthetic compounds are attractive to male midges in the field. The pheromones were collected by trapping of volatiles which proved far more effective than gland extraction methods used previously. Even so the single component of the pheromone of *D. mali* was present at only 2 pg per female. This was detected by electroantennography (EAG) linked to gas chromatographic (GC) analysis, and identified by interpretation of the mass spectra and comparison with synthetic standards. A rubber septum impregnated with 1 μg of the synthetic, racemic pheromone is highly attractive to male *D. mali*, and release rate measurements indicate this lure will remain active for several years in the field. Pheromone components of *R. theobaldii* were collected in larger amounts and could be detected by comparison of GC analyses of collections from females and males as well as by linked GC-EAG analyses. Four components were identified by interpretation of their mass spectra and comparison with synthetic standards. Only one enantiomer of each of the chiral components is produced by the insect, as determined by GC analysis on a chiral cyclodextrin column. However, the synthetic racemate of the major component is attractive to male *R. theobaldii* in field trapping tests, and the effect of addition of minor component is being investigated. The enantiomers of the major components of the pheromones of both species are being separated by liquid chromatography for field testing and they are also being synthesised to determine their configurations. These compounds are variations on a new class of pheromone structure that is currently the subject of a patent application but which will be described in the oral presentation.

Female sex pheromone of the dogwood borer (DWB) *Synanthedon scitula*

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The female sex pheromone of dogwood borer (DWB) *Synanthedon scitula* (Harris) (Lepidoptera: Sesiidae) was determined to be a blend of (*Z,Z*)-3,13-octadecadienyl acetate (*Z,Z*-3,13-ODDA), (*E,Z*)-2,13-octadecadienyl acetate (*E,Z*-2,13-ODDA), and (*Z,E*)-3,13-octadecadienyl acetate (*Z,E*-3,13-ODDA) by gas chromatographic-electroantennographic detection (GC-EAD) and gas chromatography-mass spectrometry. The ratio of the pheromone components was estimated to be 88 : 6 : 6 (v/v) of the *Z,Z*-3,13-ODDA, *E,Z*-2,13-ODDA, and *Z,E*-3,13-ODDA (trinary blend), respectively. Preliminary field assays demonstrated that the major sex pheromone component, *Z,Z*-3,13-ODDA (single-component), itself was attractive. A blend of *Z,Z*-3,13-ODDA with 1~3% of *E,Z*-2,13-ODDA (binary blend) was significantly more attractive than the single-component. A third component, *Z,E*-3,13-ODDA, was sometimes observed in GC-EAD and significantly enhanced attraction of the binary blend under most conditions. Traps baited with the trinary and binary blends captured significantly more male dogwood borers than traps baited with the best commercially available lure, with greatest captures in traps baited with the trinary blend. Species-specificity of the trinary and binary blends was very high; >97% of all moths captured were dogwood borer, compared with 6-74.4% for the commercial lure. Male dogwood borer showed a concentration-dependent response to traps baited with different source concentrations of the trinary blend at all locations, and of the binary blend at most locations. The concentration threshold can be as low as 100-µg per rubber septum per trap. Male dogwood borer did not discriminate between the trinary and binary blends at distances of 3 and 30 m, but did discriminate between the trinary blend and the commercial lure at distances of 100 m. Lures containing 1 mg of binary and trinary blends attracted 18 and 28 times more male DWB moth, respectively, than caged virgin females in field trials. However, attraction to the trinary lures could be significantly antagonized by addition of as little as 0.5-1% of a geometric isomer, *E,Z*-3,13-octadecadienyl acetate (*E,Z*-3,13-ODDA) (pheromone antagonist). In a period of 12 wk in 2004, more than 60,000 males were captured in Pherocon 1C and Delta (Trécé Inc., Salinas, CA) traps baited with synthetic pheromone blends in six apple orchards in Virginia, West Virginia, and North Carolina.

Characterisation of olfactory pathways involved in pheromone and interspecific signal information in three heliothine species

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The sub-family Heliothinae comprises more than 80 species, distributed in all five continents. Especially fascinating is the fact that heliothine moths communicate, not only within, but also across the species. Certain pheromone substances produced by the females of one species may act as a behavioural antagonist on males of sympatric species. Thus, the males have evolved receptor neurons detecting pheromones leading to attraction and sexual behaviour, as well as neurons detecting interspecific signals leading to interruption of attraction. From an evolutionary perspective, it has been interesting to compare the structure and function of the male specific neural pathways across heliothine species, particularly of the genera *Helicoverpa* and *Heliothis*. We have made three-dimensional reconstructions of the male specific macroglomerular complexes (MGCs), processing information about insect produced compounds, in three heliothine moths, *Heliothis virescens*, *Helicoverpa assulta*, and *Helicoverpa armigera* (Berg et al. 2002; Skiri et al. 2005). Furthermore, we have studied the peripheral compartmentalization of the male specific neuron types as well as their axonal projections to the MGC units. Particularly interesting is the characteristics of *Helicoverpa assulta*, since this species is unique as concerns the composition of the pheromone blend. The switch in using a different major pheromone component than the other species studied is reflected in a different tuning of the glomeruli comprising the MGC (Berg et al. 2005). Based on similarities and differences across the species, general principles are discussed.

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Olfactory receptor neuron responses of Asian and European corn borer hybrid males to parental pheromone components.

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We recorded responses from single olfactory receptor neurons (ORNs) in the trichoid sensilla of males of the univoltene Z strain European corn borer (*Ostrinia nubilalis*), the Asian cornborer *(O. furnacalis*), and their bidirectional F1 hybrids. The pheromone components tested include those of the respective parental species: Z/E-11- and Z/E-12-tetradecenyl acetate, as well as the behaviorally antagonistic compound (Z)-9-tetradecenyl acetate. As previously shown (Hansson et al., 1987; Roelofs et al., 1987) , ORNs in the European corn borer exhibited three distinct spike sizes and selective responses to Z11-, E11-, and Z9-14:OAc, respectively. Asian corn borer sensilla had a large-amplitude –spiking ORN responsive to both Z12- and E12-14:OAc, a much smaller- spiking ORN stimulated selectively by E12-14:OAc, and an intermediate spike-sized ORN tuned to Z9-14:OAc. The ORNs of both parental species were unresponsive to the respective cross-specific pheromone components. ORNs in the hybrid sensilla responded to a broader range of parental pheromone components in a manner that was largely consistent with the ORN-specific patterning of both parents. However, the spike amplitude relationships differed from those of both parents. Like the Asian corn borer parents, hybrids usually had two distinctly sized spiking ORN that were sensitive to E12-14:OAc. However, the smaller-spiking ORN became larger in hybrids and now showed sensitivity also to E11-14:OAc, whereas the larger-spiking E12- and Z12-14:OAc-sensitive ORN responded now also to Z11-14:OAc. Hybrid ORNs were thus more broadly tuned, but the responses to all compounds were less intense than those of both parental-type ORNs. In hybrids there was also a distinct intermediate-sized spiking ORN with a selective response to the behavioral antagonist Z9-14:OAc. This antagonist-related hybrid ORN was unresponsive to any of the parental-species' pheromone components, thus being similar to the antagonistic ORNs of both parental species. Any differences in the upwind flight behavior of hybrid males compared to those of *O. nubilalis* and *O. furnicalis* would therefore not seem to be attributable to the activity of the Z9-14:Ac-sensitive ORN.

Antagonistic activity of pheromone responses in Lepidopteran males by esterase inhibitors

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Trifluoromethyl ketones (TFMKs) are a family of compounds able to reversibly inhibit a number of esterases and proteases, particularly the antennal esterases present in insect olfactory tissues. These are key enzymes for a rapid degradation of pheromone esters, thus maintaining a low stimulus noise level in sensory hairs, which is crucial for a successful attractant and mating behavior. Possibly as a consequence of their esterase inhibition effect, these chemicals also display a remarkable antagonistic activity of the pheromone responses in some Lepidoptera in addition to other electrophysiological effects on receptor neurons tuned to pheromone components.

Following our current research on this topic, we present herein an overview of the effects of these compounds on the Mediterranean maize pests *Sesamia nonagrioides* and *Ostrinia nubilalis*, both in the laboratory and in the field, and their prospectives as new pest control agents. In both insects the TFMK analogues of the main component of the pheromone displayed an antiesterase activity on male antennae esterase extracts with IC_{50} values in the micromolar range. In addition, these compounds elicited a remarkable disruptant effect in a wind tunnel, particularly on close approach and source contact behavior. In the presence of the antagonist, males displayed erratic flight tracks with frequent counter turns and intersections with the plume when attracted to virgin females or synthetic pheromone lures. In different experiments in the field, the TFMK analogues resulted effective antagonists of the pheromone action when mixed with the natural attractant in 10:1 ratio, the effect being dose dependent. It should be noted that only TFMKs very closely structurally related to the major component of the pheromone are significantly active, so that homologous compounds with one carbon less in the chain are remarkably less active, particularly as esterase inhibitor.

The effects displayed by these chemicals in conjunction to the previously noted low toxicity to mice, as a consequence of their reversible activity, provide valuable data for their putative utilization as new biorational pest control agents (1).

(1) A. Guerrero, M.P. Bosch, G. Rosell, M. Riba and A. Sans, Patent pending Spain 200301667 (2003).

Elongases in *Drosophila* : role in fatty acid and sex pheromones metabolism.

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Species and sex recognition in *Drosophila melanogaster* are mediated by cuticular contact pheromones. These molecules are long chain hydrocarbons which are synthesized from fatty acids through a series of elongation and desaturation steps and a final decarboxylation. We investigated the role of several elongases and characterized one of them, *elo68*: this gene was selectively expressed in males (testis and ejaculatory bulb). When expressed in yeast, it could elongate myristoleic and palmitoleic acids, therefore sharing an Elo1 specificity. We isolated and generated *Drosophila elo68* mutants; one mutant line with only 1% *elo68* showed decreased levels of vaccenyl acetate, a male pheromone produced in the ejaculatory bulb. The induction of *elo68* expression at 21°C was also paralleled with higher vaccenyl acetate production. Although these effects could be indirect, the data suggest that *elo68* might play a role in vaccenyl acetate biosynthesis.

The chemistry of collembola

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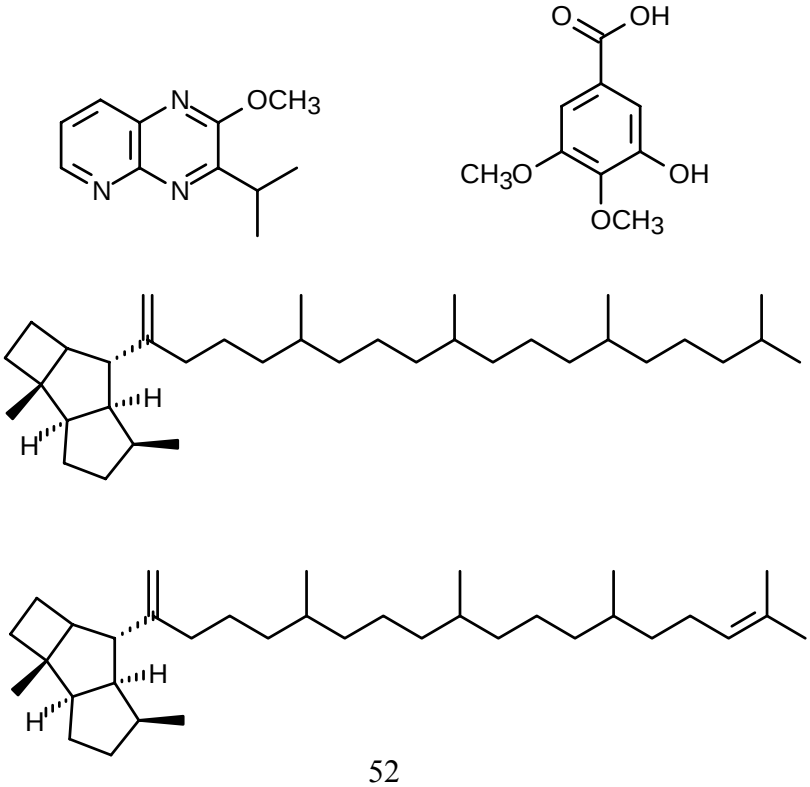
Chemical defense and chemical communication plays an important role in collembolans. The furca of hemi- and euedaphic collembola is often reduced or absent and thus they are unable to escape predators by jumping. In the presentation we will discuss different aspects of their chemistry. Several species show only low amounts of typical insect cuticular hydrocarbons. Instead unusual tetraterpenes and norprenyltetraterpenes are present, which are unique in the animal kingdom. They can be regarded as oligoprenylated sesquiterpenes, a rare compound class. Some analytical procedures for their detection are presented.

Several compound classes are used as defensive compounds by collembola. These include alkaloids like pyridopyrazines, benzoic acid derivatives, amides, and aldehydes. New results on the biosynthesis of these compounds and their bioactivity will be reported. Furthermore we will report on the identification of new chloro-containing metabolites from the springtail *Ceratophysella sigillata.* This species is active in the winter and can sometimes found in large quantities on snow. The analysis of an extract of this species revealed the presence of a metabolite with high chlorine content. The unusual structure of this compound was elucidated by thorough NMR investigations, IR spectroscopy, chemical derivatization, and ab-initio calculations as well as by the use of other chemoinformatic tools like COCON. This compound, which we propose to call sigillin, is not related to other known insect metabolites. The elaborate structure of this compound suggests an important function of sigillin for the collembola.



Characterization of early stages of the iridoid biosynthesis in larvae of the leaf beetle *Phaedon cochleariae*

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Insects developed an impressive diversity of toxins to defend themselves against predatory attacks. Some species owe their defense to iridoids which can be sequestered or autogenously synthesized. Larvae of certain Chrysomelina species *de novo* synthesize iridoids via the mevalonate pathway and release them in case of danger from nine pairs of dorsal glands. In the species *Phaedon cochleariae* it has been demonstrated that the conversion of glucosidically bound 8-hydroxygeraniol to chrysomelidial occurs in the glandular reservoir. However, the location of the precursor's biosynthesis has remained elusive. Here we present that the fat body is involved in the synthesis of glucosidically bound 8-hydroxygeraniol. In our studies we compared *P. cochleariae* and *Chrysomela populi*, a non-iridoid producing species belonging also to the subtribe Chrysomelina. Using Real Time PCR we determined a high mRNA level of the gene encoding the 3-hydroxy-3-methylglutaryl-CoA reductase - a key enzyme in the mevalonate pathway - only in the fat body of the iridoid producing species in comparison to gut, Malpighian tubules, glands, head and to the tested tissues of *C. populi*. Moreover, we determined a significant geranyl pyrophosphate (GPP) synthase activity in the fat body of *P. cochleariae* compared to the non-iridoid producing species where no GPP-synthase activity was detectable. These results correlate with the identification of glucosidically bound 8-hydroxygeraniol in the fat body of *P. cochleariae*. The compound could not be found in other tissues and not in any tested *C. populi* tissue. Thus, we propose that in *P. cochleariae* glucosidically bound 8-hydroxygeraniol might be released from the fat body and transported via the hemolymph into the glandular reservoir where the compound is further metabolized.

An update on the juvenile hormone regulation of pheromone production in bark beetles.

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Juvenile hormone III (JH III) regulates the de novo biosynthesis of monoterpenoid aggregation pheromones in midgut tissue of males in many bark beetle species. cDNA microarrays of *Ips pini* genes showed that all of the represented mevalonate genes were coordinately up-regulated in midgut tissue by feeding and JH. Real time PCR demonstrated that males had higher basal levels of the mevalonate pathway mRNAs in midgut tissue than females, with geranyl diphosphate synthase (GPPS) and a putative cytochrome P450 having 400- and 500-fold higher levels. GPPS cDNA from *I. pini* was isolated, expressed and modeled. It is the first animal GPPS isolated and is located almost exclusively in midgut tissue, consistent with its role in pheromone production. The expressed enzyme produced GPP as its primary product. The substrate binding pocket of GPPS is smaller than that of *I. pin*i farensyl diphosphate synthase (FPPS) so that FPP is not well accommodated in the substrate binding pocket of GPPS. Enzyme studies showed that HMG-R, HMG-S and GPPS activities were up-regulated by both feeding and JH in *I. pini*, whereas HMG-R and GPPS enzyme activity were only up-regulated by feeding in *I. confusus*. Using the *I. pini* microarray, the expression levels of mevalonate pathway genes were shown to be also up-regulated in *I. confusus*. Finally, proteomic studies are underway to examine the effect of feeding and JH III on protein levels in both *I. pini* and *I. confusus*.

Plant chemistry-based host specificity of *Oxyops vitiosa*, a weevil introduced for weed biological control of *Melaleuca quinquenervia*

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The safety of weed biological control depends upon the selection and utilization of the target weed by the control agent while causing minimal harm to non-target plant species. However, the mechanisms used by weed biological control insects to select the correct host plant while sparing desired plants is poorly known. Host specific insects, like those used for weed biological control have sensory neurons that are specifically tuned to components of the volatile profile of their host plant. The compounds detected by these neurons facilitate the decisions of where to lay an egg and begin feeding. By identifying the tuning of the receptor neurons of potential biological control agents to volatiles collected from test plants, we can assist in the prediction of an agent's potential host range. The objective of this study was to develop techniques to screen potential biological control agents for specificity by determining the volatile compounds that elicit receptor neuron responses. The results of this research will enhance current host testing methods by developing techniques that determine the sensitivity of potential agents to the compounds produced by the target weed and the test plants. This research will increase our understanding of this process and thereby improve the predictability of host plant selection by weed biological control agents.

Results indicate that host selection by adults of the weed biological control agent *Oxyops vitiosa* (Coleoptera: Curculionidae) can be explained by the presence of specific monoterpenoids in the *Melaleuca quinquenervia* (Myrtaceae) host foliage. Detection of these compounds by *O. vitiosa* adults was found with EAG, EAD, and SSR analysis. Dual-choice bioassays with adults confirmed the significance of these compounds. These same compounds found in two congeneric and one unrelated species explain their laboratory use in quarantine. These results indicate that when compiling a list of test plants for risk assessment of potential biological control agents, not only taxonomy, but similar chemistry should be considered. A major contribution of this research is to increase our understanding of the process of host selection in specific insects and thereby improve the predictability of specificity of weed biological control agents.

Antifeedants in the faeces of the pine weevil *Hylobius abietis*: identification and biological activity.

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Egg-laying females of the pine weevil, *Hylobius abietis* (L.), regularly deposit faeces adjacent to each egg. Egg cavities are gnawed in the bark of roots of recently cut conifer trees. After egg deposition, the cavity is sealed by faeces and a plug of macerated bark. Root bark containing egg cavities with faeces is avoided as food by pine weevils, which indicates the presence of natural antifeedants. Here we present the first results of the isolation and chemical analyses of antifeedant compounds in the faeces of *H. abietis*. In a feeding bioassay, the methanol extracts of the faeces revealed strong antifeedant properties. The methanol extract was fractionated by MPLC chromatography and the antifeedant effect was mainly found in the fractions of highest polarity. Volatile compounds in the active fractions were identified by GC-MS and the non-volatile compounds were characterized by pyrolysis GC-MS. Based on the mass spectra, a number of compounds with various chemical structures were selected to be tested for their antifeedant properties. Antifeedant effects were found among compounds apparently originating from lignin: e.g. guaiacol, veratrol, dihydroxybenzenes and dihydroconiferyl alcohol. A weak effect was found for long chain fatty acid derivatives. The types of naturally occurring antifeedant compounds identified in this study may become useful for future chemical design of molecules active in protection of planted conifer seedlings against damage by *H. abietis*.

Structure-activity relationships of benzoic acid derivatives as antifeedants for the pine weevil *Hylobius abietis*

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Aromatic organic compounds present in the faeces of the pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) have been shown to evoke antifeedant effects on this species, which is a serious pest of planted conifer seedlings in Europe. Here we evaluate 55 benzoic acid derivatives as antifeedants for *H. abietis*. Structure – activity relationships are identified by bioassaying related compounds obtained by rational synthesis of functional group analogs and structural isomers. Five main criteria of efficiency as antifeedants among the benzoic acid derivatives are identified. By predicting optimal structures for *H. abietis* antifeedants we attempt to find a commercial antifeedant to protect conifer seedlings against pine weevil damage in forest regenerations. Methyl 2,4-dimethoxybenzoate and isopropyl 2,4-dimethoxybenzoate are two new candidates for practical use among several potent antifeedants identified.

Nematicidal Activity of Quinolizidine Alkaloids and Field Tests to Control Pine Wilt Disease with Aloperine

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Pine wilt disease, transmitted by the longhorn beetle (*Monochamus alternatus* Hope), which carries the causal agent, pine wood nematode (*Bursaohelencus xylophilus* (Stener et Buhrer) Nicker), is a serious problem of pine forests in Asia. Since the disease invaded China in 1982, it quickly has spread to six provinces and continues to spread rapidly. Aloperine, cytisine, N-methylcytisine, and matrine are the alkaloids monomers in *Sophora alopecuroides* L., which is a common shrub species grown in desert areas of North-West of China.

Nematicidal activity of aloperine, cytisine, N-methylcytisine, and matrine was bioassayed by the agar medium method and the cotton ball method. The corresponding results from the two methods showed that the nematicidal activity of aloperine was the strongest. Its activity, expressed as log(1/ID50) was also the strongest (8.67) in comparison to the previously reported nematicidal activity of seven other quinolizidine alkaloids bioassayed by the same method. It is hypothesized that the nematicidal activity of quinolizidine alkaloids is determined primarily by the types of functional group pairs and types of functional group in their molecular structures. Based on this hypothesis, alkaloids with strong nematicidal activity can be predicted on the basis of their molecular structure. Further bioassay of dehydroaloperine showed its nematicidal activity was weaker than aloperine, just as that predicted by the hypothesis. Field test was done in a forest of 6-7years old pine trees (*Pinus Thunbergii)* near Nanjing, China, with trunk injecting a dose of aloperine 0.21g per tree two weeks before inoculation of 5000 pine wood nematodes. In the next spring results of the field test showed survival rate of treated tree was 93.8% and that of the corresponding control was 20%. The result indicated there was potentials for use of aloperine as a nematicide to control pine wilt disease.

Control of Western Pine Beetle, *Dendroctonus brevicomis*, populations using aerially applied verbenone flakes.

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In a field assay to assess efficacy for Western Pine Beetle control, the antiaggregation pheromone verbenone was applied in a laminated flake formulation (Hercon Environmental, Emigsville, PA) from fixed-wing aircraft to *Pinus ponderosa* stands in Northern California. Ten 20.2 ha plots were selected in the Big Valley Mountains in Lassen County, CA, then five of those were chosen at random to receive treatment and the other five served as controls. The flakes were applied at the rate of 2.5 kg flakes/ha or 375 g active ingredient/ha. Eight Intercept® traps were placed on each plot, and each trap was baited with one Western Pine Beetle tree lure (Phero Tech, Inc, Delta, BC). Four trees per plot were also baited with the same lure; thus, each plot contained 12 lures, which were replaced at 30-day intervals. Several response variables were measured, including numbers of beetles trapped in baited Intercept® traps, numbers of attacks per tree, numbers of successful attacks, and rates of tree mortality. The treatments reduced the numbers of beetles caught in baited traps for 14 weeks, after which point snowfall appeared to shut down beetle flight. Treated plots showed fewer attacks per tree by both *Dendroctonus brevicomis* and *Dendroctonus valens* when compared to untreated control plots at four weeks post-treatment. At the end of the 2004 season, the number of "faded" baited trees in treated stands was only 20%, whereas in control stands it was 75%.

Reexamination of the pheromone system of the southern pine beetle, *Dendroctonus frontalis.*

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The semiochemistry of southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae: Scolytinae) was studied intensively from the late 1960s through the 1970s, and five compounds with differing behavioral activities were identified as pheromones for this species. However, evidence suggests that the pheromone system of this insect has not been adequately characterized. We applied research tools not available to the original researchers (including GC-EAD and chiral/capillary GC-MS) to a reexamination of the pheromone system of *D. frontalis*. GC-EAD analyses of extracts of adult beetles revealed ten compounds not previously identified as pheromones for this species that elicited antennal responses in conspecifics. Eight of these were identified by GC-MS and were subsequently found to alter *D. frontalis* responses to traps baited with an attractant. Acetophenone, fenchyl alcohol, myrtenal, 2-phenylethanol, and *cis*- and *trans*-myrtanol reduced attraction of one or both sexes while cis-verbenol enhanced attraction of females. These eight compounds were generally present in greatest quantities in recently emerged beetles, with substantially smaller amounts produced post-attack. While these newly-identified *D. frontalis* semiochemicals clearly have potential for use in management of this serious pest, the appropriateness of classifying these compounds as pheromones is uncertain.

Additionally, we used chiral capillary GC-EAD analyses to identify differential activities for the enantiomers of known semiochemicals for *D. frontalis*. An exceptional degree of enantiomeric discrimination was observed for the male-produced compound *endo*-brevicomin, with the (+)-enantiomer eliciting antennal responses at concentrations five orders of magnitude lower than the (-)-enantiomer. Chiral GC/MS analyses of aerations/extracts of individual males indicated that they produce only the (+)-enantiomer, which has been reported to be a potent attractant for *D. frontalis*. We present data from initial field trials conducted with >99% pure *endo*-brevicomin enantiomers, and, in light of these findings, we reassess the previous characterization of *endo*-brevicomin as a multi-function pheromone for *D. frontalis.*

Area-wide effects of a plant volatile-based attract and kill formulation against *Helicoverpa armigera* and *H. punctigera* in Australia

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Magnet® is an attract-and-kill formulation based on plant volatiles which is currently undergoing product evaluation trials on about 20,000 ha of cotton, and smaller areas of other crops, in Australia. It is based on a blend of five plant volatiles, previously shown in olfactometer studies to be attractive to both sexes of *Helicoverpa armigera* and *Helicoverpa punctigera*. These noctuid moths are key pests of cotton, and also affect many other crops, in Australia.

This presentation will discuss the area-wide effects of treating individual fields, or contiguous areas of cotton, with Magnet® containing small quantities of the insecticides methomyl and thiodicarb. Methods used to estimate the numbers of moths killed, and simple models to predict the impacts on oviposition, will be described. *Helicoverpa* spp. moths are extensively mobile within and between fields, and treated areas appear to act as population sinks, over distances of several km. Substantial reductions in oviposition can be obtained, not only in treated fields but in fields some distance away. Magnet® is applied to less than 2% of the area of treated fields, thus allowing beneficial predators and parasitoids to survive in the larger untreated area of the field. Attract-and-kill with formulations based on plant volatiles offers considerable promise as a component of area-wide integrated pest management for *Helicoverpa* spp. Potential applications in resistance management for transgenic crops will also be discussed.

Pheromones and chemosensory investigative behavior by African elephants

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Chemical signals play vital roles in elephant interactions. The release, detection and response of such signals are affected by the sex, age and reproductive state of the senders and receivers. Because adult male and female elephants live in quite different social settings, the development of chemocommunication should reflect the differential endpoints. We are pursuing the identity of specific pheromones, most notably an estrous pheromone, using bioassays with captive and wild elephants coupled with a variety of analytical chemical procedures. We are examining the developmental pattern of chemocommunication in field studies at Addo Elephant National Park, South Africa and in northern Tanzania at Ndarakwai Ranch. We perform focal animal observations with continuous recording on male and female elephants over the range of age classes from calf to adult. In bioassays, adult male African elephants responded more to preovulatory than luteal phase urine. In field studies, male elephants show an increasing interest in elephant urine and feces with age, and are more likely to show higher rates of response of key chemosensory behaviors compared to females. These tendencies become apparent in the first ten years of life although males do not typically breed until they are over twenty years. Sexual dimorphism in chemocommunication appears to reflect social, physical and reproductive dimorphism in elephant society.

Does chemical signature reflect genetic relatedness between population and colonies in termites?

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Cuticular hydrocarbons constitute the chemical signatures that form the bases for recognition processes in social insects such as subterranean termites of the genus *Reticulitermes* that present specific population and colony structures. In a first study we tried unsuccessfully to correlate variations in chemical signature with population and colony structure in species *Reticulitermes grassei* living in 3 different natural sites in S/W of France. In a second study we attempted to correlate the composition of chemical signatures and genetic relationships among and within colonies in species *R. santonensis* living in an urban setting, i.e., in and around the city of Paris, France. We performed GC analyses of cuticular hydrocarbons (HCs) from 10 different workers collected from 14 colonies and determined genotypes of the same individuals using 10 DNA microsatellite loci. Multivariate analysis showed that variations in HCs among colonies followed almost the same genetic pattern. Using redundancy analysis to combine chemical and genetic data, we found that a few hydrocarbons accounted for most of the genetic variations among colonies. We also found a strong positive correlation between chemical and genetic distances between colonies. This finding suggests a genetic basis for HCs but also that environmental sources cannot be ruled out. A special feature of *R. santonensis* is that its colonies are always open (non-aggressive) colonies. This is certainly due to invasive character of the species that may have been imported from North America.

Identification of queen sex pheromone components of the bumblebee *Bombus terrestris*

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We investigated the origin and chemical composition of the queen sex pheromone of the primitively eusocial bumblebee *Bombus terrestris*. Physiologically and behaviorally active compounds were identified by coupled gas chromatography electroantennography (GC-EAD), gas chromatography-mass spectrometry (GC-MS), and behavioral tests in the laboratory. In biotests, frozen virgin queens were highly attractive to males. Dummies impregnated with head extracts and surface extracts obtained from virgin queens were significantly more attractive to males than odorless dummies. Our results prove that male mating behavior is stimulated by components of cephalic secretions that are smeared onto the cuticle surface by the queen. Overall, 21 compounds present in cuticle extracts and head extracts evoked electroantennographic responses in male antennae: saturated and unsaturated fatty acids, ethyl- and methyl esters, heptacosene and 2-nonanone. Behavioral tests with synthetic copies of the electrophysiologically active compounds elicited typical male mating behavior. Since solvent impregnated dummies were approached by the males but did not release copulatory behavior, the initial step in stimulating male mating behavior seems to be visually based while close range olfactory signals are important for releasing male mating behavior as well as for species recognition. In further behavioral test series, we investigated the influence of different storage conditions on the attractiveness of a frozen virgin queen. The attractiveness of a female decreased when storage times were prolonged. Therefore, the chemical composition of the sex pheromone may change during freezing as biological active compounds may decompose.

Chemical cues for nestmate recognition in *Acromyrmex landolti*

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Eighteen volatile organic compounds were isolated from mandibular gland of different castes of the leaf-cutting ant *Acromyrmex landolti* using headspace analysis combined with solid phase microextraction (HS-SPME) and gas chromatographic analyses (GC). Eight major compounds were identified by coupled gas chromatography - mass spectrometry (GC-MS): 4-methyl-3-heptanone, 4-methyl-3-heptanol, 3-octanone, 3-octanol, 2-nonanone, 2-nonanol, 2-undecanone and 2-undecanol, being six of them novel compounds to *Acromyrmex*. Another four compounds were tentatively identified by comparison of structure-related compounds using retention index system: 3-heptanone, 4-methyl-3-octanone, 3-decanone, and 3-undecanone. Compounds present in mandibular gland content have been reported as responsible for nestmate recognition, alarm and mating. Significant differences between the relative amount of five compounds and the castes were found. Behavioral bioassays were performed to determine the source of nestmate recognition signal in *A. landolti*: two worker ants (a nestmate and an alien) were placed on the central axis of an active trail, and three types of treatments were used: whole bodies, body parts and gland extracts. Workers recognized conspecifics from other colonies as aliens, from body parts, showing that recognition signals are found over the whole body. Nestmate recognition is achieved apparently by mandibular gland volatiles and PPG hydrocarbons.

Structural Complexity of Chemical Recognition Cues Affects Perception of Group Membership in Ants.

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In social insects, suites of hydrocarbon molecules present on the cuticle, known as hydrocarbon profiles, act as multi-component recognition cues. Social insects use variation in hydrocarbon profiles as recognition cues in order to recognize group membership of other individuals they interact with. Group membership may include colony-membership, species membership or, within colonies, task membership or reproductive status. Recognition responses vary according to context, although the recognition of a non-member of a colony is often met with aggression by members of the colony. Although growing evidence has shown that cuticular hydrocarbons are used as recognition cues, we know little about the mechanism by which they communicate nestmate, species, or task group status. For example, is the relevant information of the cue present in the entire hydrocarbon profile, or only in specific pieces? I studied the species recognition response of two ant species, *Linepithema humile* and *Aphaenogaster cockerelli*, to examine what role structural complexity plays in ant recognition responses mediated by hydrocarbon recognition cues. A bioassay was employed that measured the species recognition response of the ants, i.e. aggressive behaviour towards heterospecific. Hydrocarbons isolated from heterospecific ants, isolated structural classes from the heterospecific hydrocarbon profiles, mixtures of the heterospecific structural classes and controls were used as experimental stimuli. The data show that the ants responded to structural complexity, a feature of complex cuticular hydrocarbon mixtures, and not simply to the presence of specific compounds or to the number of hydrocarbons in a mixture in their species recognition response. A combination of at least two hydrocarbon structural classes was necessary to elicit an aggressive species recognition response. However, no one class of hydrocarbons was more important than the others in eliciting a response. Structural complexity in hydrocarbon recognition cues appears to provide a chemical context for a recognition response, allowing individuals to perceive species membership.

Sponge-microbe symbioses: model systems for integrating molecular and chemical ecology

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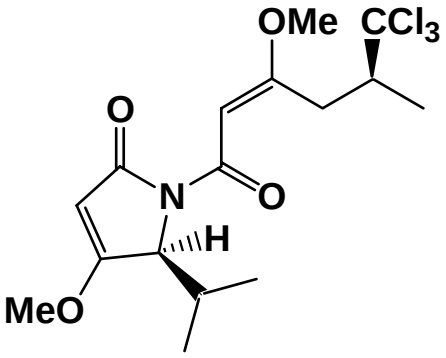
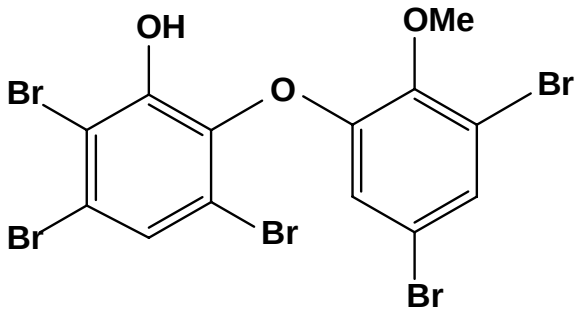
Marine sponges can obtain up to 80% of their daily carbon and nutritional needs through symbiotic associations with blue-green algae (cyanobacteria) and bacteria. Phylogenetic studies of sponges and their associated cyanobacteria reveal that some symbionts show patterns of cospeciation with their hosts, while other symbionts are found in a diverse array of hosts. These symbioses appear to span the range of mutualistic, commensal, and parasitic interactions.

Many natural products with pharmaceutical applications have been described from the sponge *Lamellodysidea herbacea*. Fluorescent *in situ* hybridization demonstrates that the biosynthetic genes that produce chlorinated lipopeptides (e.g., compound 1) are located in a cyanobacterial symbiont, *Oscillatoria spongeliae*. A closely related sponge, *Phyllospongia papyracea*, hosts a genetically distinct strain of *O. spongeliae*. Although several researchers have implicated cyanobacteria in the production of brominated diphenyl ethers (e.g. compound 2) by this sponge-symbiont association, shading experiments demonstrated little impact of light availability on the production of these compounds. Since chlorinated lipopeptides and brominated diphenyl ethers may affect the structure of symbiotic bacterial communities, current research focuses on whether sponges contain random samples of bacteria from ambient seawater or highly structured communities of coevolving microbes.



Compound 1: Dysidin



Compound 2

Seagrass-pathogen interactions: attack by the wasting disease pathogen, *Labyrinthula* spp., causes the "pseudo-induction" of phenolics.

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Protists of the genus *Labyrinthula* are omnipresent in coastal ecosystems and periodically cause outbreaks of the seagrass wasting disease. Infection leads to the formation of necrotic lesions on leaves and has been associated with regional die-offs of eelgrass, *Zostera marina* and turtlegrass, *Thalassia testudinum*. Recent experiments suggested a link between disease outbreaks, declining environmental conditions, and levels of (poly)phenolic-based natural products known to inhibit *Labyrinthula* growth in culture. We tested this hypothesis in populations of eelgrass and turtlegrass acclimated to a broad range of environmental conditions and inoculated with various strains of *Labyrinthula*. Results confirm that the spread of the disease can be promoted or inhibited by specific environmental conditions, but indicate that disease resistance is only occasionally correlated with levels of phenolic acids or condensed tannins in leaves. For example, low salinities prevent wasting disease symptoms in both eelgrass and turtlegrass, however they may do so with or without a corresponding change in phenolic metabolites, by affecting the pathogen directly. Our results also cast doubt on the proposed role of phenolics as effective inducible defenses against this pathogen *in vivo*. For example, in turtlegrass we found that *Labyrinthula* attack induces rapid accumulations of phenolic acids above, but not below, necrotic lesions. Since leaf sections located above necrotic lesions are rapidly lost from the plants it is unlikely that (poly)phenolics accumulated in these tissues represent an effective induced defense against *Labyrinthula*. We propose that (poly)phenolics accumulate above wound-sites simply because lesions disrupt resource translocation within leaves, creating local areas of carbohydrate overabundance and phenolic biosynthesis. Thus, necrotic lesions cause an ineffective "pseudo-induction" of phenolic acids in soon-to-be lost tissues, in accordance with the predictions of the sink-source model of plant defense. Taken together, results indicate that wasting disease outbreaks are likely to be promoted by specific environmental conditions (e.g., high salinities, low irradiances) but do not support the view that (poly)phenolics in seagrass leaves are the primary factor determining disease resistance.

The tale of an ordinary bryozoan, its symbiont, and an anticancer compound: the chemical ecology and biosynthesis of the bryostatins

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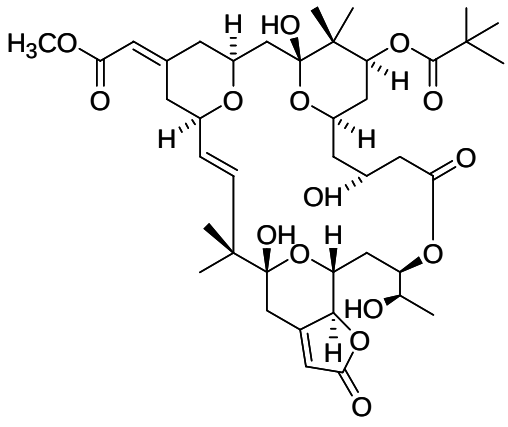
Despite recent evidence demonstrating that microbial symbionts often produce secondary metabolites found in marine invertebrates, there are few documented instances of these symbiont-synthesized metabolites playing a role in the survival of the host. Larvae of the sessile marine invertebrate *Bugula neritina* (Bryozoa) are protected by an effective chemical defense. From the larvae, we isolated three bryostatin-class macrocyclic polyketides, including the novel bryostatin 20 (**1**), that deterred feeding by a common planktivorous fish that co-occurs with *B. neritina*. A unique bacterial symbiont of *B. neritina*, *Endobugula sertula*, was hypothesized as the putative source of the bryostatins. We established that (i) bryostatins are concentrated in the larvae of *B. neritina* and protect them against predation by fish, (ii) bryostatin concentrations decrease as *B. neritina* ages, and (iii) *E. sertula* produces bryostatins. In addition, we have isolated a polyketide synthase gene cluster from symbiont-enriched samples of *B. neritina* that may biosynthesize the bryostatins. This cluster has several enzymatic domains that indicate that it is responsible for making characteristic additions to the pre-bryostatin polyketide chain. This study represents the first example from the marine environment of a microbial symbiont producing an anti-predator defense for its host, and in this case, specifically for the host's larval stage, which is exceptionally vulnerable to predators.



1 bryostatin 20

Sensory biology and ecology of chemosensory foraging: a tale of two predators (and their prey).

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Chemical ecology in aquatic (and terrestrial) communities has largely focused on the nature and ecological effects of deterrent compounds contained within prey species. Yet, many organisms in aquatic (and terrestrial) environments acquire chemical information via fluid-borne chemical cues. These odor plumes mediate attraction to food and mates, and also aversive responses. The ability of animals to respond to fluid-borne signals from predators, prey or mates is affected by both perceptual mechanisms, and turbulent mixing processes that determine the chemical signal properties contained within the plume. We have explored the implications of information acquisition by examining a trio of common aquatic consumers and prey that find food, and evade predation by sensing aquatic odor plumes. Our approach involves an analysis of fluid flow, odor signal properties, behavior and manipulative field experiments. Blue crabs detect chemical signals from bivalve prey, and rely on their ability to sense coherent odor filaments contained within odor plumes. Turbulent mixing that reduces the coherence and intensity of odor plumes diminishes blue crab search success and efficiency. It is likely that other highly mobile animals rely on the detection of discrete odor filaments, as evinced in studies of mate location in terrestrial insects. These terrestrial and aquatic animals sample odor properties coarsely, which dictates their guidance strategies and responses to turbulent mixing. Simple models of temporal and spatial sampling properties of animals relative to scales of variation in an odor plume suggest a range of possible ways animals can acquire information during chemosensory navigation. Indeed, slow moving animals such as whelks seem to use a time-averaging approach to navigate towards attractive odor sources. These animals are minimally affected by turbulent mixing, which affects instantaneous odor properties such as filament intensity, but not average odor distributions within the plume. The existence of alternate tactics employed by potential predators to sense their prey suggests that turbulence is a relevant niche dimension of the olfactory environment even for animals that hunt the same prey. Manipulative field experiments that examine predation intensity in environments with different turbulence levels indicate that predation success by blue crabs is reduced when turbulence is increased beyond a critical threshold, whereas whelks continue to forage effectively. The distribution of these competitors, and the predation intensity attributable to a given consumer, may therefore be at least partially determined by the prevailing fluid environment. Like their predators, bivalve prey also detect chemical cues, and respond to water-borne metabolites from potential predators by reducing their apparency to avoid discovery. Bivalves that detect predator scents cease feeding in an effort to avoid releasing odor cues into the water. Turbulence alters the reactive distance of bivalve prey in a predator-specific manner; bivalves in mildly turbulent flows are less able to perceive blue crabs from a distance, whereas whelks remain detectable even in substantially turbulent conditions. Thus, the balance between perceptual abilities of prey and predators may determine the nature and impact of predation in a given environment. Direct, lethal effects of predators (consumption of prey) will occur where predators have a sensory advantage, whereas indirect effects (reduced growth) will be more important where prey have a sensory advantage. We are currently performing field experiments to examine whether, as expected, the flow environment affects the magnitude of direct vs indirect effects of predation in natural communities.

Chemical ecology of plant defenses against insects and pathogens

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Substances in the oral secretions of caterpillars induce plants to synthesize and release volatile organic compounds that attract natural enemies of the caterpillars. These substances have been identified as fatty acid amides and are synthesized by enzymes found in the membranes of the caterpillar gut walls. In addition to recruiting natural enemies of insect herbivores, volatile organic compounds released by damaged plants also act as plant-plant signals. Green leafy volatiles (GLV), 6-carbon aldehydes, alcohols and esters, prime the defenses of neighboring undamaged plants, so that when insect herbivores attack, the primed plants produce volatiles and the hormone jasmonic acid much more rapidly and in significantly greater amounts than plants that have not previously been treated with GLV.

Plant-insect interactions in Arabidopsis: From transcript profiling to induced resistance

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Transcriptional changes after herbivore attack are triggered in response both to physical damage as well as to biological components displayed or released by the attacker. Using DNA microarrays we characterized gene expression in Arabidopsis leaves in response to a specialist insect, *Pieris rapae*. Groups of insect-responsive genes potentially important for defense were identified, including genes involved in the synthesis of toxic compounds like indole glucosinolates, genes inhibiting insect digestion, or genes implicated in detoxifications processes. The contribution of signalling pathways was investigated and we found that three quarter of inducible genes are regulated by the jasmonate pathway. When analysing Arabidopis response to larvae of the generalist insect *Spodoptera littoralis* we observed a strikingly similar transcript pattern, as compared to *P. rapae*. The specific role of insect-induced genes on larval performance is currently under investigation.

Funded by the Swiss State Secretariat for Education and Research SER (QLK3-CT-2002-02035).

Genetically silenced defense responses: Consequences for the herbivore community composition on *Nicotiana attenuata*.

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The scientific value of using genetically silenced plants to study ecological interactions in nature remains to be realized. The mechanisms of induced plant defense responses have mainly been studied through the application of synthetic plant hormone compounds or inhibitors of their biosynthesis. This approach, although revealing the involvement of individual signaling cascades in the activation of plant defenses, is very limited. Their main limitations are unspecific elicitation (or inhibition) by synthetic elicitors (or inhibitors), and the spatial and temporal uncoupling of the elicitation from the direct interaction between the plant and its 'environment' including the arthropod community. We manipulated the defensive capability of the wild tobacco plant *Nicotiana attenuata* by genetically silencing specific defense traits or the endogenous production of the plant hormone jasmonic acid which is involved in the activation of these defense responses. Comparative analysis of the performance of these differentially disarmed genotypes of *N. attenuata* in their natural habitat revealed .the crucial role of herbivore-induced plant defense traits for the composition of the associated arthropod community and the herbivores' host plant choice. These effects exceed the expected reduction in plant resistance against well known herbivores of *N. attenuata* and include the attraction of previously unobserved opportunistic herbivores and changes in the attractiveness of attacked plants for predators.

Physiological and molecular adaptations stabilizing symbiotic ant-plant mutualisms

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Ant-plant mutualisms are very common and differ widely in their specificity, thus being particularly suitable for studying biochemical mechanisms that determine species-specific interactions. While foraging ants are attracted by plant-derived food rewards in facultative (myrmecophilic) interactions, obligate ant-plants (myrmecophytes) house and nourish specialized ant colonies, which defend their hosts against herbivores, pathogens, and competing vegetation. *Acacia* ant-plants provide their ant mutualists with extrafloral nectar (EFN). During the evolution of myrmecophytism, EFN secretion has shifted from being an inducible to a constitutive trait.

EFN generally is a highly attractive source of carbohydrates, constitutively secreted EFN thus could be the target of exploitation by insects parasitizing the system. We found, however, that EFN secreted by myrmecophytic *Acacia* species lacks sucrose, a disaccharide usually present in nectars and accounting to a high degree to its attractivity to ants. Generalist ants therefore preferred EFN of myrmecophilic *Acacia* species (which contains high amounts of sucrose) over EFN of myrmecophytes, while *Pseudomyrmex* ants specifically inhabiting *Acacia* myrmecophytes preferred EFN of myrmecophytes. These ants lack invertase, the sucrose-cleaving enzyme, and thus apparently are not able to digest sucrose. Secreting sucrose-free EFN represents a filter preventing this food source from exploitation by competitors and parasites.

The lack of sucrose is a consequence of high invertase activity in the EFN and thus of a post-secretory regulation. Few enzymes are known from any type of nectar, and those that have been characterized so far function in the antimicrobial defense of floral nectars. The invertase present in the EFN of *Acacia* myrmecophytes, in contrast, "pre-digests" the EFN in order to be suitable for its specialized consumers. The absence of sucrose from the EFN of myrmecophytes is an illustration of what had been called "adaptive specialization", i.e. an evolved trait that excludes less desirable partners in a multispecies association. Such simple compounds may have easily been overlooked in previous studies, if only because of their ubiquity, and should be considered in future studies.

Signals, effects, and specificity of volatile-induced plant defense responses

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Plants under insect herbivore attack have evolved various mechanisms to counteract this threat. Besides direct defense measures like production of proteins that block digestion or disrupt intestinal tissue and the production of defense-related (toxic) secondary metabolites the release of volatile organic compounds (VOC), a mixture of volatile secondary metabolites from various pathways, can serve as signals not only to attract predators and parasites of attacking herbivores, but also can be recognized by neighboring plants resulting in defense-related gene expression. Among those volatiles are the so called green leafy volatiles (GLV), which are rapidly emitted during herbivory. We have shown that GLV can act as priming signals preparing receiver plants against impending herbivory. Structure/function analysis of natural GLV as well as synthetic analogs clearly showed certain structural requirements, but excluded α,β-unsaturated carbonyls as active centers. Jasmonic acid (JA) and other lipid-derived compounds (oxylipins), which are activated by wounding and insect elicitors, also represent important signals in this process. A comparison of gene expression after wounding, wounding with application of crude regurgitant elicitors (CRE), and exposure to Z-3-hexenyl acetate (Z-3-HAC) further demonstrated the specificity of the GLV signal in plant-plant communication through selective activation of genes involved in JA biosynthesis. In conclusion, inter-plant communication via GLV results in an enhanced preparedness specifically directed against insect herbivore attack mediated by specific activation of distinct parts of the octadecanoid signaling pathway.

Chemical Signaling in the Microbial Community of the Lepidopteran Gut

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Pathogens must breach the barrier presented by native communities to colonize and infect their hosts. The structure of many microbial communities is consistent across space and time despite continually changing biological and physical conditions, suggesting that these microbial communities are intrinsically robust. Little is known, however, about the basis for robustness. Our hypothesis is that communication networks are essential to robustness of microbial communities. Communication may enable members of the community to respond to conditions in a coordinated fashion, enhancing the success of species that depend on each other for survival. Our goal is to define the chemical and genetic network that unifies a microbial community and perturb the network to determine its role in community robustness.

Our model system is the midgut of the gypsy moth larvae. This insect can be reared from eggs on artificial media in the laboratory, thereby providing large numbers of larvae and control over their diet. The microbial community in the gypsy moth gut is relatively simple compared with other gut environments, but contains sufficient diversity to provide a manageable system for exploring community dynamics. When the insect is reared on sterile diet, the gut contains 10 bacterial species. Four are as yet unculturable and six are readily culturable. Members of the low-GC Gram positive and Proteobacteria phyla dominate the community.

We have begun to dissect the entire community using metagenomics, or genomic analysis of the entire assemblage in the gut. We constructed libraries with microbial DNA extracted directly from the gut community. In a search for new signal molecules, we screened the metagenomic library with an “intracellular” screen for genes encoding products that induce quorum sensing. In this screen, the metagenomic DNA is in the same cell as a biosensor that is triggered by small molecules that induce quorum sensing. When the biosensor is triggered, the cell fluoresces and can be captured by fluorescence-activated cell sorting or identified by fluorescence microscopy. This assay led us to a metagenomic clone that carries a gene encoding a putative monooxygenase that directs the synthesis of a collection of small molecules. When the gene encoding the monooxygenase was overexpressed, the clone induced quorum sensing and is highly toxic to gypsy moth larvae. The role of this signal in community structure and robustness will be discussed.

Xenobiotic metabolism by caterpillars: inductions and deductions

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At risk of attack by a broad spectrum of consumers, including pathogens and herbivores, plants utilize multiple signaling molecules to activate biosynthetic pathways for production of defense compounds. These signaling pathways interact to induce different suites of defense genes in response to different consumers. Crosstalk among these pathways promotes cross-resistance to multiple enemies and may be functionally adaptive because damage associated with herbivores can predispose plants to fungal infection. *Helicoverpa zea* (corn earworm) is a major pest of corn and other crops. Losses to *H. zea* are exacerbated by accompanying infection by *Aspergillus* spp. and concomitant production of aflatoxins, highly mutagenic contaminants of grain costing millions of dollars in crop losses annually. *H. zea* upregulates detoxificative cytochrome P450 *CYP6B* monoxygenase (P450) genes upon detecting the plant signaling substances jasmonic acid and salicylic acid. Under certain circumstances this ability to "eavesdrop" on plant defense response activity is adaptive in that it allows corn earworms to reduce the risk of exposure to toxic concentrations of defense compounds. Eavesdropping, however, comes at an ecological cost in that induction of P450-mediated metabolism can in some cases result in bioactivation rather than detoxification. Upon P450-mediated metabolism, aflatoxins can be converted to active metabolites with enhanced toxicity and mutagenic activity. Aflatoxin B1 administered in artificial diet to ultimate instar *H. zea* larvae at concentrations of 1 to 3 ug/g results in significantly reduced growth rate and pupal weight. Although salicylate and methyl jasmonate alone have no effect on growth rate or survivorship, their presence in combination with aflatoxin B1 effectively synergizes its toxic effects significantly; P450-mediated bioactivation is the presumed mechanism underlying this synergism. Because it serves as the plant signal substance regulating many biosynthetic responses to pathogen infection, salicylate likely co-occurs with aflatoxin in plants infected with *A. flavus*. Insects that respond to plant signal substances by upregulating detoxificative P450s are thus likely to experience greater mortality than those with autoinducible P450s upregulated only by their substrates.

MecWorm, a novel tool to study plant-herbivore interactions

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Herbivory elicits defence responses in the infested plants, including the emission of volatile organic compounds (VOCs) that can serve as indirect defense signals. The contribution of plant tissue wounding during the feeding process in the elicitation of defense responses is not clear up to now. For example, in Lima bean (*Phaseolus lunatus*) the composition of the volatiles induced by both the insect caterpillar *Spodoptera littoralis* and the snail *Cepaea hortensis* is very similar. Thus, a mechanical caterpillar has been designed and used in this study which very closely resembles the herbivore-caused tissue damage in terms of a similar physical appearance and a long lasting wounding period on defined leaf areas. This mode of treatment was sufficient to induce the emission of a VOC blend qualitatively similar to that as known from real herbivore feeding, although there were significant quantitative differences for a number of certain compounds. Moreover, both the duration and the area that has been mechanically damaged, contribute to the induction of the whole volatile response. Based on those two parameters, time and intensity, which can replace each other to some extent, a damage level could be defined. That damage level exhibits a close linear relationship with the accumulation of fatty acid-derived volatiles and monoterpenes while other terpenoid volatiles and methyl salicylate respond in a non-linear manner. The results strongly suggest that the impact of mechanical wounding on the induction of defence responses during herbivore feeding was underestimated up to now. This controlled and reproducible mechanical damage that strongly resembles the insect's feeding process represents a novel and valuable tool to analyze the role of various signals involved in the induction of plant reactions against herbivory.

Emission of herbivore-induced volatiles in absence of a herbivore – response of *Zea mays* to green leaf volatiles, terpenoids, and ethylene

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Green leaf volatiles (GLV), a series of saturated and monounsaturated six-carbon aldehydes, alcohols, and esters of the latter are emitted by plants upon mechanical damage. Evidence is increasing that intact plants respond to GLV by activating their own defense mechanisms. Maize plants exposed to GLV respond by the emission of a volatile bouquet consisting mainly of terpenoids which are typically associated with insect feeding and have been shown to attract carnivores and deter herbivores from oviposition. We investigated the role of GLV dose and structure (i.e. functional group, presence/configuration of a double bond, chain length) on volatile emission of exposed maize plants and compared different maize cultivars with respect to their responsiveness to GLV. Furthermore, we examined the role of the phytohormone ethylene on volatile emission in maize after exposure to GLV. Finally, we studied whether also externally applied terpenoids are able to increase the emission of inducible volatiles in maize. Our results [1] demonstrate that all tested GLV irrespective of presence and configuration of a double bond are able to induce volatile emission in maize. (*Z*)-3-configured GLV induced stronger responses than (*E*)-2- and saturated derivatives, the functional group of the GLV (aldehyde, alcohol, or ester) had no significant effect on the total amounts of emitted volatiles. Leaf alcohol (*Z*)-3-hexen-1-ol induced volatile emission at doses between 0.05 and 1.0 μmol. The naturally occurring chain length of six carbon atoms induced stronger responses than shorter and longer chains. Different maize cultivars showed significant differences in their responsiveness to GLV. Ethylene drastically synergized volatile emission in maize after exposure to GLV but had no volatile inducing effect on its own [2]. In contrast, exposure of maize plants to terpenoids [β-caryophyllene, (*E*)-β-farnesene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene] did not significantly increase the total amount of other inducible volatiles in maize [1]. Our results suggest that GLV not only can alert neighboring plants, but may facilitate intra-plant information transfer and help to mediate a rapid systemic defense response in a plant. We furthermore propose that ethylene plays an important role in this interaction as a synergist.

[1] Ruther J and Fürstenau B (2005) Emission of herbivore-induced volatiles in absence of a herbivore – response of *Zea mays* to green leaf volatiles and terpenoids. *Z. Naturforsch. 60c* (in press).
[2] Ruther J and Kleier S (2005) Plant – plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (*Z*)-3-hexen-1-ol. *J. Chem. Ecol.* DOI: 10.1007/s10886-005-6413-8

Above and below ground tritrophic signaling in maize

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Plants under attack by arthropod herbivores have been shown to employ a strategy of indirect defence by attracting natural enemies of the herbivores. Such interactions also occur below ground when insects-damaged roots release compounds that attract entomophatogenic nematodes. We investigated such root signals for maize plants under attack by larvae of *Diabrotica virgifera virgifera*. With the use of a newly developed below ground olfactometer we found that the nematode *Heterorhabditis megidis* was highly attracted to *Diabrotica*-damaged maize roots, compared to mechanically damaged roots or healthy roots**.** Additional experiments showed that *D.v.virgifera*-damaged roots produce large quantities of the sesquiterpene (E)-β-caryophyllene. This sesquiterpene was indeed found to be a key attractant for the nematode. Interestingly, most North American maize varieties do not emit this signal, which results in dramatic differences in the attractiveness between different maize lines and in larval-infection rates under field conditions. We are currently studying the cross effects between the above and below ground tritrophic interactions.

Elucidation of resistance and disease mechanisms in roots using sugar beet and sugar beet root maggot as a model system

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U.S. sugar beet (*Beta vulgaris* L.) production is plagued by the sugar beet root maggot (SBRM, *Tetanops myopaeformis* Röder), the most devastating insect pest of sugar beet. To study the interactions between sugar beet roots and SBRM, we developed an *in vitro* bioassay using seedlings and hairy root cultures of susceptible (F1010) and moderately resistant (F1016) sugar beet germplasm. SBRM larvae aggregated and fed on F1010 roots but moved away from the F1016 tissues. Damage to F1010 roots included rasping, tunneling and severed roots and was similar to damage observed in SBRM-infested fields. We collected infested F1010 and F1016 root and hypocotyl tissues at 24 and 48 h to identify genes that are regulated by SBRM feeding. The Suppressive Subtractive Hybridization (SSH) method was used to compare infested and uninfested plants of the same genotype. Infested samples of the two genotypes were also compared in order to identify genes up-regulated in one genotype while down-regulated in the other. Over 1000 cDNA fragments were cloned and approximately half of them have been confirmed to be differentially expressed in response to SBRM infestation. Further characterization is ongoing and includes DNA sequence determination, functional annotation and expression profiling following various plant stresses. Functionality of genes with potential roles in resistance or susceptibility will be analyzed *in vitro* using sugar beet hairy root cultures. Given that root defense responses have not been well characterized, identification of the genes involved will yield a better understanding of root-insect interactions for devising effective insect control measures.

Jasmonate in lepidopteran eggs and neonates

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Jasmonic acid (JA) is a key molecule initiating plant defensive responses to attack by pathogens and herbivores. This phytohormone is produced at sites of insect damage and is ingested by feeding insects, but its occurrence in insects remains to be studied. We report the presence of JA in eggs and neonates of all nine lepidopteran species that we screened, representing four superfamilies and five families of Lepidoptera. Concentrations of JA in some lepidopteran species far exceeded those found in most plant species. Levels of JA varied significantly among species and between eggs and neonates of the same species. In some cases, eggs contained significantly more JA than neonates, but for at least one species (*Lymantria dispar*) neonates had significantly more JA than their eggs despite lacking food upon emergence. The presence of JA in eggs and neonates across a wide taxonomic range may indicate that JA has an as yet undescribed function in insects.

The influence of the introduced pathogen *Phytophthora ramorum* on saprotrophic beetle (Coleoptera: Scolytidae) host selection behavior

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The forest disease known as sudden oak death is caused by an introduced oomycete, *Phytophthora ramorum*. Although the host range of this pathogen is broad, coast live oak (*Quercus agrifolia)* is among the few tree species that are consistently killed. Up to six ambrosia and bark beetle species (Coleoptera: Scolytidae) selectively tunnel into the bark directly overlying the disease cankers. In the absence of *P. ramorum* infection, these beetle species are characteristically restricted to severely weakened or freshly killed oaks. However, in our disease progression studies, approximately 50% of the *P. ramorum*-infected trees in Marin County, California forests were under beetle attack each year. Although the functional role of the beetles in disease progression and tree death is unclear, the mortality rate of infected trees is considerably elevated once beetles attack the bleeding cankers. Also, living infected trees that are colonized by beetles are subject to significantly increased structural failure rates. Evidence suggests that the interaction of the pathogen with the host tree produces volatile compounds that lead to beetle attacks and accelerate tree death. We are attempting to understand the factors influencing this shift in beetle host selection behavior from weak or moribund trees to discrete infection sites on generally vigorous trees. In July 2002, healthy trees were selected for three treatments, inoculation with *P. ramorum* (80), wounded without inoculation (40), and controls (40), then half of each group was treated with insecticide to prevent beetle attacks. In 2003, traps were placed on the insecticide-treated trees to monitor beetle responses to infection. Infected trees trapped more beetles (97% of the total) than did wounded trees throughout 2003. The trees with the most advanced disease status in December 2003 had suffered the greatest number of beetle attacks early in the year. We used SPME fibers to collect headspace gasses from inoculated and wounded trees. Three volatile phenolics were found to be associated with the infected trees. Field trials are under way to evaluate the activity of these compounds in the attraction of scolytid beetles to *P. ramorum*-infected coast live oaks.

Peripheral and central shift in the olfactory circuitry mediate preference for toxic fruit in *D. melanogaster* sibling *D. sechellia*.

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Drosophila sechellia is a specialist on Morinda fruit, a smelly fruit toxic to its sibling species. How this has affected its olfactory circuitry is poorly studied. Here we report on shifts at various levels in the olfactory circuitry, which are in part adaptive. Combined gas chromatography and Electro-Antenno Detection (GC-EAD) and GC- MS (mass spectrometry) revealed that both *D. melanogaster* and *D. sechellia* antennae respond strongly to the fruit's characteristic hexanoates. Acids, which dominate the fruit's headspace elicited very little antennal responses. Further single sensillum screening of antennal sensory neurons revealed that in *D. sechellia* large basiconic sensillae type 3 (AB3) were overrepresented (approximately 3.5x times more) on the costs of AB2 (not found) and AB1 sensillae (50-70% fewer). AB3 sensilla responded down to femtogram quantities of its key ligand methyl hexanoate. Concordantly, we found that neuronal projections of large AB inhabiting neurons had undergone substantial rewiring in the antennal lobe, creating two enlarged glomeruli receiving input from the AB3 type sensillae. The physiological and morphological changes are reflected in shift in *D. sechellia*'s behavior. Behaviorally *D. sechellia* is attracted to lower concentrations of hexanoates, than its sibling *D. melanogaster*, whereas no tapering was observed at high concentrations. However, whereas *D. sechellia* was behaviorally also more sensitive to the fruit's acids, particularly caproic acid, no evidence for a peripherally mediated shift was found on antennae or palpae. Several classes of olfactory sensillae responded to hexanoic acid, but no obvious changes in either frequency, their distribution or sensitivity were observed. Clearly, the shift accounting for the acid preference is located downstream at a higher level of integration. These findings uniquely indicate how evolution can act at several levels of the olfactory circuitry in mediating the fly's unique preference for fruit that kills its sibling species.

Plant latex and its diverse ingredients protect plants from insect herbivory: tales of cysteine proteases in papaya and fig latex and sugar-mimicking alkaloids in mulberry latex.

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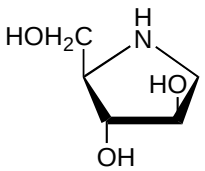
Plant latex is widely found in plant species (ca. 12,000-35,000 species). Some chemical ecologists proposed a hypothesis that the role of latex is defense against insect herbivory, and the story of cardenolides in milkweed latex is a very well known example that supports the hypothesis. However, biological roles of latex and their material bases have not been studied in most cases. We here show two novel examples from our studies where latex ingredients, proteins and chemicals, play crucial roles in plant defense against insect herbivory.

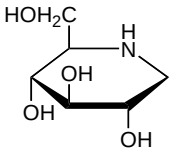
The first story is about cysteine proteases in papaya and fig latex and its crucial role against caterpillars. We found that leaves of the Papaya tree (*Carica papaya*, Caricaceae) and a wild Fig tree (*Ficus virgarta*, Moraceae) are strongly toxic against lepidopteran larvae such as oligophagous *Samia ricini* (Saturniidae) and two notorious polyphagous pests, *Mamestra brassicae* (Noctuidae) and *Spodoptera litura* (Noctuidae), though no apparent toxic factors from these species have been reported. When the latex was washed off, the leaves of papaya lost toxicity. Latexes of both papaya and the wild fig were rich in cysteine-protease activity. E-64, a cysteine protease-specific inhibitor, completely deprived the leaves of toxicity when painted on the surface of papaya and fig leaves. Cysteine proteases, such as papain, ficin, and bromelain, all showed toxicity. The results suggest that plant latex and the proteins in it, cysteine proteases in particular, provide plants with a general defense mechanism against herbivorous insects.

The second story is about sugar-mimicking alkaloids in mulberry latex. Since leaves of the Mulberry trees (*Morus* spp.) have been used to rear the silkworm, *Bombyx mori*, and the silkworm grows well on them, their toxicities and defensive activities against herbivorous insects have been neglected. However we found that mulberry leaves are highly toxic to caterpillars other than the silkworm, *B. mori*, (such as *S. ricini* and *M. brassicae*), due to the ingredients of the latex. The toxicity of mulberry leaves was lost when latex was washed off, and latex-added artificial diets showed toxicity. Mulberry (*M. australis*) latex contained very high concentrations of alkaloidal sugar-mimic glycosidase inhibitors reported to have anti-diabetic activities, such as 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1), 1-deoxy nojirimycin (DNJ), and 1,4-dideoxy -1,4-imino-D-ribitol. Their concentrations, altogether, in latex reached 1.5-2.5% (8-18% to dry weight) in several mulberry varieties, which were 100 times the concentrations previously reported from whole mulberry leaves. These sugar-mimicking alkaloids showed toxicities to caterpillars, but not to the silkworm, *B. mori*. Our results suggest that sugar-mimicking alkaloids play key roles in defense of mulberry against insect herbivory.

These two stories may suggest that plant latexes are treasuries of bioactive substances (chemicals and proteins) that play key roles in plant-insect interactions.



D-AB1



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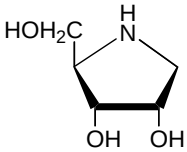

1,4-dideoxy-1,4-imino-D-ribitol

Geographic variation in response of *Dendroctonus valens* to host volatiles of *Pinus* spp.: a holarctic perspective

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Abstract- Bark beetles (Coleoptera: Scolytidae) have specialized feeding habits and attack one or a few closely related host-trees in their host range. Monoterpenes are the dominant source of volatiles in conifers and are attractive to several scolytid species. The red turpentine beetle, *Dendroctonus valens* LeConte, has a very wide distribution in North America and responds to host odors for host location. Attractive semiochemicals have been locally investigated for *D. valens* in North America and its introduced range in China and yielded regional differences. Testing host volatiles as attractants for *D. valens* provides an important opportunity to evaluate whether local adaptation to host compounds occurs or which host compounds govern host selection in its native and exotic habitats. Further, understanding of the ecological aspects of host selection is particularly important for the development of semiochemicals for control of bark beetles. Our objective was to investigate variation in the behavior of *D. valens* to various monoterpenes at seven sites across North America and two sites in China in 2004. Semiochemicals were selected based on previous work with *D. valens*: ((*R*)-(+)-α-pinene, (*S*)-(-)-α-pinene, (*S*)-(-)-β-pinene, (*S*)-(+)-3-carene and a combination of three compounds (1:1:1 ratio of ((*R*)-(+)-α-pinene:(*S*)-(-)-β-pinene:(*S*)-(+)-3-carene) and a blank control. (+)-3-Carene was the most attractive monotepene tested throughout its native range and in China. The importance of monoterpenes in attraction of *D. valens* and other insect species and in general plant-insect interactions will be discussed.

Posters

Olfactory and behavioral responses of females *Salaria pavo* (Pisces: Blenniidae) to putative pheromones of conspecific males and of the closely related *S. fluviatilis* males

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Salaria pavo is small fish living in the rocky littoral zone of the Mediterranean and adjacent Atlantic coast. The closely related freshwater species, *S. fluviatilis*, inhabits rivers and lakes in the vicinity of the Mediterranean. In both species, the mating system is promiscuous. Males occupy holes or crevices in the hard substrate where females come to spawn and the males subsequently guard the eggs. The males of both species develop seasonally anal glands (AG) in the first two rays of the anal fin concurrent with development of the testis, and for *S. pavo* it has been suggested that the AG is the source of a pheromone that attracts females. These are good model species to investigate specialization of sex pheromone production in fish. Male blennies may be "active signallers" in contrast with known hormonal pheromone systems in teleosts where receivers are "chemical spies" detecting non-specific gonadal steroids or prostaglandins passively excreted by females into the water *via* the urine or gills.
In this study we investigated the olfactory and behavioral responses of females *S. pavo* to putative pheromones of conspecific males and of *S. fluviatilis* males. The male-related chemical substances (putative pheromones) were extracted by solid phase extraction (SPE; C2/ENV+ sorbent) from water conditioned by: **a)** males *S. pavo* or *S. fluviatilis* with fully developed anal glands (FD AG); **b)** the same males after the anal glands were excised; **c)** males *S. pavo* with partially developed anal glands (PD AG); **d)** the same males after the anal glands were removed. The olfactory potency of each SPE extract was tested by recording of electro-olfactograms (EOG). Water conditioned by males *S. pavo* or *S. fluviatilis* with FD AG had similar olfactory potency and both evoked larger EOG amplitudes than the water conditioned by the same males after excision of the AG. Water conditioned by males *S. pavo* with PD AG evoked smaller EOG amplitudes than those of water conditioned by the male blennies with FD AG, and after excision of the PD AGs the olfactory potency of the corresponding male-conditioned water was not significantly affected.
The effect of water conditioned by conspecific males, before or after excision of the FD AG, or water conditioned by males *S. fluviatilis* with FD AG on the swimming behavior of females *S. pavo* was measured in a flow through aquarium. The results showed that females were attracted to the water conditioned by conspecific males with FD AG but not to the water conditioned by the same males after excision of the AG or to the water conditioned by males *S. fluviatilis* with FD AG.
Taken together these results suggest that the FD AG of males *S. pavo* is the source of a putative pheromone that attracts females, providing olfactory information related not only with the male's reproductive condition but also its specific identity.

This study was supported by an FCT Grant (POCTI/BSE/45843/2002) and a CRUP/British Council Grant (14/03 & 90/04).

Molecular Characterization of Pheromone Biosynthesis Activating Neuropeptide from the Diamondback Moth, *Plutella xylostella* (L.)

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Pheromone biosynthesis activating neuropeptide (PBAN) produced in the subesophageal ganglion stimulates pheromone production in the pheromone gland of many lepidopterans. A cDNA isolated from female adult heads of the diamondback moth (DBM, *Plutella xylostella* (L.)) encodes 193 amino acids including PBAN, designated as Plx-PBAN, and four other neuropeptides (NPs): diapause hormone (DH) homologue, α-NP, β-NP and γ-NP. All of the peptides are amidated in their C-termini and shared a conserved motif, FXPR(or K)L structure, as reported from other PBAN cDNAs. Plx-PBAN consists of 30 amino acids, the shortest PBAN so far reported. Plx-PBAN exhibited below 50% homology, compared with other known PBANs. The Plx-DH homologue is structurally different from DH of *Bombyx mori*. The length of Plx-β-NP (16 amino acids) was the shortest and showed relatively low similarity, whereas γ-NP (10 amino acids in length) was the longest among γ-NPs examined. When female adults were injected with synthetic Plx-PBAN, pheromone production showed a maximal increase 1 hr post-injection. RT-PCR screening revealed that Plx-PBAN cDNA was expressed in all examined body parts, with the highest expression level in the head of female adults. Analysis of RT-PCR products indicated that the Plx-PBAN sequence was identical in all examined body parts of both sexes. Phylogenetic analysis revealed that the Plx-PBAN gene is distantly related to other PBANs, demonstrated by the relatively low homology.

Attraction and trapping of female Noctuidae moths *Autographa californica* (Alfalfa looper) and *Helicoverpa zea* (Corn earworm) to feeding attractants derived from moth-visited flowers; obliteration via attract-and-kill.

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Both sexes of *Autographa californica* adults were attracted (upwind flight with a zigzagging flight pattern and contact with the source) to a floral double-component chemical lure on a flight tunnel. *Helicoverpa zea* moths of both sexes were captured on Universal Moth Traps™ baited with a floral chemical lure. Female *Autographa c.* moths were attracted to a double component lure containing phenylacetaldehyde and beta-myrcene. Studies were conducted on a Plexiglas flight tunnel. Moth attractants were dispensed from polypropylene vials that provide controlled release rate for extended periods of time. A killing station is being tested in the field for use in combination with these lures as an attract-and-kill system for deployment on agricultural settings. Field experiments demonstrated significant reduction of female *Autographa c.* captures on plots treated with bait station compared to untreated plots. Female and male *Helicoverpa z.* moths were captured with Universal Moth Traps™ baited with phenylacetaldehyde and methoxy-2-methyl-benzoate. Experiments were conducted in a corn growing region of Washington State during the 2003 and 2004 seasons. Lures were dispensed with polypropylene vials that provide controlled release rate for extended periods of time (similar lure dispenser used to attract *Autographa c.*).

Species 1: Lepidoptera Noctuidae *Autographa californica* (alfalfa looper)
Species 2: Lepidoptera Noctuidae *Helicoverpa Zea* (corn earworm)

Induction of host specificity in the larvae of *Manduca sexta:* a molecular approach

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Larvae of the moth *Manduca sexta* are facultative specialists on the plants in the family Solanaceae. A few days after hatching, larvae feeding on solanaceous foliage become specialist feeders, while those feeding on non-solanaceous foliage or artificial diets remain polyphagous. The induced feeding preference of *M. sexta* larvae involves the formation of a recognition template to the host recognition cue indioside D, a steroidal glycoside so far only found in Solanaceae. The induction of host specificity in *M. sexta* is coincident with a chemosensory tuning to indioside D for half of the taste receptor cells located in two pairs of taste sensilla, the lateral and medial sensilla styloconica. The information transduced by these taste receptor cells is sufficient and necessary for the feeding preferences of host-restricted *M. sexta* larvae. We hypothesize that these changes in test receptor responses are at least partly due to changes in gene expression in these cells. To test this hypothesis, two populations of larvae were reared to their fifth stadium, one polyphagous population reared on wheat germ artificial diet, and the other specialist population reared on potato foliage. Two types of tissues were collected from each of the fifth instars: the maxilla, containing the sensilla styloconica as well as the maxillary palp, and the dorsal abdominal horn as a control tissue, which does not contain chemoreceptors. Total RNA was extracted from each tissue type, in each population, and turned into cDNA. The profile of cDNAs generated in each tissue and population was compared by differential display. Differentially expressed cDNAs were extracted from polyacrylamide gels, reamplified by PCR and sequenced. Here we present our results and place them in context of taste transduction and feeding behavior.

Cuticular hydrocarbons in *Anopheles gambiae*: preliminary results and future perspectives

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The mosquito *Anopheles gambiae* s.s. is the most efficient vector of human malaria, responsible of more than 100 millions human cases and over 1 million deaths each year in the Afrotropical region. This great efficiency in malaria transmission is due to the very high degree of ecological flexibility, synanthropy (i.e. the ability to exploit anthropogenic breeding sites, feeds almost exclusively on humans and bites and rests indoors) and genetic variability shown by this species. Chromosomal [Coluzzi *et al*. (1985); (2002)] and molecular discontinuities [della Torre et al., 2001] within *An. gambiae* s.s. have been shown, which have led to the proposal of the existence of an incipient, ongoing speciation process. Two "molecular forms" (provisionally named with a non-Linnean nomenclature as M- and S-form) have been defined on the basis of single nucleotide differences in rDNA regions and by the virtual absence of hybrid rDNA genotypes in nature. Although these forms do not show significant differences with regard to sporozite rate and antropophily, this ongoing speciation process increases the overall flexibility of the vector system in exploiting the environmental resources and would likely result in an increase of the overall vectorial capacity.

Cuticular hydrocarbons (CHCs) generally represent the most abundant epicuticular lipids in insect and have been investigated as taxonomic and systematic characters in several taxa, among which mosquito sibling species complexes. Although no inter-specific qualitative differences in the composition of the CHC profile have been ever shown, the relative abundance of some compounds has been found to differ among closely related Anopheline species, such as *An. gambiae* and its sibling species *An. arabiensis* [Carson *et al.*, 1980]. Studies on mosquitoes have also shown the existence of high levels of variations in the relative abundance of CHCs correlated to geographic and physiological parameters, such as age [Brei *et al*., 2004; Gerade *et al*., 2004]. We have characterised by Gas Chromatography-Mass Spectrometry analysis the whole CHC profile of *An. gambiae*, which is shown to be composed by 14 *n*-alkanes, 16 monomethyl alkanes, 13 dimethyl alkanes, 5 alkenes, with main-chain lengths ranging from C_{17} to C_{47}. Moreover, we have carried out Gas Chromatography analyses of the CHC profile of field and laboratory specimens of *An. gambiae* s.s. molecular forms and *An. arabiensis* with the aim to: 1) evaluate whether the 3 taxa differ in the CHC profile and whether these differences may be linked to pre-mating isolation barriers, and 2) analyse age-related changes and evaluate whether these can be used to develop a model to predict the age of field specimens, which could be used for the correct age determination in vector populations.

The preliminary results of these analyses will be presented and future perspectives of this research field will be discussed.

Role of host cues and oviposition-induced plant signals on the host location behaviour of the egg parasitoid *Trissolcus basalis*.

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During the host selection process insect parasitoid make flexible use of chemical information which can be directly or indirectly associated with the presence of their hosts. It was hypothesized that stimulus wasp's response should be inversely related to its potential response and a change in wasp's reaction can be the result of associative learning. In this view, influence of host indirect cues on the host location behaviour of the egg parasitoid *Trissolcus basalis* was investigated. *T. basalis* host selection behaviour is mediated by chemical cues which are both directly or indirectly associated with the presence of host egg mass of *Nezara viridula*. Volatile compounds emitted by plants as a consequence of herbivore activities play a noteworthy role. In fact, it was shown that the annual plants *Vicia faba* and *Phaseolus vulgaris*, under the combined feeding and oviposition activity by *N. viridula*, a piercing/sucking herbivore, emit volatiles that attract *T. basalis*. As a result of the polyphagous feeding habitat of *N. viridula*, wasp females likely face complex and unsettled habitats which likely result in emission of different chemical cues. Then, once on the plants, host footprints left on the substrate are known to play a role as indirect host-derived cues inducing an arrestment response in the parasitoid. Nevertheless, differences in the wasp's intensity responses correlate to different host stages and/or host physiological conditions have been observed, e.g. naive *T. basalis* females showed the stronger arrestment response when in contact with substrate contaminated by *N. viridula* host mated females in a pre-ovipositional condition. Wasp preferences for host mated female traces has been interpreted as a wasp innate reaction to a cues more correlated with the host egg presence. We hypothesized that females which experienced the presence of host footprints kairomone without finding the host eggs should be less likely to respond to the same stimulus in the near future, while females that successfully located host egg masses would have shown reinforced responses. Results support our hypotheses. The significance of these findings for the manipulation of adult parasitoid behavior to enhance pest control and their implications for host selection by egg parasitoids are discussed.

Pheromonal and seasonal activity of three *Cydia spp.* in France.

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Pheromone attraction of *Cydia splendana* was studied in 2003 in two chestnut orchards in France (Dordogne and Gard), using blends containing 10 µg of E8,E10-12:Ac (*E,E*) alone or combined with 0.5, 2 or 10 µg of Z8,E10-12:Ac (*Z,E*). An additional blend containing 2 µg of *E,E* and 10 µg of *Z,E* was also tested. All these blends were renewed after 2 weeks and retested during two additional consecutive periods. Irrespective of the region or the flight period, the addition of *Z,E* to *E,E* stimulated the attraction of *C. splendana* males in a dose-dependent manner. However, when the ratio of *E,E*/*Z,E* was 2:10, the attraction of *C. splendana* males was strongly inhibited. Whether the *Z,E* isomer is synthesized by *C. splendana* females remains to be determined. Two other species, *C. ulicetana* (Dordogne) and *C. fagiglandana* (Gard), representing together 4 % of the total catch, were also attracted to these blends. While *C. ulicetana* responded equally to all blends, most *C. fagiglandana* were attracted to the *E,E* isomer as blends containing *Z,E* were inhibitory. Not only did the three *Cydia* species differ in their pheromonal response but they also appeared to be temporally separated since *C. fagiglandana* became less abundant as the flight activity of *C. splendana* progressed, while the opposite was observed for *C. ulicetana*. Three successive peaks of *C. splendana* males, observed towards the end of the flight period in Dordogne, were probably the result of moth invasions, an aspect that deserves consideration when interpreting male trap catches of this important chestnut pest.

Sexual mimicry in the German cockroach, *Blattella germanica*

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German cockroach males exhibit a typical courtship behavior upon contacting a mature and sexually receptive female. The male behavior includes an unambiguous elevation of the wings, which occurs only during courtship. The female contact pheromone that elicits this behavior is a mixture of methyl ketone hydrocarbon derivatives, four of which have been identified. Surprisingly, teneral females, males and nymphs produce the same behavioral effect on adult males. We found that, unlike males, who lose their ability to stimulate courtship behavior in mature males within a few hours after adult eclosion, nymphs retain this capacity throughout their development. The semiochemicals responsible for this phenomenon are currently being investigated using behavioral assays to guide their isolation from nymph cuticular lipid extracts, separated by flash chromatography and HPLC. It was found that both male and female nymphs posses a behaviorally active compound that is different from the known components of the adult female contact sex pheromone. In addition, female nymphs posses at least 3 components that are not distinguishable from the adult female components, yet their chemical structure awaits confirmation.

Although the adaptive significance of this behavior remains to be elucidated, it may offer insight into the evolution of the sexual communication system of the German cockroach.

Environmental and genetic influences on chemical defense in *Piper imperiale*

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Plants defend themselves against herbivores and pathogens with a suite of different types of morphological, biochemical, and biotic defenses, each of which may vary within and among species. Variation in defense may be a result of interactions between plant genetics, environmental variables, the predictability of herbivore or pathogen attack, and the costs of producing defenses. In order to examine the causes and consequences of defensive variation at different scales, we compared different defensive strategies and their effectiveness against herbivores across plant species and focused on a single species for a detailed examination of the influence of environmental and genetic variables on plant defense and the resultant effects on herbivores. Previously, we have shown that shrubs in the genus *Piper* differ significantly in their defensive strategies, which include combinations of ant mutualism, secondary compounds, and leaf toughness. These strategies vary in their effectiveness against herbivores, so that herbivory varies greatly across plant species. Here, we present results from a field experiment examining the influences of genetic and environmental variables on defenses in *Piper imperiale.* We planted clones of 8 individuals in the wet forest under varying conditions of light, soil moisture, soil type, and soil nutrient availability and measured leaf toughness, secondary metabolite content, and herbivory. Previous data shows that increased production of secondary metabolites in this species costly in that it is associated with trade-offs in allocation of resources, probably N, to growth vs. defense, but that increased defense in associated with a substantial reduction in herbivory.

Fatty Acid and Sex Pheromone Responses to PBAN stimulation in the Female Pheromone Gland of the Moth *Heliothis virescens*

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The pheromone biosynthesis activating neuropeptide (PBAN) is known to stimulate pheromone biosynthesis in many species of moths. PBAN is thought to work by controlling a particular biosynthetic enzyme, either during the synthesis of saturated fatty acids or the metabolism of fatty acids. If PBAN activates a single enzyme step, it should be possible to observe which step is affected by following the dynamic changes in fatty acid titers in the gland. In time-course studies we have observed the effect of PBAN injection on pheromone, total fatty acid titers and acyl-CoA thioesters in the pheromone gland of female *H. virescens*. Following a single PBAN injection, pheromone [major component (*Z*)-11-hexadenal] is produced fairly rapidly (within 0.5 h) and reaches peak titer by 2.5 h, before it declines rapidly. In contrast, the total titer of the pheromone precursor acid, (*Z*)-11-hexadecenoate, reaches a maximum at 5.5h following injection, before declining. There are no significant changes in total titers of hexadecanoate or octadecanoate (both pheromone intermediate acids) following PBAN injection. However, the patterns observed for the total titers of these fatty acids changed when titers of only their acyl-CoA thioesters were determined. Although the thioester of (*Z*)-11-hexadecenoate still reached maximal titer at 5.5 h titer, its titer did not decline thereafter. Furthermore, the thioesters of both hexadecanoate and octadecanoate both showed increase up to 2.5 h and then significant declines. These data are discussed in terms of the effects of PBAN on fatty acid synthesis and metabolism.

Oviposition stimulation of the banded sunflower moth, *Cochylis hospes*, by diterpenoids from sunflower heads.

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The banded sunflower moth, *Cochylis hospes* (Walshingham) (Lepidoptera: Cochylidae)(BSFM), oviposits preferentially on the bracts of sunflower heads (*Helianthus* spp.). Testing of sunflower head crude extracts, in an egg-laying bioassay, indicated the presence of chemical oviposition stimulants for BSFM. Fractionation of crude extracts by silica gel flash column chromatography, then bioassay of fractions, showed ovipositionally active compounds were concentrated in two groups; one of relatively non-polar and one of more polar compounds. Further bench column fractionations on silica gels and by lipophilic Sephadex gel permeation chromatography, followed by HPLC, allowed purification of two bioactive diterpenoid alcohols from the non-polar group, and three bioactive diterpenoid acids from the polar group. Chemical characterization, and an indication of the final purity of the compounds used in bioassays, was carried out by GC-MS of TMS derivatives, and NMR spectroscopy. Purified compounds were tested individually in dose response bioassays with 0, 2, 10, or 50 μg applied to a 1cm^2 area of chromatography paper. At 50 μg /cm^2, the five bioactive diterpenoids stimulating significantly more eggs to be laid than on the solvent-only control. Comparison of the chemical structures of active versus inactive diterpenoids indicates a possible structure-activity relationship, with an alcohol group on ring D of the diterpenoid structure necessary for activity.

Genetic and ontogenetic variation in the defensive chemistry of tiger swallowtail caterpillars

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Chemical defenses are common in larval Lepidoptera and fall into two major categories: sequestered and glandular defenses. Based on a comparison of the average logP of compounds, glandular defenses tend to be less polar in nature. These compounds are biosynthesized by the insect in specialized glands and may be subject to dietary, developmental and genetic effects. One example of a glandular defense is the osmeteria; eversible glands located dorsally between the head capsule and thorax swallowtail butterfly caterpillars. It is well known for several *Papilio* species that the osmeterial chemistry changes between the fourth and fifth instars, and that fifth instar *P. glaucus* predominantly produce a mixture methyl and isobutyric acids. Our analysis of the early instar constituents revealed a complicated mixture of at least 50 different mono- and sesquiterpene compounds that changed quantitatively throughout the first four instars. In addition, substantial genetic and maternal effects also influenced the composition of the secretions. Preliminary field studies indicate that these genetically based variations have implications for the survivorship of different lines. The biosynthetic complexity of the secretions and underlying developmental and genetic variation may provides the raw material for directional selection of the composition of these secretions in populations of swallowtail butterflies and warrants further investigation.

Constitutive and jasmonate-inducible traits of *Datura wrightii.*

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Plants in the family Solanaceae possess numerous traits that are induced by damage by herbivores. Many of these traits also can be induced by exposing plants to the plant hormone, jasmonic acid, or its volatile ester, methyl jasmonate. Methyl jasmonate also induces an increase in densities of leaf trichomes in some crucifer species. Recent studies on a mutant tomato genotype that is insensitive to jasmonic acid (*jai1*) showed abnormal trichome development on both leaves and fruits. *Datura wrightii* (Solanaceae) is dimorphic for leaf trichome morphology in most southern California populations. Within populations, some plants produce leaves with dense, nonglandular trichomes, while other plants produce leaves with longer, less dense glandular trichomes. The glandular trichomes secrete esters of glucose and aliphatic acids that confer resistance to a number of herbivorous insect species. Trichome morphology in *D. wrightii* is governed by a single gene, and the glandular trichome condition is dominant. Moreover, all plants produce glandular trichome while young, but the homozygous recessive plants switch from producing glandular to nonglandular trichomes during the first 10 – 12 weeks of growth. This study addressed two major objectives. The first was to determine if exposure of seedlings to methyl jasmonate during the period of trichome differentiation altered either the type or the density of trichomes that mature plants expressed. The second was to determine if mature plants with glandular or nonglandular trichomes responded differentially to methyl jasmonate.

Methyl jasmonate induced the production of proteinase inhibitors and increased the activity of polyphenol oxidase similarly in plants expressing either glandular or nonglandular trichomes. Methyl jasmonate exposure, however, did not increase the activity of peroxidase or the concentration of scopolamine or hyoscyamine, the two major alkaloids of *Datura.* Exposure to methyl jasmonate during trichome differentiation did not affect either the final type or the density of either type of trichome. For plants with glandular trichomes, those exposed to methyl jasmonate produced significantly higher concentrations of acylsugars than unexposed plants. We conclude that *D. wrightii* shares inducible proteinase inhibitors and inducible polyphenol oxidase activity with other better-studied solanaceous species. *D. wrightii* differed from tobacco in that alkaloid concentrations were constitutive, and not inducible. Because trichome phenotype was not inducible in *D. wrightii,* and because both trichome phenotypes showed similar increases in proteinase inhibitors and polyphenol oxidase activity, the methyl jasmonate-induced responses of *D. wrightii* are not correlated with the variation in frequencies of the glandular and nonglandular trichome phenotypes among *Datura* populations.

Transformation of plant acquired pyrrolizidine alkaloids into insect-specific necine esters by polyphagous arctiids: mechanistic and functional aspects.

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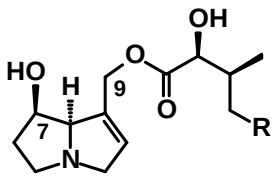
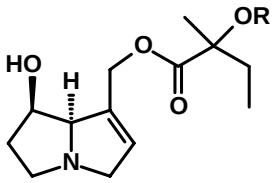
Two highly polyphagous arctiids, *Estigmene acrea*, known to produce the male courtship pheromone, hydroxydanaidal, and *Grammia geneura* not known to do so, were studied [1, 2]. Larvae of both arctiids sequester plant pyrrolizidine alkaloids (PAs) of various structural types. In both arctiids varying proportions of these PAs were unaltered carried through all life stages, whereas the remaining parts were hydrolyzed and the resulting necine bases transformed into insect-specific retronecine esters, i.e. creatonotines or callimorphines:



R = H Creatonotine A
R = CH_3 Creatonotine B



R = H Deacetylcallimorphine
R = Acetyl Callimorphine
R = Propionyl Homocallimorhine

E. acrea synthesizes exclusively creatonotines (formed in larval stage), whereas *G. geneura* synthesizes first creatonotines (early pupal stage) which are subsequently (late pupal stage) converted into callimorphines. In *E. acrea* males the creatonotines are the common precursor for the formation of hydroxydanaidal which is produced as a PA-signal from any of the sequestered 7-hydroxylated plant PAs. Both arctiids are able to convert various necine bases, obtained from plant acquired PAs into the respective insect-specific necine esters. Evidence is presented that the major function of the insect-specific necine esters is to salvage plant acquired PAs that cannot *per se* be carried from larvae via pupae to the adult stage. These PAs are hydrolyzed and the resulting necine bases are converted into the respective insect-specific necine esters that are maintained through all developmental stages. It is suggested that the insect-specific necine esters mimic plant derived PAs of the lycopsamine type to which the arctiids are well adapted.

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Changes in secondary metabolite production and transcriptome complexity in response to artificial predation in a benthic soft coral, *Sinularia polydactyla*

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Sinularia polydactyla, an abundant soft coral species in Guam, exhibits biochemical phenotypic plasticity. Preliminary evidence indicates that observed phenotypic plasticity in secondary metabolite production is related to predation intensity. However, it is unclear if these changes in secondary metabolite content represent an inducible defense response in *S. polydactyla*. To investigate both chemical changes and differences in mRNA diversity in response to predation stress, a set of artificial predation experiments were conducted *in situ* on colonies of *S. polydactyla*. These experiments were designed to mimic predation by butterflyfishes, the main predators of *S. polydactyla*. Tissues were collected and preserved for both molecular and chemical analyses. RNA extracts were used to construct cDNA libraries using a nano-scale synthesis strategy. The cDNA libraries were used to measure changes in transcriptome complexity using a Cot-type, reannealing assay that employs an informatics-based analysis of kinetic profiles. This method allows for quick, high-throughput analysis of sequence complexity and has been used to uncover significant transcriptome-level differences in *S. polydactyla* during previous *in situ* grazing experiments. In conjunction with the molecular assays, analyses of known *S. polydactyla* marker compounds were carried out using LC-mass spectrophotometry and HPLC. By examining both molecular and chemical data together we hope to gain a better understanding of secondary metabolite production and induction in response to predation stress.

Studies on the mechanism of the production of sternal gland secretion of the predatory stink bug, *Eocanthecona furcellata* (Wolff)

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Production of the secretion from the male sternal gland of the predatory stink bug, *Eocanthecona furcellata* (Wolff), is affected by the rearing condition. The amount of the male specific compound produced by bugs kept individually is about a hundred fold of the amount secreted by the bugs kept in groups. Possible cues including visual, olfaction, and physical contact were examined. Preliminary results indicate that olfaction alone will not affect the production of the secretion. Vision alone does not affect the production. Physical contact with live bugs is necessary in inhibiting the production of the male specific compound.

Gustatory receptor neurones in contact chemosensilla on the antennae and the proboscis of *Heliothis virescens* project in close, but distinct areas of the CNS

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In the ultimate decision of rejection or acceptance of a host plant, the moth *Heliothis virescens* uses the contact chemosensilla located on several appendages of the body. In addition, host plant selection is influenced by experience. Appetitive learning is demonstrated by the use of the proboscis extension response. Stimulation with sucrose (unconditioned stimulus) reflexly elicits proboscis extension. By pairing an odour stimulus (conditioned stimulus) with sucrose stimulation and allowing feeding, the moth extends the proboscis when subsequently stimulated with the odour alone. In order to reveal the neuronal connection between the olfactory and gustatory pathways we have traced the projections in the CNS of the gustatory receptor neurones on the antennae and on the proboscis.

Fluorescent dyes were applied to the antennal (*s. chaetica*) and the proboscis (*s. styloconica*) contact chemosensilla. Examination in a confocal laser scanning microscope (CLSM) revealed the labelled primary axons in the CNS. The axons from *s. chaetica* ran tightly together, bypassed the antennal lobe posterior-laterally and terminated ipsilaterally in a fan-shaped pattern in the antennal mechanosensory and motor centre (AMMC) in the deutocerebrum, and in a finger-like pattern in the dorsal SOG. Projections of a single fibre extending to the ventral SOG were also found in some preparations. Axons of the receptor neurones in *s. styloconica* on the proboscis entered the SOG via the maxillary nerve and terminated in the SOG/ tritocerebrum in a pattern parallel, anterior and medial to the projections of the antennal taste neurones. A few single fibres projected both ipsi- and contralaterally in the SOG. Three-dimensional reconstructions of the brain and the SOG with precise location of the projection areas were made by the use of the software AMIRA. This is the first study showing projections of antennal and proboscis gustatory receptor neurones in the SOG/ tritocerebrum of an adult lepidopteran species.

The present study also includes electrophysiological recordings from the receptor neurones in *s. chaetica* during mechanical and chemical stimulation. The results showed responses of one mechanosensory and of several gustatory neurones. Separate receptor neurones responded excitatory to sucrose and sinigrin. The behavioural effect of sucrose and sinigrin on the proboscis extension reflex was tested by repeated stimulations with solutions of the two compounds. Whereas sucrose elicited extension in 100% of the individuals in all repetitions, sinigrin elicited extension in fewer individuals, a number that deceased with repeated stimulation. This demonstrated the role of sucrose as a phagostimulant and of sinigrin as a deterrent.

Geometric isomers of methyl 2,4,6-decatrienoate: Synthesis and attractiveness to stink bugs and tachinid flies

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All possible geometric isomers of methyl 2,4,6-decatrienoate, some as targeted sting bug pheromones and others as reference compounds, were synthesized from accessible starting materials by fully exploiting Wittig-type olefinations. The aggregation pheromone of the brown-winged green bug, *Plautia stali*, methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (also a cross-attractant for the brown marmorated stink bug, *Halyomorpha halys*, which recently invaded the Northeast U.S. and poses a potential threat to many commercial crops and ornamental plants), was expediently produced in two easy steps from (*E*)-4,4-dimethoxy-2-butenal in 55% yield. The same starting material was used to prepare another stereoisomer of a potential importance, methyl (*Z*,*E*,*Z*)-2,4,6-decatrienoate, which was electrophysiologically active for the male *H. halys* but has never been isolated from the bug. The sex pheromone of the red-shouldered stink bug, *Thyanta pallidovirens*, methyl (*E*,*Z*,*Z*)-2,4,6-decatrienoate, was conveniently synthesized from 2,4-octadiyn-1-ol in 32% yield using *in situ* manganese dioxide oxidation-Wittig condensation in a key step.

While the origin of the cross-attraction of *H. halys* to methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate and methyl (*Z*,*E*,*Z*)-2,4,6-decatrienoate is under a collaborative investigation with Rutgers University, New Jersey, we have made two additional observations in Maryland field-tests indicating kairomonal properties of trienoates. First, we found that methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate attracted *Gymnosoma fuliginosa*, a native tachinid fly, which could thus potentially parasitize the invasive brown marmorated stink bug. Second, we observed that both *Z,E,Z* and *E,Z,Z* esters synergized attraction of the dusky stink bug, *Euschistus tristigmus*, to its main male-specific pheromone component, methyl (*E,Z*)-2,4-decadienoate. The identification of the later pheromone was conducted in this laboratory over a decade ago, and possibly needs revisiting to see if one or both trienic esters are part of the pheromone blend.

Study on the Feeding Stimulant of the Asian Comma Butterfly, *Poligonia c-aureum* L. (Lepidoptera: Nymphalidae)

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In order to develop an artificial diet of *Polygonia c-aureum* for continuous indoor-rearing system, we searched for phgostimulatory substance from the host plant, *Humulus japonicus.* The dried leaves of *H. japonicus* were subjected to methanol extraction. The Me-OH extracts were futher separated by four solvents, and then the attractant activity was investigated. The attractant activity of *n*-BuOH, *n*-Hexane, $CHCl_3$, and EtOAc was 47%, 19%, 12%, and 0%, respectively. These results demonstrate that butanol fraction of the extract contains potent phagostimulatory substance of *P. c-aureum*. On the other hand, the attractant activity of Linalyl acetate, known as phagostimulatory substance of Lepidoptera, was 48% in the *P. c-aureum*. And ß-sitosterol, known as feeding stimulant of Lepidoptera, also stimulated feeding of *P. c-aureum.* Further study is hoped to find final pure phagostimulatory substance of *P. c-aureum*

Lima bean neighbourhood watch – Herbivore-induced volatiles induce an indirect defence in neighbouring plants

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Many plant species are known to respond to herbivore damage with an increased emission of volatile organic compounds that attract carnivorous arthropods and thereby function as an indirect defence. Whether also neighbouring plants 'eavesdrop' on these airborne signals and tailor their defences accordingly has been debated intensively during the last years. The most frequent criticism of the plant-plant communication hypothesis was the fact that most studies regarding this phenomenon have been conducted under artificial laboratory conditions to increase the probability of detecting a response, thereby sacrificing ecological realism.

During field experiments at the natural growing site of the Lima bean (*Phaseolus lunatus*), we could show for the first time that herbivore-induced volatiles of wild growing Lima bean plants induce the secretion of extrafloral nectar in neighbouring, conspecific plants. Extrafloral nectar secretion, which represents another indirect defence strategy, could also be induced by an artificial blend of volatiles resembling the naturally released blend. Pairwise, single-compound comparisons with eight main constituents of the full herbivore-induced volatile blend identified the green leaf volatile (*Z*)-3-hexenyl-acetate as one elicitor of the defence reaction.

In a further experiment we compared the defensive effect of EFN only with the effect mediated by volatiles (EFN induction plus attraction of plant defenders). Bean tendrils that had received either volatiles or EFN benefited almost equally of the respective treatment as compared to controls. The strong similarity in the responses of these two treatment groups in terms of both performance of several fitness-relevant plant parameters measured (e.g., herbivory rate, leaf number and number of inflorescences) as well as number of putative plant defenders attracted (ants and wasps) indicated that the observed beneficial effects may have been rather due to an increased attraction of protective arthropods to EFN than to volatile chemicals in the headspace of the so treated tendrils.

To our knowledge, these results represent the first report on an induction of an indirect herbivore defence by herbivore-induced volatiles. Considering the widespread taxonomic distribution of both extrafloral nectaries and the ability of plants to emit volatiles upon herbivore damage, this phenomenon may be of potential ecological and economic importance.

Attraction of the parasitic wasp *Cotesia plutellae* to the synthetic HIPV blend under both laboratory and field conditions

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The diamondback moth larvae, *Plutella xylostella*, are specialist herbivores of cruciferous plants. When the larvae attack their host plants, the plants emit a specific blend of volatile compounds, called herbivore-induced plant volatiles (HIPV), that attract *Cotesia plutella*, the specialist larval parasitoid of *P. xylostella*. Headspace analysis using a gas chromatograph-mass spectrometer (GC-MS) revealed that four compounds were emitted as HIPV from cabbages only slightly infested by the larvae. In a choice-chamber (ca. 30 x 30 x 30 cm) bioassay, an uninfested plant treated with an artificial blend of the four compounds was preferred by the wasps to the untreated plant. In a climate-controlled room (ca. 3 x 3 x 3 m), uninfested plants treated with the blend attracted more wasps than those without the blend. The attractiveness of the uninfested plants treated with the blend was equal to that of infested plants. Females of the diamondback moth showed an equal oviposition preference between the plants treated with the blend and the control plants. The attractiveness of the blend to the parasitic wasps was also examined in the field. The possible use of the HIPV blend in the pest management of *P. xylostella* in agroecosystems will be discussed.

The contact chemosensilla *Sensilla styloconica* on the proboscis of *Heliothis virescens* and the projection pattern of the associated receptor neurones in the central nervous system

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Stimulation of the taste sensilla with sucrose elicits extension of the proboscis in nectar feeding insects, including Heliothine moths. This proboscis extension reflex (PER) is used to study appetite learning, where sucrose stimulation represents the unconditioned stimulus and odours the conditioned stimulus. In trying to determine the neuronal connection between the taste and the olfactory pathways in Heliothine moths, we are studying the projections of the taste receptor neurones in the central nervous system. We here present the morphology of the taste sensilla (*s. styloconica*) on the proboscis and the projections in the central nervous system of the associated receptor neurones. The morphology of these sensilla was studied by light and electron microscopy (SEM, TEM). The sensilla are 80□m long and 15□m in diameter. Each sensillum contains 3 or 4 sensory neurones, one mechanosensory and two or three chemosensory. The tip of the sensillum has a single pore allowing the taste chemicals to enter the sensillum lymph and contact the taste neurones. The receptor neurones were stained with Neurobiotin tracer combined with avidin-fluorecein conjugate, and the projections were viewed in a confocal laser scanning microscope (CLSM). The stained axons entered the suboesophageal ganglion (SOG) via the maxillary nerves and were divided into two categories based on their projection pattern. Category one projected exclusively ipsilaterally in the dorsal SOG/tritocerebrum and is assumed to be taste neurones. Category two projected bilaterally in the SOG confined to the frontal surface of the neuropil and is assumed to be mechanosensory. These neurones had also one additional branch terminating in the ipsilateral SOG/tritocerebral area.

Behavior associated with pheromone release in the cerambycid beetle *Neoclytus acuminatus acuminatus*

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We describe a male-specific calling behavior associated with the release of the aggregation pheromone (2*S*,3*S*)-hexanediol in the cerambycid species *N. a. acuminatus*. Males release pheromone when they raise the anterior half of the body above the substrate. We confirmed that this behavior is associated with pheromone release by sampling the pheromone-producing surface with Solid-phase microextraction (SPME). To examine the adaptive significance of the behavior, we identified the site of pheromone release with chemical, histological, and morphological techniques and demonstrated that the posture increases pheromone dissemination.

Functional Organization of the Antennal Lobe of the moth, *Helicoverpa zea*

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The larvae of corn earworm (*Helicoverpa zea*) are serious crop pests of corn and cotton in North America. Adult female moths produce *(Z)*-11-hexadecenal (Z11-16:Ald) and *(Z)*-9-hexadecenal (Z9-16:Ald) as major and secondary sex pheromone components, respectively. Males have three different types of pheromone-related olfactory sensilla, which are characterized by electrophysiological responsiveness to different conspecific pheromone components or behavioral antagonists, the pheromone components of other sympatric heliothine species.

We examined the morphological and physiological characteristics of pheromone-component-sensitive olfactory receptor neurons (ORNs) of male *H. zea* in order to delineate the glomeruli in the antennal lobe to which each type of ORN projects its axons. Using the single-sensillum recording technique and a neuronal staining technique, we examined electrophysiological characteristics of ORNs residing in the olfactory sensilla on the male antennae and visualized their glomerular destinations in the olfactory lobe by track-tracing. In addition, we used calcium-imaging to corroborate activity-dependent topography by the populations of active ORNs that project to glomeruli similar to those recorded from and stained individually. We found that the ORNs of each sensillum type exhibited consistent neuronal pathways to specific glomeruli in the olfactory lobe. We confirmed the results from these individual tracings by recording the activities of populations of these same ORN types using calcium-imaging of glomerular activity during antennal excitation with these same compounds. Furthermore, we recognized a distinct glomerular complex structure that had been previously undescribed and appears to be associated with *H. zea* pheromone olfaction.

Analysis of pheromone production by the red turpentine beetle, *Dendroctonus valens*

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The red turpentine beetle, *Dendroctonus valens*, is a pine bark beetle that is broadly distributed in North and Central America. It has also been accidentally introduced and established in 1998 in eastern China. As its name suggests, the beetle is well known for its response to host pine volatiles (monoterpenes), but little is known of its pheromone biology. Several workers have reported the presence of *trans*-verbenol in female hindgut extracts, but no other candidate pheromone components have been studied. Head-space volatiles from small groups of JH III-treated or phloem-fed (Monterey pine, *Pinus radiata*) male and female *D. valens* were analyzed by GC-MS and GC-FID. Volatiles collected from females between approx. 22 and 68 hrs after JH III treatment contained significant amounts of the bicyclic acetal frontalin. The first trace of frontalin was detected by SPME sampling 14 hrs after JH III treatment and maximum release occurred between 24 and 64 hrs. Males and females in this experiment also released *cis*-verbenol, *trans*-verbenol, and verbenone, which are typical semiochemicals of other *Dendroctonus* spp. Head-space volatiles from large groups of male and female *D. valens* feeding in cut logs of *P. radiata* showed only trace quantities of frontalin in the female sample and none in the male sample. GC-EAD analyses showed stereospecific antennal responses by both sexes to synthetic (–)-frontalin and to synthetic versions of other frequently occurring *Dendroctonus* spp. pheromone compounds. Antennal responses of both sexes to the large-group extract from infested cut logs were nearly identical and directed primarily to the host monoterpenes present in the extract from the logs.

Are sex pheromones effective reproductive isolating mechanisms in garter snakes?

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Sex pheromones are considered to be potent reproductive isolating mechanisms. Reproductive isolation between sympatric taxa can be maintained by specific mate-recognition behaviors or by ecological divergence that reduces interspecific contact during reproduction. Common garter snakes, *Thamnophis sirtalis* and plains garter snakes, *Thamnophis radix*, are sympatric over large parts of their range, but morphological data suggest that prezygotic isolation between these two species partially breaks down in an area of overlap at the extreme northern and eastern extents of their respective ranges in Manitoba, Canada. Males of both species selectively court and mate with females of their own species, but male *T. radix* are less choosy than male *T. sirtalis*. Hexane extracts of female skin lipids that contain the sex pheromones also elicited courtship behavior from males even in the absence of the female herself. Certainly this male preference for species-specific pheromones contributes to species isolation, however, it is not strong enough to completely isolate the two species. The absence of hybridization over most of the sympatric range may depend on differences in the timing of mating and the lack of geographical overlap in overwintering sites or hibernacula. At the extreme edges of the two species ranges in Manitoba, the relative lack of suitable hibernation sites and the timing of spring emergence from winter hibernation are severely constrained by the very brief optimal spring emergence temperatures. This may be responsible for the apparent breakdown of the temporal and geographic isolation of these two species. Although speculative, global climate change may be having a disproportionate effect on the breakdown of species-isolating mechanisms in species at extreme latitudes. Long-term reproduction data sets from field work on organisms like these garter snakes (35+ years) may prove to be especially compelling for insights into global climate change effects.

Biosynthesis of 2-alkanones and mandibular acids in social hymenopterans.

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In three Japanese *Bombus* species, 2-alkanones (C7 and C9) and 3-hydroxyalkanoic acids (C8 and C10) were identified in worker mandibular glands. In *Apis* species, the Japanese honey bee, *Apis cerana japonica*, gave 3-hydroxyotanoic acid as a forager-specific major compound with a small amount of 2-heptanone, whereas *Apis mellifera* gave about 1 microgram of 2-heptanone and a trace of 3-hydroxyoctanoic acid. Application of deuterated 3-hydroxyalkanoic acids onto mandibular glands resulted in detection of deuterated 2-alkannones. These results supports that 2-alkanones are biosynthesized through oxidative decarboxylation from corresponding 3-hydroxyalkanoic acids in the mandibular glands.

In order to test a hypothesis that 3-hydroxyalkanoic acids are produced from longer straight chain fatty acids, deuterated palmitic and stearic acid were prepared and applied to mandibular glands of these social bees. After incubation, the mandibular glands were extracted with ether, analyzed by GC/MS for deuterated 3-hydroxyalkanoic acids.

Biochemical crypsis by insect herbivores

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We explore the ability of the lepidopteran herbivore *Heliothis subflexa* to reduce its vulnerability to natural enemies through adaptation to a feature of its host plant, *Physalis angulata*. The fruits of this plant lack free linolenic acid, which is required for the development of most insects and is a key compound involved in the elicitation of plant defense mechanisms. By overcoming this nutritional deficiency, *H. subflexa* larvae are able to avoid the induction of some plant defenses and simultaneously render themselves nutritionally unsuitable as hosts for natural enemies that require linolenic acid for their own development.

Sex pheromone of the citrus leafminer, *Phyllocnistis citrella*

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The citrus leafminer is an important vector of citrus canker in many of the major citrus production areas of the world. (7*Z*, 11*Z*)-Hexadecadienal was reported as a sex attractant for this insect in the 1980s, based on trap catches during pheromone screening trials in Japan. However, attempts to reproduce this work in other areas of the world have not been successful. We report here that (7*Z*, 11*Z*)-hexadecadienal is only one component of the pheromone, with the other critical component being the analogous trienal, (7*Z*, 11*Z*, 13*E*)-hexadecatrienal. Both compounds were identified in the effluvia from live female moths by coupled gas chromatography-electroantennography, using nonpolar and polar GC columns, and the identifications were confirmed by comparisons of mass spectra with those of authentic standards. In field trials, each compound alone was not attractive to male moths, but blends of the two compounds were highly attractive, with thousands of insects being caught per trial. Addition of the isomeric (7*Z*, 11*Z*, 13*Z*)-hexadecatrienal inhibited attraction to the 2-component blend.

Key Words – sex pheromone, (7*Z*, 11*Z*)-hexadecadienal, (7*Z*, 11*Z*, 13*E*)-hexadecatrienal, 5, 7, 11-hexadecatrienal, citrus leafminer, (7*Z*, 11*Z*, 13*Z*)-hexadecatrienal

Response of larvae of *Culex quinquefasciatus* and *Liriomyza trifoli*, to extracts and compounds isolated from *Alchornea glandulosa* and *Muntingia calabura*.

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Mosquitoes of the *Culex* group have been turned into severe social problem to inhabitants near Muña dam on the highlands of Colombia. The major strain has been described as belonging to *Culex quinquefasciatus* Say (Diptera: Culicidae), vector of filariasis and encephalitis. In our continuing search for biologically active compounds from native plants, the structures of two triterpenoids derivatives isolated from the apolar extract of fresh leaves of *Alchornea glandulosa* Endl & Poepp (Euphorbiaceae**)** and two chalcones separated from the apolar extract from leaves of *Muntingia calabura* L. (Elaeocarpaceae) growing in Colombia, were determinate. These compounds not were reported previously in these species and they present notable larvicide activity against two pests: 3rd instar larvae of *C. quinquefasciatus* and the leafminer *Liriomyza trifolii* L.(Diptera:Agromyzidae) which affects seriously the *Gypsophila paniculata* (Cariophyllaceae) cultivars. For *C. quinquefasciatus*, extracts were applied in field bioassays (houses near lake borders) as formulations recently prepared, affording good results as repellent and insecticide agents with residual effects until six hours in some areas. For *L. trifolii* the preparations were applied twice in greenhouse cultivars during sixteen weeks, by sprinkling solutions of different concentrations on plants and evaluating the damage level as a defined protection index (PI), using as controls one synthetic product and one neem commercial formulation, and they gave good results as plant protectors.

The identification of flavonoids (Su et al., 2003) and pentatronol and related derivatives (Rasool et al, 1992) have been achieved using chromatographic and spectroscopic methods, mainly IR, MS and NMR- ^{1}H , ^{13}C-NMR of 1D and 2D. Details of this study will be presented.

Acknowledgments

This work have been developed with the financial support of the Universidad Nacional de Colombia proyecto DIB y la Universidad de Magallanes. We are grateful with the Departamento de Parasitología de la Facultad de Medicina Veterinaria y Zooteccnia de la Universidad Nacional Bogotá, by affording the *C. quinquefasciatus* larvae, and Flores Tiba for plants and insects for the bioassays.

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Taste in the proboscis of adult nymphalid butterflies: adaptation to ethanol and acetic acid for foraging rotting foods

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Adults of several nymphalid butterflies forage for exuded tree sap and rotting fruits as well as flower nectar. These rotting foods contain a remarkably lower concentration of total sugars (approx. 3%) than flower nectar (more than 15% in general). Furthermore, they are found to have several fermentative products such as ethanol (1%) and acetic acid (0.5%), that are absent in flower nectar. Thus, the butterfly species feeding on rotting foods are considered to have a gustatory sense specifically adapted to such chemicals. In this study, two flower-visiting nymphalid butterflies, *Vanessa indica* with a feeding habit for rotting foods and *Argyreus hyperbius* without such a feeding habit, were examined for their behavioral and electrophysiological responses to three omnipresent sugars (sucrose, fructose, and glucose), ethanol, and acetic acid. Behavioral responses were determined as the degree of feeding behavior by individuals when the tip of their proboscis was brought into contact with test samples. Electrophysiological responses were recorded using the tip recording method from the sensilla styloconica (SS) on the terminal part of the proboscis. Among the sugars tested, sucrose and fructose highly stimulated behavioral and electrophysiological responses from both species, while glucose was apparently less active than these sugars. As to the response to the same sugar, *A. hyperbius* was slightly more sensitive than *V. indica* in behavioral responsiveness but the level of SS sensitivity was almost similar in the two species. Ethanol and acetic acid elicited no feeding responses from individuals tested. The electrophysiological responses to these components were not usually observed or were sometimes in burst when examined at higher doses. Moreover, these fermentative products were found to suppress behavioral and electrophysiological responses to sugars. When mixed with a 10% sucrose aq. solution (a concentration sufficient to elicit feeding behavior), ethanol suppressed sugar responses of *A. hyperbius* at the concentration of more than 30% but little affected those of *V. indica*, whereas acetic acid deterred sugar responses at the concentration of more than 1% for *A. hyperbius* and 5% for *V. indica*, respectively. It demonstrated that acetic acid is more effective as a feeding deterrent than ethanol. These results suggest that (1) sugar reception in the proboscis is comparatively similar in both butterflies irrespective of their feeding habits. (2) Since *A. hyperbius* is more susceptive than *V. indica* to ethanol and acetic acid (especially acetic acid deters *A. hyperbius* from feeding at the natural concentration level), the butterfly species feeding on rotting foods are more tolerant to these fermentative products than those using only flower nectar.

Presence of two sympatric strains in Kanzawa spider mites *Tetranychus kanzawai*: difference in their ability to induced defensive gene expressions in lima bean leaves

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Spider mites *Tetranychus kanzawai* are polyphagous herbivores that feed on various plant families including legume. Scars made by Kanzawa spider mites on lima bean leaves (*Phaseolus lunatus*) were classified into two types; white scars and red scars. We obtained two strains to select the mite individuals based on the color of the scars. One strain inducing white scars was called 'White' and another strain inducing red scars was called 'Red'. When plants are infested with herbivores, some defense responses are induced in the plants. We assumed that pigmentation of leaf scars by Kanzawa spider mites was the results of the defense responses of lima bean plants. Thus, we analyzed expression of genes for pathogenesis related (PR) proteins that are related to the induced defense response in plants. It has been known that the expression of basic PR gene is related to activation of jasmonate-related signaling pathway and the expression of acidic PR gene is related to that of salicylate-signaling pathway. There was no difference in the expression of basic PR gene in lima bean leaves infested with either White or Red. The expression of acidic PR gene was, however, observed only in Red-infested leaves. In addition, levels of endogenous salicylate in the Red-infested leaves were significantly higher than those in the White infested leaves. Thus, it was suggested that the infestation by Red activated both jasmonate-related and salicylate-related signaling pathways, while the infestation by White activated mainly jasmonate-related signaling pathway. Based on these data together with headspace analysis of infested lima bean leaf volatiles, we will discuss the reasons why Red and White are co-exist even on the same leaf.

Unusual Natural Products in Beetles: How Are Anthraquinones Produced in the Tansy Leaf Beetle (*Galeruca tanaceti*)?

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Chrysomelid beetle of the taxon Galerucinae, tribe Galerucini, contain 1,8-dihydroxylated anthraquinones (chrysazin, chrysophanol) as well as anthrones (dithranol, chrysarobin) in all developmental stages, especially in the egg and larval stage. The host plants of these species do not contain anthraquinones. We studied the question whether Galerucini are able to produce anthraquinones by themselves or whether these components are produced with the help of endosymbiotic microorganisms (bacteria, fungi). The tansy leaf beetle, *Galercua tanaceti*, was used as a model species for Galerucini. Specimens treated with antibiotics and antimycotics during larval development and adult stage still lay eggs containing anthraquinones in the yolk. It might be objected that the treatment did not kill all the potential endosymbionts. Since putative anthraquinone-producing endosymbionts might be transferred to the next generation via the egg stage and since eggs contain anthraquinones within the yolk, we hypothesized that endosymbiotic bacterial or fungal DNA should be detectable within the eggs. However, with the exception of *Wolbachia pipientis*, no other bacterial (16s rDNA) and no fungal DNA (fungal specific 18S rDNA) was found within the eggs. *Wolbachia* is unknown so far to produce polyketides. Furthermore, *W. pipientis* was also found in eggs of *Agelastica alni*, which belongs to the tribe Sermylini of the taxon Galerucinae. Eggs of *A. alni* do not contain anthraquinones. Thus, we conclude that *Wolbachia* is no anthraquinone-producer in the eggs of *G. tanaceti*. So far, all our results suggest that the anthraquinones in *G. tanaceti* are not produced by endosymbiotic microorganisms, but instead by Galerucini-specific enzymes. Such enzymes could be polyketide synthases (PKS), since 1,8-dihydroxylated anthraquinones in plants, bacteria, and fungi are known be biosynthesised *via* the polyketide pathway. While PKS have been studied for these latter organisms, no animal PKS genes have been decoded so far. Nevertheless, we are currently screening the beetle´s genome for PKS sequences.

From Courtship Behavior to Field Evaluation: Sex Pheromone of the Brazilian Population of Citrus Leaf Miner, *Phyllocnistis citrella*

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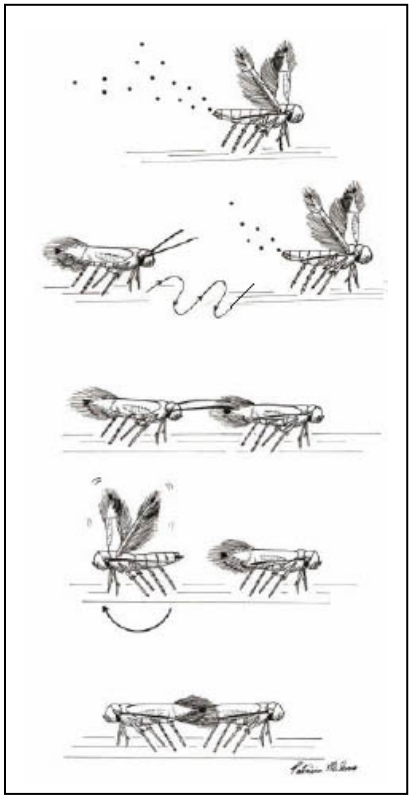
Behavioral studies aimed at laying the foundation for the isolation, identification, and synthesis of the complete sex pheromone system of citrus leaf miner, *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae) indicated that mating occurred in a time window of 2h, starting 1 h before the onset of photophase. The large majority of tested insects mated in the first two days after emergence, with no significant difference between mating at day-1 and day-2. Following a stereotype courtship (inset), copulation (when successful) was recorded in average for 49.6 min. In Y-olfactometer tests conducted at the time of mating activity, males were strongly attracted to caged virgin females as well as to extracts from putative pheromone glands (Parra-Pedrazzoli et al. 2005). Using male antenna as the sensing element, three EAD-active peaks were detected from these pheromone gland extracts. Based on GC-MS and GC-IR data the semiochemicals were tentatively identified as a novel pheromone, (*Z,Z,E*)-7,11,13-hexadecatrienal, a previously identified attractant, (*Z,Z*)-7,11-hexadecadienal (Ando et al. 1985), and (*Z*)-7-hexadecenal in a ratio of 30:10:1, respectively. Identification was confirmed with synthetic compounds, which gave retention times identical to those of the natural products on three capillary columns with polar and non-polar phases (Leal et al. 2005). While traps baited with the previously identified attractant alone did not catch any males in Brazil, binary and tertiary mixtures with the major constituents caught significantly more male moths than traps baited with five virgin females



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Why are the mini plants not attacked by the large pine weevil? Emissions from mini plants and 1-year-old seedlings of *Picea abies*.

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Field tests in Sweden showed that mini plants (10-12 week old) of pine and spruce had a larger survival rate than conventional 1-year-old plants, mainly due to less attack by the pine weevil, *Hylobius abietis* [1]. The volatile blends of mini plants and 2-year-old seedlings of Norway spruce, *Picea abies*, were studied. Mechanical injury mimicking gnaw of the pine weevil was made and head space samples were collected by solid phase micro extraction (SPME), and analysed by gas chromatography and mass spectrometry (GC-MS). Two-dimensional gas chromatography (2DGC) was used to determine the enantiomeric compositions of monoterpenes known to attract or repel the pine weevil. The older unwounded plants generally emitted larger amounts than the mini plants and had a blend more dominated by sesquiterpenes than monoterpenes. The repellent (-)-limonene were present in the odour from all the plants while the attractive (+)-α-pinene were only detected in the odour from the older plants. While the older plants emitted mainly monoterpenes from the phloem when wounded, the mini plants emitted mainly GLV:s (green leaf volatiles) and thus smelled more like grass than conifers. This delay in biosynthesis of monoterpenes in the phloem of the mini plants can be discussed in the light of coevolution.

A novel microbial DPS-protein contributes to the homeostasis of *N*-acyl amino acids in the caterpillar gut

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N-Acyl amino acids have been isolated from microorganisms since at least two decades ago. Because of their amphiphilic nature, they are widely accepted as biosurfactants (Boulton 1989). Recently, a similar group of compounds was identified in insect regurgitants which can induce defense reactions in plants (Alborn et al., 1997). The compounds are synthesized in the insect gut utilizing food derived lipids and insect produced glutamine (Pare *et al*. 1998). Interestingly, some bacteria living commensally in the insect gut had been shown to be able to catalyze the amide bond formation between externally supplied free fatty acids and amino acids (Spiteller *et al*., 2000). On the other hand, a crude protein fraction from *Manduca sexta* alimentary tissues has also been demonstrated to rapidly catalyze amide bond formation between free fatty and amino acids (Lait *et al*. 2003).

We have successfully purified an active protein from the lysate of a strain of *Microbacterium arborescens* isolated from the gut of *Spodoptora exigua*. Most of the catalytic properties of this protein are similar to the crude protein fraction reported for *M. sexta*. It catalyzes reversible condensation of many amino and fatty acids to the corresponding amides. The ratio of incorporation of the tested fatty acid substrates is coincident with that of their conjugates in insect regurgitant. The temperature and pH optima of this protein make it suitable for working in the poikilothermal and alkaline caterpillar digestion tract. Two reaction optima were found around pH 8 and pH 10, respectively. The enzyme tolerates temperatures as high as 48 °C. The protein is homologous to bacterial Dps proteins (DNA-binding protein from starved cell), but without DNA binding activity. Freshly prepared native protein is a homo-dodecamer. The molecular weight of a protomer is 17.2 kD measured by MALDI-TOF. It binds up to 10 atoms of iron per monomer. Our data suggests that this protein plays a role in the homeostasis of *N*-acyl glutamines in the insect gut.

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Tergal glands of female primary reproductives of the Formosan subterranean termite: Structure and possible function

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The Formosan subterranean termite *Coptotermes formosanus* is a major urban pest attacking wooden structures and live trees in several southern states and Hawaii. Each mature colony can have several million individuals. Every year from April-June, these colonies produce swarms of winged adults. After a brief flight, the adults land, drop their wings and form tandem pairs with female in the lead and male maintaining contact with the posterior end of the female. Initial contact between sexes may be accidental and does not involve a long range sex attractant. Once a suitable place is found, these primary reproductives build a nuptial chamber, mate and lay eggs.

The adult females have a pair of tergal glands below the 9th and 10th tergites. The larger gland is 1.1 mm long and 0.18 mm at its widest and both have a segmented appearance. Situated just below the cuticle, the glands have two distinct regions. The outer layer consists of type I cells with a characteristic layer of microvilli. These cells possibly open to the outside through narrow cuticle lined ducts. The lower layer consists of glandular cells, packed with electron dense granules measuring an m in diameter. Comparison of HPLC-APCI-MS of extracts of average of 0.9 9th-10th abdominal segments of both female and male showed a peak present only in the female extracts. APCI-MS of the peak showed ions for the triacylglycerol, trilinolein. Males have no tergal glands and no trilinolein.

Newly molted, pre-flight females contain negligible amounts of trilinolein, which gradually increases to about 800 ng in post-flight females. Trilinolein titer in both virgin and mated females decreases gradually reaching about 20 ng in 42 days. Structurally, the glands of newly molted females have electron lucent granules in place of electron dense granules. These may represent a precursor of trilinolein. On the other hand females, seven days after mating, show breakdown of type I cells and appearance of large number of vacuoles. The electron dense granules coalesce to form large granules that appear to move towards the intersegmental membrane for possible release. The males which have originally no trilinolein, acquire increasing amounts of it, apparently from the female as a nuptial/companionship gift. The trichoid sensilla near the tip of the maxillary palps of males did show significant increase in the electrical activity in response to trilinolein. However, the exact role of trilinolein in sexual behavior is not fully understood. There is also the possibility for the presence of a close range sex pheromone emitting from the fine openings in the cuticle above the tergal glands. This needs further investigation.

Acknowledgments: We like to thank Christopher Florane for outstanding technical assistance, Eric Erbe for scanning electron-microscopy and J. C. Dickens for electrical recordings.

Taxonomic patterns in use of long-range pheromones within the longhorned beetle subfamily Cerambycinae

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Male-produced long-range pheromones recently have been identified for a number of species of longhorned beetles in the tribe Clytini of the Subfamily Cerambycinae. We have discovered that pheromone glands of one species are located in the pronotum of males, and are associated with pores that are absent in females. Previous research suggests that species in other cerambycine tribes apparently do not use long-range pheromones. We used scanning electron microscopy to examine prothoraces of males and females of 50 species in 20 genera of the Clytini and related tribes to examine the taxonomic distribution of gland pores. Micrographs revealed pores or clusters of pores in males of some species that were absent in females, indicative of male-produced pheromones. Distribution and location of pores on the prothorax varied greatly between and within genera. This work is part of a larger project currently underway that surveys taxonomic groups throughout the Subfamily Cerambycinae for presence of the pores to provide a framework for research on long-range pheromones.

Defense induction in marine macroalgae: a common phenomenon?

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Resisting against herbivory is one of the major challenges algae have to face. Chemical defense is one strategy to reduce grazing pressure and this adaptation may be regulated 'on demand'. Induction of defense is widely known in terrestrial plants, but only few examples exist in marine algae. Furthermore, these are restricted to brown algae.
To check whether this surprising pattern results from research bias or represents a typical marine feature, identical experiments were conducted in 9 countries. Sixty-five alga-herbivore combinations from tropical, subtropical, and temperate regions were used, to test for the capacity of macroalgae to regulate their anti-herbivore defense. Thirty percent of the tested algae reduced their palatability after grazing exposure relative to ungrazed conspecifics. Additionally, the frequency of defense induction was evenly distributed among brown, red, and green algae. In contrast to recent theories, we found no evidence for latitudinal differences in the frequency of macroalgae with inducible defenses.
We conclude that defense induction in marine algae is taxonomically and geographically widely distributed and its ecological relevance has to be re-evaluated.

Drowned in sorrow: surfactants and other compounds in lepidopteran regurgitate act as anti-predator defence

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The discharge of regurgitate is a common anti-predator defence among herbivorous insects. Several studies using generalist herbivores have demonstrated that the defensive effect of regurgitate is dependant on the secondary chemistry of the host plant [1]. We have tested this hypothesis of host-plant dependent defence for the polyphagous moth *Spodoptera exigua* (Lep., Noctuidae).
Caterpillars were reared on *Lycopersicon esculentum*, *Apium graveolens* or artificial diet and were subsequently exposed to ants. Ants that came in contact with regurgitate stopped their attack and groomed themselves. The duration of grooming was independent of the caterpillar's diet. In no-choice feeding tests with regurgitate from caterpillars reared on either diet, no deterrent effects on ants were found when compared to sucrose solution. Therefore, an effect of secondary metabolites from the host plant was excluded. Since ants, which possess a highly hydrophobic cuticle, were easily wetted by regurgitate, we hypothesized that the highly amphiphilic property of regurgitate was important for the defensive effect. Contact angle measurements of regurgitate on silanized glas confirmed a high degree of amphiphilicity (contact angles: 65° vs. 102, regurgitate vs. water). We tested the importance of amphiphilicity by applying droplets of water, regurgitate or water containing Tween 20 (surfactant, adjusted to c.a.: 65°), respectively, on single ants. Ants groomed significantly longer when wetted by regurgitate than by aq. Tween 20. No grooming was observed when ants were treated with water. This result suggests that i) surfactants are a prerequisite for regurgitate to act as a mechanism of defence and ii) that other non-deterrent plant- or insect-derived compounds may add to this effect. Within this context we are currently investigating the role of the regurgitate-derived surfactant *N*-linolenoyl-glutamine.

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Phloem Monoterpene Contents of Four Pine Species and Relative Susceptibility to the Attack of *Tomicus* spp.

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In Portugal, pine forests constitute one of the most important renewable resources, for timber, pulp, paper and chemical industries. Among insect pests, bark beetles cause important damage to pine stands. The Mediterranean pine shoot beetle, *Tomicus destruens*, is an endemic species, the larvae of which excavate galleries in the phloem of trees and freshly cut logs, while the adults must feed on shoots, to complete sexual maturation. Although at low densities *T. destruens* does not attain economic importance, at epidemic levels, which frequently occur after forest fires, it becomes more aggressive causing the death of healthy trees.

The characterization of volatiles of different *Pinus* species is an important tool to decode the process of host tree selection, by herbivore insects using olfactory communication. Over the last decades several works suggested that this bark beetle uses volatile stimuli to locate suitable hosts. Furthermore, a growing body of evidence indicates that females do not use pheromones either for male attraction, or for host location, but rely instead upon tree volatiles, namely terpenic compounds, that act as kairomones.

However, the relative susceptibility of host trees varies widely, geographically, among pine species, and even within monospecific stands.

Since terpenic compounds constitute the dominant group in the volatile fraction of pines, the monoterpenes present in phloem samples of *Pinus halepensis*, *Pinus sylvestris*, *Pinus pinaster* and *Pinus pinea* were identified and characterized. The contents of the volatile fraction of the phloem, as well as the enantiomeric ratio of the monoterpenes, were determined by high-resolution gas chromatography using both flame ionization (GC-FID), time of flight (GC-TOF-MS) and mass (GC-MS) detectors, after head space solid phase micro extraction (SPME). The estimated relative composition was used to perform a discriminative analysis between species by means of cluster and principal component analysis.

Results were further related to laboratory host preference tests, aiming at the detection of possible combinations of host-plant attractants for this bark beetle.

Chemical mimicry of honey bee pheromone in flower aroma of oriental orchid (*Cymbidium floribundum* Lindl.).

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Social behaviors in honey bees can serve as models for investigating recognition and communications among intra- and interspecies as well as those towards environmental conditions. Thus, it is important to know the semiochemicals which play crucial roles in social behaviors.

Flowers of an oriental orchid, *Cymbidium floribundum* Lindl. (Kin-ryou-hen in Japanese), blooms in spring and specifically attracts the Japanese honey bee (*Apis cerana japonica* Rad.) for pollination. The European honey bee (*Apis mellifera* L.) is not attracted. The flowers do not have nectars and pollinia will attach onto the back of the thorax when visited by the bees. So, the pollination occurs successfully but there seems no reward for bees. Moreover, the flowers attract not only workers but also drones and sometimes whole swarming colonies of *Apis cerana japonica*. This project was intended to reveal the chemical basis of this strong attraction by the orchid by analyzing flower aroma and honey bee pheromone gland components by GC/EAD, GC/MS and by bioassays.

So far, we have reported that (1) Semiochemicals in the honeybee exocrine glands were species specific. Components of the aggregation pheromone from Nasonov glands were totally different between *Apis cerana* and *Apis mellifera*. (2) Flower aroma contained the same components that can be found in honeybee aggregation pheromone, especially those found in the Japanese honeybee, *Apis cerana japonica*, in large quantity. (3) Flower aroma did not contain honey bee queen mandibular substances. (4) Presence of drone aggregation pheromone was suggested to exist in both the mandibular gland of drone *Apis cerana japonica* and the flower aroma. (5) The Chinese *Cymbidium floribundum*, probably the origin of Japanese one, strongly attracted the Asian honeybee (*Apis cerana cerana*) with the flower aroma by having several common components in the aggregation pheromone of *Apis cerana cerana*.

This presentation will deal with the reason for the specific attraction of worker, drone, queen, the swarming colony of the Japanese honeybee and chemical cues for social behaviors.

How to spoil the taste of insect prey? A novel feeding deterrent against ants released by larvae of the alder leaf beetle, *Agelastica alni* (Coleoptera, Chrysomelidae)

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Larvae of the alder leaf beetle *Agelastica alni* release a yellow fluid from tubercles, located pairwise dorsolaterally at the first to eighth abdominal segments, when attacked by ants or other predators[a]. This larval fluid has a high feeding deterrent activity towards the ant *Myrmica rubra*[b]. The fluid keeps its bioactivity also after heating, treatment with proteinase K, or size-exclusion chromatography of molecules > 3 kD. The combined samples of the fluid from 804 larvae (L3) were separated by RP-HPLC-DAD into 5 fractions. Only one of these fractions showed a highly significant feeding deterrent activity. Two additional HPLC separations with different eluents were necessary to obtain a highly active fraction, showing strong feeding deterrent activity. This fraction, containing only one component, was analysed by NMR. One- and two-dimensional ^{1}H and ^{13}C NMR experiments suggested a dipeptide structure, 2-furyl-*N*-(glutam-5-yl)alanine. This compound was synthesized and proved to be identical to the natural component. Currently the absolute configuration of this compound is investigated. To the best of our knowledge, 2-furylalanine has not been described as a naturally occurring amino acid before. Furthermore, no dipeptides were known up to now as defensive components for the leaf beetle taxon Galerucinae, to which *A. alni* belongs. However, related glutamic acid dipeptides with an unusal γ-peptide bond such as conjugated with (*S,Z)-N*-(glutam-5-yl)-2-amino-3-hexenoic acid or (*S,Z)-N*-(glutam-5-yl)-2-amino-3,5-hexadienoic acid have been described as defensive components in the sister taxon, the Chrysomelinae[c]. While polypeptides such as defensins are well-known for numerous insects, interorganismic activity of insect dipeptids are hardly known.

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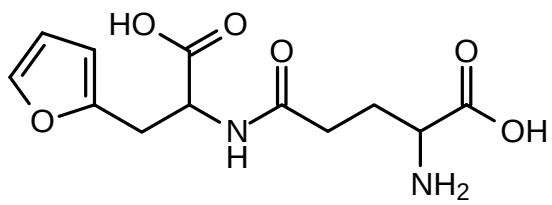

The chemistry of bacterial communication

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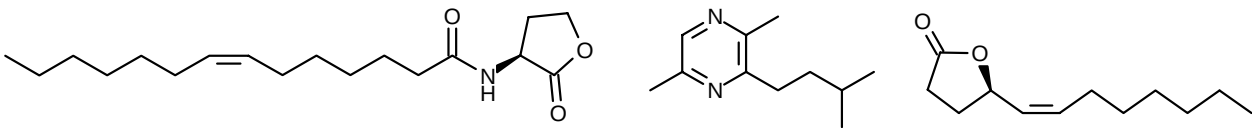
Bacteria secrete small signal molecules called autoinducers into the environment, which regulate gene expression in a concentration and thus population density dependent way. This type of cell-to-cell communication, known as quorum sensing, was discovered as the regulatory mechanism responsible for luminescence in the marine symbiont *Vibrio fisheri*. It is now known to control widely different traits, including the expression of virulence factors, production of antibiotics, and the formation of biofilms. In Gram negative Proteobacteria *N*-acyl homoserine lactones (AHLs) represent a group of well studied autoinducers. AHLs directly bind a luxR type transcriptional regulator with high specificity. This specificity is associated with different acyl side chains. The pattern of AHL molecules produced by a certain organism is stable, but different species produce different patterns of AHLs. Thus, AHLs are thought to mediate species specific signalling. Autoinducers are present in culture media in nM concentrations or less, and their identification by chemical methods is difficult. Therefore, biosensor strains are usually used for screening purposes. They do not produce AHLs but sense their presence through the transcriptional response of a luxR-controlled promoter fused to a gene coding for an easily detectable output signal, e.g. luminescence, fluorescence, or β-galactosidase production. Although the sensitivity of these constructs matches the physiological concentrations of AHLs, the chemical identification of the triggering compounds remains a problem. Here we report a systematic screening of a large number of phylogenetically well characterized isolates from different marine habitats for AHL activity using reporter strains, followed by non-target GC-MS analysis, identification of the AHLs, and chemical synthesis. We used XAD resin as a new method to extract AHLs from culture supernatants. Surprisingly, we found long-chain AHLs in more than half of the Alphaproteobacteria investigated (59 %). AHLs were produced by strains isolated from such diverse habitats as dinoflagellates, marine snow, picoplankton, and sediments. Overlapping patterns of AHLs were present in distantly related genera, challenging the assumption that these compounds mediate species-specific communication. The analytical constraints on detection of quorum sensing compounds may be responsible for a currently biased view of this mechanism, which might be almost ubiquitous and not necessarily restricted to symbiotic interactions.

In a different research program we screened about 90 different bacterial strains for the production of volatiles by analysis of agar plate cultures. Predominant classes of volatiles produced are fatty acid metabolites, terpenes, aromatic compounds, sulfur compounds, and pyrazines. Surprisingly, several strains produced known insect pheromones, some of them requiring elaborate biosynthetic machinery, like the spiroacetals. The function of these compounds in the ecology of the bacteria is largely unknown, but a communicative role seems plausible.



Profiling of labile plant oxylipins by *in situ* derivatization with pentafluorobenzyl-hydroxylamine

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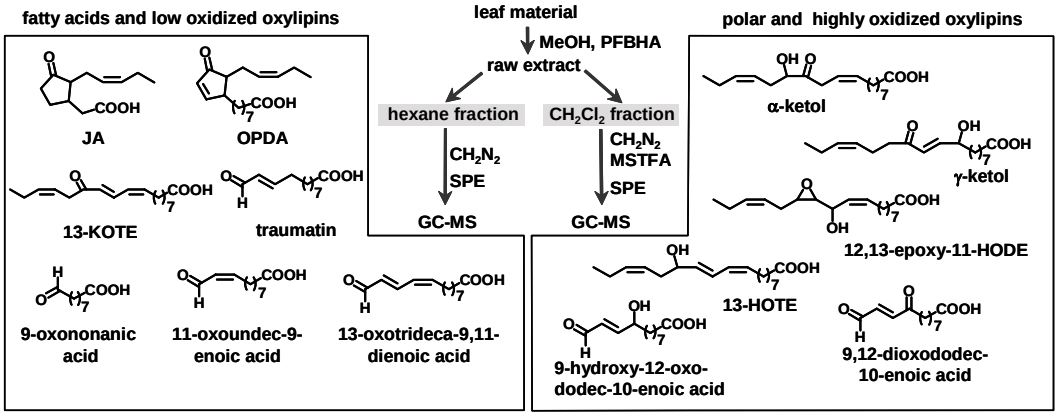
Signaling compounds derived from unsaturated membrane fatty acids play an important role during plant development and stress response. Although the group of oxylipins covers a broad range of chemically diverse compounds, current studies often focus only on the analysis of known octadecanoids like jasmonic acid (JA) and 12-oxophytodienoic acid (12-OPDA).

To establish the role of other oxylipins during the plant's response to abiotic and biotic stress, we developed a rapid and reliable GC-MS-based method for their identification and quantification. The approach is based on *in situ* derivatization of leaf extracts with pentafluorobenzyl hydroxylamine (PFBHA) yielding stable PFB-oximes. The raw extract is subsequently partitioned with hexane and CH_2Cl_2 to separate less polar and polar compounds. These can be analyzed with high sensitivity by negative chemical ionization mass spectrometry (NCI). The PFB-oximes exhibit characteristic mass spectra and, consequently, the identification of unknown oxylipins in complex matrices is greatly facilitated. New structures can be proposed without time consuming isolation, and can be verified by synthetic reference compounds (Scheme 1). Additionally, the *in situ* derivatization completely suppresses the isomerization of *cis*-JA to the less active *trans*-JA and makes a kinetic analysis possible.



Scheme 1: Isolation of labile oxylipins from plant leaves. Identified compounds are displayed in their corresponding extract fraction.

The above analytical approach enables us not only to identify novel oxylipins, but also to study temporal and spatial changes in the oxylipin pattern of stressed plants. High levels of oxylipins were observed locally in caterpillar damaged leaf tissue. Some of the oxylipins were already produced after mere mechanical wounding; others required an additional interaction of the leaf biochemistry with salivary components of the herbivore. The time course of JA-production after mechanical wounding could be followed. Initially, only *cis*-JA is produced due to *de novo* biosynthesis *via* the octadecanoid pathway. Then, *cis*-JA is slowly converted into its less active *trans*-isomer until the resting level is reached. Although substantial differences in physiological activity of various JA isomers have been reported, it is not clear to which extend the epimerization is under enzymatic control or occurs spontaneously due to acidic- or basic conditions in the tissue.

Real-time analysis of alarm pheromone emission in pea aphids (*Acyrthosiphon pisum).*

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Release rate of the major constituent of the alarm pheromone in pea aphids, (*E*)-ß-farnesene, was measured as aphids were attacked by lacewing (*Chrysoperla carnae)* larvae. Volatilization of (*E*)-ß-farnesene from aphids under attack was quantified continuously at 2 minute intervals for all life stages, providing release quantity, degradation rate and signal duration. Alarm signal emission patterns differ significantly between different instars with second instar aphids producing a more concentrated signal over a shorter period of time than 4th instar and adult aphids.

How does utilization of sugars and amino acids by ant communities relate to extrafloral nectar compositions of a myrmecophyte?

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Several studies on the composition of extrafloral nectar (EFN) have revealed it to be a rich source of sugars and amino acids for ants, which are its most common consumers. However, studies that examine the rules that shape the intricate relationship between plant species and their ant partners are scarce. The preferences that diverse ant species exhibit toward different concentrations and types of sugars and amino acids may play an important role in defining the composition of EFN. The extent to which ant species in turn depend upon EFN may be related to its nutritional value and composition.

While the response of floral visitors has been correlated with the concentration and composition of sugars, the main nutrient component in floral nectar, few studies have examined EFN composition and utilization. Although studies have examined sugar and/or amino acid preferences of isolated ant species, only a small number of studies have investigated preferences for these nutrients at a community level, especially in the tropics. Tropical ant communities are very diverse and involve many ant species that co-occur at the same plant or source of nutrition and whose interactions could influence EFN utilization.

This study examines utilization of sugars and amino acids by ants, by presenting artificial nectar solutions to multispecies ant communities in their natural habitat, in an attempt to understand the interaction of ants with the semi-myrmecophytic tree *Humboldtia brunonis* (Fabaceae). *H. brunonis* produces EFN from nectaries present on the sepals and along the margin of its young leaves. This understorey tree species offers a good model system for two reasons. Firstly, *H. brunonis* is not a specialist myrmecophyte; it interacts with different assemblages of ants in different regions of the Western Ghats, India. Secondly, it is very abundant in the Western Ghats and the EFN that it produces is probably one of the most important sources of nutrition for ants in that region. We have examined sugar and amino acid utilization by ants at three sites dominated by *H. brunonis* at the same elevation and along a latitudinal gradient. We examined this utilization by performing experiments in which we varied firstly, sugars at the same concentration and secondly, concentrations of the same sugars. We have tested concentrations of sucrose, glucose and fructose, since these are the most common sugars in a wide range of extrafloral nectars. We also examined utilization of varied combinations of twenty essential and non-essential amino acids.

Preliminary results indicate that dominant ant species at each site show a strong preference for sucrose and may out compete less dominant ants in access to this sugar. The non-dominant ants tend to distribute themselves equally across the other tested sugars. No clear pattern has yet emerged for the amino acid utilization although individual ant species at each site utilize certain amino acid combinations differentially. There appears to be inter-site variation in EFN composition. How this relates to EFN utilization is being explored.

Context-dependent behavioral effects and neurophysiological responses to selected deterrents by gypsy moth larvae, *Lymantria dispar* (L.)

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We tested the deterrent effects of eight alkaloids on larvae of a polyphagous lepidopteran species, gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), using two-choice feeding bioassays. Plants containing alkaloids are generally unfavored and typically avoided by these larvae. Each alkaloid was applied to glass fiber disks and leaf disks from red oak trees (*Quercus rubra*), a plant species highly favored by these larvae. All eight alkaloids tested on glass fiber disks were deterrent to varying degrees. When these alkaloids were applied to red oak leaf disks, however only seven were still deterrent. Of these seven, five were less deterrent on red oak disks compared with glass fiber disks, indicating that their potency was dramatically reduced when they were applied to red oak leaf disks. The reduction in deterrency may be attributed to the phagostimulatory effect of red oak leaves, which suppress the negative deterrent effect of these alkaloids, suggesting that individual alkaloids may confer context-dependent deterrent effects in plants in which they occur. Lepidopteran larvae bear taste cells housed in the galeal styloconic sensilla located on the mouthparts. These sensilla are involved in food plant recognition. Electrophysiological tip recordings from these sensilla revealed that medial styloconic sensilla responded to several alkaloids that deterred feeding in our bioassays and to a sugar alcohol. Lateral sensilla contained a cell that responded to several sugars. The different taste cells exhibited characteristic temporal firing patterns and dose-dependent responses. Thus, this study provides correlative insights into the feeding behavior and taste physiology of this larval insect.

Communication Between Sagebrush Individuals: Ecological Relevance and a Means of Overcoming Limited Vascular Architecture

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Airborne communication between sagebrush individuals can cause plants to become more resistant to damage by herbivores when a neighbor has been experimentally clipped. The ecological relevance of this result has been in question since individuals may be too far apart for this interaction to affect many plants in natural populations. Here we found that pairs of sagebrush plants that were up to 60 cm apart were influenced by experimental clipping to a neighbor. Furthermore we observed that most individuals had conspecific neighbors that were much closer than 60 cm. Air contact was essential for communication; treatments that reduced airflow between individuals, either because of wind direction or bagging, prevented induced resistance. Air flow was necessary for systemic induced resistance among branches within an individual as well as induced resistance resulting from communication between individuals. Reports from the literature and experiments with dyes that move through vascular connections both indicated that sagebrush is highly sectorial. Branches within a plant do not freely exchange material via vascular connections and probably cannot rely on an internal signaling pathway for coordinating induction of resistance to herbivores. This hypothesis provides a proximal explanation for why sagebrush does not demonstrate systemic induced resistance and why airborne communication induces resistance between branches.

The role of secondary chemical compounds of *Aristolochia* in the host shift of Troidini butterflies

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Butterflies of the tribe Troidini (Papilionidae, Papilioninae) feed almost exclusively in plants on the genus *Aristolochia* (Aristolochiaceae). These plants contain characteristically aristolochic acids (AAs), aristolactams, benzylisoquinoline alkaloids, and mono-, sesqui-, di- and triterpenes, including derived diterpene labdanoic acids (LAs). AAs are suggested to be responsible for the chemical defense of Troidini, although field bioassays showed that AAs do not deter predation. These compounds can also act as feeding stimulants, enhancing the growth of Troidini larvae, even though they are deleterious to non-AA specialists; both AAs and benzylisoquinoline alkaloids have a strong deterrent effect on non-*Aristolochia* feeding herbivores. Here, the role of AAs and of sesquiterpenes in the host shift of Troidini butterflies was analyzed along a phylogenetic hypothesis proposed to troidines of SE Brazil. The presence of AAs and LAs were optimized onto the phylogeny of Troidini, and a chemogram obtained with the sesquiterpene similarity of *Aristolochia* acquired by GC and GC-MS was compared with the phylogeny of the butterflies. Considering only the preferred host-plant associations between troidines and *Aristolochia*, the chemical similarity among the host-plants may has constrained the evolutionary host shift of Troidini butterflies. However, the present pattern of host-plant use by Troidini does not seem to be constrained by the secondary chemicals in these plants. In general, the butterflies studied here eat in species with or without AAs in their leaves, and even in species showing only LAs. In addition, the troidines accept equally well *Aristolochia* species showing distinct sesquiterpene patterns. Since AAs do not deter predation in field, they may not be constraining host shift in Troidini and other chemical compounds could be responsible by defense in these butterflies. The current host-plant use in Troidini seems to be simply opportunistic, with species with a wide geographical range using more species of host-plants than those with a more restrict distribution.

Effects of infection, salinity, and light on the production of phenolic compounds in *Thalassia testudinum*

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In the fall of 1987, several areas of Florida Bay were severely affected by the sudden die-off of the seagrass *Thalassia testudinum* Banks ex Konig (turtle grass). Although the cause is still unknown, several factors were suggested as influencing the on-set of the die-off event including increased salinty, light stress due to self-shading and disease. Blades of seagrass plants found in the die-off were infected by *Labyrinthula sp*, a pathogenic slime mold. A similar die-off occurred in another species of seagrass, *Zostera marina*, in the 1930's that was attributed to the pathogenic slime mold *Labyrinthula zosterae*. *Zostera marina* produces inhibitory phenolic acids in response to infection by *L. zosterae*, a response that is diminished in plants exposed to low light and high temperature. *Thalassia testudinum* produces phenolic acids similar to those produced by *Z. marina*. We examined the differences in phenolic content of healthy and infected *T. testudinum* leaf blades in laboratory cultures to determine if *T. testudinum* produces a chemical defense against pathogens similar to that of *Z. marina.* We found that levels of PHBA (parahydroxybenzoic acid), vanillic acid and caffeic acid as well as total phenolics were reduced in plants that had been exposed to infection for one week. This is contrary to the results found in *Zostera*, which showed an increase in caffeic acid in response to infection. This decrease may be evidence of a stress response to the presence of *Labyrinthula* as opposed to the expected phytoalexin response. We also examined the increased susceptibility of turtle grass to *Labyrinthula sp*. infection under high salinity and low light. The qualitative and quantitative effects on phenolic content of *T. testudinum* leaf blades in relation to the three various stresses will be discussed.

Sex pheromones of *Creontiades dilutus* (Stål) (Hemiptera: Miridae): identification and field tests

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The green mirid, *Creontiades dilutus*, is one of the important sucking pests of Australian field crops like cotton, lucerne, sunflower, safflower, and legumes such as soybeans, pigeon peas and mung beans. In cotton, green mirids are abundant during the early to mid-season causing localized leaf damage, terminal wilting of young plants, and shedding of squares and bolls. Green mirids in conventional cotton are usually controlled with insecticides used for *Helicoverpa* spp. With more farmers planting transgenic cotton varieties, however, green mirids could become more significant pests in cotton. Sex pheromones for monitoring, mating disruption or attract-and-kill might be useful in the management and control of these pests.

Identification of the sex pheromone components of *C. dilutus* was done by means of air collection and whole body extracts of both sexes. A two-component blend consisting of hexyl hexanoate and (E)-2 hexenyl hexanoate, in a 5:1 ratio was found to be attractive to green mirid males in field trapping experiments. The major component, hexyl hexanoate was present in the whole body extracts and air collections of both sexes, while the minor component, (E)-2-hexenyl hexanoate was detected in the female air collections only. No sex-specific compounds were found in the whole body extracts of males and females. Preliminary field trials in cotton have indicated that attract-and-kill and mating disruption using this two-component blend might also work for this species.

Nineteen types of plant odour receptor neurones characterized in female heliothine moths using GC-SCR and GC-MS

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Moths of the subfamily Heliothinae (Lepidoptera: Noctuidae) are used to compare mechanisms evolved for detecting and processing plant odour information among monophyletic species. In addition, the pest status of the polyphagous *Heliothis virescens* and *Helicoverpa armigera* makes it important to identify the odorants the females use to locate the host plants. These experiments started out trapping headspace samples of various host and non-host plants to stimulate the olfactory receptor neurones on the antennae. Essential oils and chemical standards were also included to increase the number of volatile compounds to be tested. Using electrophysiological recordings from single receptor neurones linked to gas chromatography (GC-SCR) and GC-MS, we have functionally identified olfactory receptor neurones and classified them into 19 types. All neurones were narrowly tuned, responding strongest to one compound (primary odorant) and weaker to a few others with related structures (secondary odorants). The neuron types were named according to the primary odorant, and the molecular receptive ranges were similar within each type. The co-location of the types was also constant among individuals within and across species. Five of the types were identified in all three heliothine species studied: *H. virescens*, *H. armigera* and the oligophagous *Helicoverpa assulta* (Røstelien *et al*. 2000a, b, Stranden *et al*. 2002, 2003a, b). Five others were found in *H. virescens* and *H. armigera*, seven only in *H. virescens*, and two only in *H. armigera* (Røstelien *et al*. 2005). The number of receptor neurone types identified in the three species (17 in *H. virescens*, 12 in *H. armigera*, and 5 in *H. assulta*) reflects the number of recordings made in each of them. The odorants, mainly being mono- and sesquiterpenes, a few aliphatic green leaf volatiles and aromatic compounds, were often present in minor amounts in the plant samples. The identified primary odorants are cadinene, (+)-3-carene, α-caryophyllene, caryophyllene oxide, *E*,*E*-α-farnesene, geraniol, (-)-germacrene D, 1-hexanol/(3*Z*)-hexen-1-ol/(3*Z*)-hexenyl acetate, (+)-linalool, methyl benzoate, *E*-β-ocimene, 2-phenylethanol, *trans*-pinocarveol, *E*-4,8,12-trimethyl-1,3,7,11-tridecatetraene, *trans*-verbenol and vinylbenzaldehyde. Three other primary odorants could not be identified. The receptor neurones tuned to six of the odorants (underlined) constituted 88% of the recorded neurones, and the (-)-germacrene D receptor neurones alone constituted 60%. The results suggest that these neurones may detect the most important odorants for the species. The behavioural effect has only been shown for (-)-germacrene D, which was attractive to mated *H. virescens* females when added to host plants not containing this odorant (Mozuraitis *et al*. 2002).
Anatomical studies of the antennal lobes of the three moth species have shown consistent numbers and positions of most glomeruli (Berg *et al*. 2002, Skiri *et al*. 2005). Interesting questions are i) whether the number of ordinary glomeruli (60-62) reflects the number of plant odour receptor neurone types, ii) whether the same odour quality is represented in corresponding glomeruli, and iii) whether the preferred host plants of the oligophagous *H. assulta* females are based on odour information mediated by the species specific glomerulus?

Berg *et al*. 2002 J Comp Neurol 446:123-134, Mozuraitis *et al*. 2002 Chem Senses 27:505-509, Røstelien *et al*. 2005 Chem Senses 30:443-461, 2000a Chem Senses 25:141-148, 2000b J Comp Physiol A 186:833-847, Skiri *et al*. J Comp Neurol 2005 in press, Stranden *et al*. 2002 Chem Senses 27:143-152, 2003a J Comp Physiol A 189:563-577, 2003b Chemoecology 13:143-154

Attraction of *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae) to pyrrolizidine alkaloids: a potential novel approach to its management

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The increasing importance of dry season populations of the African polyphagous grasshopper, *Z. variegatus* as a pest in agriculture and forestry has been attributed to the sequestration of pyrrolizidine alkaloids (PAs) from the flowers of the exotic noxious weed *Chromolaena odorata* for defense against natural enemies and protection of its diapausing eggs. This phenomenon is, however, seen as a novel opportunity to lure the insect with PAs for the development of PA-based attracticides for its management. This, notwithstanding, there is no empirical data on the efficacy of the available PA containing plants and the stage (s) of *Z. variegatus* worth targeting for the development of an integrated management strategy. Four categories of the growth stages of *Z. variegatus* were evaluated for the extent of migration to the roots of *C. odorata*, *Heliotropium indicum* and *Crotalaria juncea* which are plants containing PAs. Subsequently, fifth instar hoppers of *Z. variegatus* were evaluated for their attraction to the dry and fresh roots and flowers of *C. odorata* with a blank control in the laboratory. The results showed that 300g of the dry chopped roots of *C. odorata* hold promise for use as PA-lures for the development of PA-based attracticides. The 3rd to 6th instar larvae of *Z. variegatus* have the highest degree of attraction to PAs. Also, the roots of *C. odorata* stored for a year are effective lures for the grasshopper while the flowers lose their attractive principle after 24 hours. The results raise hope for the cost efficient and sustainable management of the grasshopper to salvage the agriculture and timber industries in Ghana and the sub region.

Neryl esters in click beetles: Sex pheromones of *Dalopius marginatus* (LINNÉ) and *Idolus picipennis* (BACH) (Coleoptera: Elateridae)

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Click beetles (Elateridae) are represented by approximately 170 species in Central Europe. Sex pheromones are only known for 6 species so far, all belonging to the agricultural important genus *Agriotes*. In all species, sex pheromones are produced by the females and are made up of one or two geranyl- and/or (*E*,*E*)-farnesyl esters of fatty acids with 2 to 8 carbon atoms. In the present study, we report the identification and synthesis of the female sex pheromones of two closely related species from different genera.

Dalopius marginatus, the only member of this genus in Central Europe, is widespread and very common and develops in forest soil. The larvae are predominantly carnivorous but occasionally cause damages on roots. In contrast, the sole central European *Idolus* species, *I. picipennis*, is a rare and threatened beetle occurring only in a few localities in the south of Central Europe.

Similar to the *Agriotes* spp., females of these click beetles show a pair of reservoir-like pheromone glands in their abdominal tip. Extracts of these glands were examined using GC-MS and GC-EAD. Surprisingly, though closely related to the genus *Agriotes*, both species show exclusively neryl esters instead of geranyl- and (*E*,*E*)-farnesyl esters as gland compounds. Main compounds (> 95 %) were neryl decanoate (*D. marginatus*) and neryl octanoate (*I. picipennis*).

In field bioassays, synthetic samples of both compounds proved to be highly attractive to flying males of the particular species. For *Dalopius marginatus*, the pheromone may be used for pest control, while in *Idolus picipennis* the pheromone might serve as a powerful monitoring tool for natural conservation purposes.

Evaluation of Allelopathic potential of *Yushania niitakayamensis*

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Yushania niitakayamensis (Hyata) Keng f. are found widespread on mountains at altitudes of 1800-3300m in Taiwan. A unique pattern of herb exclusion by a dominant species of *Y. niitakayamensis* is found throughout the alpine herbaceous zone. It was found that aqueous extract of fresh leaves but not fallen leaves exhibited a significant suppression in radicle growth of lettuce. Bioactivity-guided fractionation of methanolic extract of fresh leaves and rhizome led to the isolation of five allelochemicals, four known compounds and one novel triterpene. Their structure were elucidated by spectroscopic methods. Those phytotoxins identified from leaves included trans-*p*-coumaric acid, 4-hydroxy-benzoic acid methyl ester, ferulic acid, indole-3-carboxylic acid and indole. Those phytotoxins may contribute to the allelopathic dominant of *Y. niitakayamensis.*

Do parasitoids discriminate between blends of HIPV that differ qualitatively and/or quantitatively?

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To investigate whether qualitative and/or quantitative differences in blends of HIPV matter to the parasitoid *Cotesia plutellae*, we offered synthetic mixtures of the volatiles that are found in the headspace of cabbage plants infested by larvae of the diamondback moth (DBM) (*Plutella sylostella*). We identified four compounds in the headspace of cabbage plants slightly infested by DBM larvae as the attractants for the parasitoid (Kugimiya et al. in this meeting). We hypothesized that adding a compound into the synthetic blend or changing the amount of each compound in the blend affects the response of the parasitoid. To test this hypothesis, we used a choice chamber (ca. 30 X 30 X 30 cm) in which two intact cruciferous plants with qualitatively or quantitatively different synthetic blends were placed. We recorded the choice of the wasps that were released between the two plants. Naive wasps did not discriminate between blends of HIPV that differ qualitatively and/or quantitatively. By contrast, after positive experiences with the qualitatively or quantitatively modified blend, the wasp showed a significant preference to the modified blend. After the negative experience with the modified blend, the wasp showed a significant repellence to the blend. The information value of the modified blend to the parasitoid will be discussed.

Does behaviour replace male scent marking in some bumble bees? Evidence of the absence of sexual marking cephalic secretion in the subgenus *Rhodobombus*

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The specific combination of scent marking and patrolling flight is of outmost importance to bumble bees as it is the first and perhaps main barrier against hybridisation. In bumble bees, males attract conspecific females by marking places with a chemical signal secreted by their cephalic labial gland. The morphology and histology of the cephalic labial glands of *Bombus* (*Rhodobombus*) *mesomelas* Dalla Torre (Hymenoptera, Apidae, *Bombus* Latreille) has shown them to be much smaller than in other species and most likely non-functional. Whereas other species are thought to use their *barbae mandibularis* to spread their secretion onto the substrate, these structures are absent in *B. mesomelas*. The chemical composition of the cephalic gland secretion in this species does not include compounds normally present in the secretions of other species. The authors never observed any marking behaviour in *B. mesomelas*. These features seem to be common to all the species of the subgenus *Rhodobombus*. Some observations of male *Rhodobombus* entering nests, as well as the prenuptial behaviour of males in the related subgenus *Fervidobombus*, suggest that the regression of marking behaviour may correlate with in-the-nest copulation.

Changes in the composition of the labial gland secretion during the life of *B. terrestris* males.

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B. terrestris is a bumblebee species whose males exhibit patrolling behavior during their mating phase of life. While patrolling, males laid scent marks on elevated spots on their flying routes to attract conspecific females. The scent is produced by the cephalic part of the male labial gland. Little is known about temporal changes of scent production during male life span. Therefore, labial glands of males of different age (from new born up to 35 days old) were dissected, extracted with hexane and analyzed. Simultaneously, glands of different age males were studied using transmission electron microscopy, EAG and GC-EAD.

Chemical analyses revealed age-dependent changes in the secretion. Very young males (0-1 day) posses only traces of compounds in the gland extracts. During 2-7 consecutive days, amounts of compounds increase rapidly and decrease slowly during the following 30 days. Terpenic alcohols (2,3-dihydrofarnesol and geranylcitronellol) appear first in the extract followed by aliphatic compounds such as ethyl esters and alcohols. Glands of old males (35 days) contained hydrocarbons and fatty acid esters of terpenic alcohols such as 2,3-dihydrofarnesyl dodecanoate.

EAG experiments were performed on antennae of virgin queens. The EAG recording showed a peak of maximum activity at 4 days followed by a slow decline. GC-EAD analysis revealed at least 9 EAD active components of the labial gland extract.

Transmission electron microscopy of the cephalic part of the labial gland showed that both changes in physiological and chemical activity closely match with histological changes within glandular structures of labial glands. The gland consists of numerous acini and the ducts connecting particular acini. Each acinus is formed by several secretory cells and a large lumen, in which the secretion is stored. As the amount of secretion increases, the volume of secretory cells decreases, they become flatter and organelles start to disintegrate. The first apoptic cells were observed in five days old and the last living cells in ten days old males. After ten days of male bumblebee life, no cells remain alive and even the volume of cell remnants gradually decreases.

Data as a whole show that pheromone biosynthesis starts immediately after eclosion and reach its maximum about 4th posteclosion day. After that the content of labial gland gradually decreases presumably due to the male marking activity.

Queen adoption in the Argentine ant: are cuticular hydrocarbon and genetic factors involved?

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The Argentine ant, *Linepithema humile*, is a highly polygynous, unicolonial widespread invasive species. Loss of intraspecific aggression in introduced populations is related to Argentine ant success as an invader and worker aggression is inversely related to genetic and cuticular hydrocarbon similarity. Reduced aggressive behavior towards queens may also play a role in shaping Argentine ant social structure as acceptance of new queens may affect colony survivorship and productivity. To better understand nestmate recognition in the Argentine ant, we compared acceptance of nestmate and non-nestmate queens in queenless and queenright colonies of this ant. Non-nestmate queen acceptance was low in queenright colonies while queenless colonies accepted more non-nestmate queens. Offspring production of non-nestmate and nestmate queens after adoption was comparable. Queen cuticular hydrocarbons were more similar between colony pairs that showed higher adoption rates, while genetic analyses revealed differentiation among colonies but no significant association with acceptance of non-nestmate queens.

Investigation of bacteria associated with the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae)

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The brown marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae) is a newly invasive species in the United States whose geographical region is expanding. *H. halys* is likely to become a significant agricultural pest in North America because it feeds on many economically valuable fruits and crops, such as apples, pears, peaches, cherry, citrus fruits, figs, green beans, and soybeans. In this study we identified two culturable bacteria in the genus of *Klebsiella* and *Serratia* in the gut of the bug. The bacteria were identified by PCR amplification of 16S rDNA, and matching the sequences obtained against a database of all known 16S rDNA sequences (GenBank); the bacterial isolates from *H. halys* produced DNA sequences that were 99% identical to sequences for the bacteria *Serretia marcescens* and *Klebsiella pneumonia*. Although *H. halys* contain well-defined gastric caeca located in four rows along the midgut, no bacteria from these caeca could be cultured in standard artificial laboratory media. Sterilization of newly deposited eggs delayed adult development, and these adults produced no progeny. This implies that the symbionts provide chemicals essential for host development and reproduction, but at this point it is not clear whether sterilization of eggs eliminated caecal or gut bacteria or both. Symbiotic bacteria may be a target for controlling this and other heteropteran pests through genetic modification of the bacteria, a process termed paratransgenesis.

Behavioral and electrophysiological response of sorghum chafer, *Pachnoda interrupta*, to natural and synthetic plant odors

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Among a wide range of insect pests on sorghum, *Sorghum bicolor (L)*, sorghum chafer, *Pachnoda interrupta* (Oliver) (Coleoptera: Scarabeidae) is a key pest in Ethiopia. The adult beetles feed on the flowers and on the grains and can cause 70% crop loss. The overall aim of this study is to develop a kairomone-based monitoring and mass trapping system for this species.

In an initial field trapping experiment, we have tested the attractiveness to sorghum chafer of five synthetic compounds: methyl salicylate, butyl butyrate, isoamyl acetate, eugenol and methyl anthranilate. The sorghum chafer is a highly polyphagous species and the selected compounds, known as attractants for other scarabs, are components of several floral and fruit odors. Field experiments were made in two seasons: In September, when the adult beetles emerge and feed on the flowering/mature sorghum before aestivating during the dry season, and in July, when the adults reappear after aestivation to mate and oviposit. In all experiments, unbaited traps were used as negative control and traps baited with mashed banana as positive control. In July, when no conspicuous food resource is available, we caught significantly more beetles than in September when the traps were placed inside or close to sorghum fields. Also, traps baited with individual synthetic compounds, notably methylsalicylate, caught significantly more beetles in July than in September compared to the banana-baited traps. This difference in trap catches may be attributed to competition with the background odor from the sorghum crop. Head-space extracts of sorghum were analyzed using GC-EAD and GC/MS. Among the GC-EAD active compounds, methylsalicylate elicited strong responses in both male and female antennae. Additional EAD active components have been identified and synthetic blends will be tested in the coming field season.

Volicitin and lipid metabolism in *Spodoptera litura* larvae

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Volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine], an elicitor of plant volatile emission that work as chemical cues for parasitic wasps to locate their hosts, was first identified in the larval regurgitants of *Spodoptera exigua*. In this study, we examined the pathway for the biosynthesis of volicitin-related compounds in larval *Spodoptera litura*.
Fifth instar of *S. litura* larvae were fed with ^{14}C-labelled glutamine or linolenic acid. The ^{14}C tracer method was used to measure the incorporation of the added isotope into volicitin-related compounds, and lipid intermediates. In the case of glutamine, most of the ^{14}C-labelled glutamine was absorbed into hemolymph and the fat body within 1 to 2 hours after feeding, while negligible incorporation into volicitin was observed during the first 6 hours. On the other hand, ^{14}C-linolenic acid was slowly absorbed through the midgut tissues, where volicitin-related compounds were actively synthesized.[1)] Within 6 hours, 65% of the labelled linolenic acid was stored in the fat body mainly as triacylglycerol and phospholipids, and over half of the rest (35%) were detected as volicitin-related compounds in the gut lumen. Volicitin and any other fatty acid amides (FAAs) were not detected in hemolymph and the fat body. These results suggested that FAAs may play a role(s) in the glutamine transportation from hemolymph into the gut lumen.

1) Yoshinaga, N., Morigaki, N., Matsuda, F., Nishida, R., Mori, N., Insect Biochemistry and Molecular Biology 35 (2005) 175-184.

Effects and mechanisms of plant signalling compounds and allelochemicals on the toxicity of aflatoxin B1 to corn earworm (*Helicoverpa zea*)

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Co-occurrence of corn earworm (*Helicoverpa zea*) damage and aflatoxin accumulation has been reported in several crops. Aflatoxins are carcinogenic mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* infecting certain grains including corn. We studied the effects of plant signalling compounds and allelochemicals on the toxicity of aflatoxin B1 to *H. zea.* Aflatoxin B1 toxicity is increased by methyl jasmonate, salicylic acid, gibberellic acid and gossypol but reduced by coumarin, chlorogenic acid and indole-3-carbinol. P450 inhibitor piperonyl butoxide reduces the toxicity of aflatoxin B_1, suggesting that aflatoxin B1 is bioactivated by P450(s). The effects on the toxicity of aflatoxin B_1 to *H. zea* by plant signalling compounds and allelochemicals are probably due to P450 induction or inhibition.

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