# INDEX


# INTERNATIONAL SOCIETY OF CHEMICAL ECOLOGY

## 22th ANNUAL MEETING

Faculty of Biology
University of Barcelona
Barcelona, Spain
15-19 July 2006

| | |
|---|---|
| Meeting Host | Angel Guerrero |
| Organizing Committee | Angel Guerrero (IIQAB-CSIC, Barcelona)<br>Gloria Rosell (Univ. Barcelona)<br>Mª Pilar Bosch (IIQAB-CSIC, Barcelona)<br>Carmen Quero (Scientific Secretary) (IIQAB-CSIC, Barcelona) |
| Scientific Committee | Walter Soares Leal (Univ. California Davis, USA)<br>Thomas C. Baker (Penn State Univ., USA)<br>Wendell Roelofs (Cornell Univ., USA)<br>Ring T. Cardé (Univ. California Riverside, USA<br>Thomas Hartmann (Tech. Univ. Braunschweig, Germany)<br>Angel Guerrero (IIQAB-CSIC, Spain)<br>Gloria Rosell (Univ. Barcelona, Spain)<br>Mª Pilar Bosch (IIQAB-CSIC, Spain)<br>Carmen Quero (IIQAB-CSIC, Spain) |
| Local Team | Lourdes Muñoz, Esmeralda Rosa, Josep Rayó, Patricia Acin, Gerard Carot, Benjamin Fürstenau |

## ACKNOWLEDGEMENTS

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C.S.I.C.

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# Program 22nd Annual Meeting ISCE

## (Faculty of Biology, University of Barcelona)
## (15-19 July 2006)

## Saturday, July 15

| | |
|---|---|
| 15.00-18.00 h | **ISCE Executive and Councilor's Meeting** |
| 17.00-19.00 h | **Registration** |
| 19.00-20.30 h | **Welcome party** |

## Sunday, July 16

| | |
|---|---|
| 8.00-8.30 h | **Registration** |
| 8.30-8.40 h | **Opening ceremony** (Aula Magna) |

**Plenary Lecture (ISCE Silver Medal Award)**

**Chair: W.S. Leal**

| | | |
|---|---|---|
| 8.40-9.30 h<br>Aula Magna | **J. Hildebrand**<br>Chemical ecology by way of the brain: Explorations of moth-host interactions | SL1 |

## Symposium 1. Biochemistry and Molecular Biology of Olfactory reception

**Invited Contributions** (Aula Magna)

**Chair: W.S. Leal**

| | | |
|---|---|---|
| 9.30-10.00 h | **R.G. Vogt**<br>Peripheral networks: The biochemistry of odor presentation | S1-IC1 |
| 10.00-10.30 h | **W.S. Leal**<br>Bug's life in the fast lane: Rapid binding, transport, release and inactivation of semiochemicals | S1-IC2 |
| 10.30-10.50 h | Coffee break | |
| 10.50-11.20 h | **R.D. Newcomb**<br>The molecular basis of pheromone and odorant reception in the tortricid moth, *Epiphyas postvittana* | S1-IC3 |
| 11.20-11.50 h | **K. Touhara**<br>Molecular mechanism underlying insect sex-pheromone reception | S1-IC4 |

| | | |
|---|---|---|
| 11.50-12.20 h | **H. Breer**<br>Receptors and binding proteins for moths' pheromones | S1-IC5 |

**Short Oral Presentations** (Aula Magna)

**Chair: R. Vogt**

| | | |
|---|---|---|
| 12.20-12.35 h | **E. Plettner**<br>Non-linear effects and the function(s) of odorant-binding proteins | S1-O1 |
| 12.35-12.50 h | **E. Jacquin-Joly**<br>Identification of olfactory genes in a crop pest, the cotton leafworm *Spodoptera littoralis*, by an expressed sequence tag approach | S1-O2 |
| 12.50-13.05 h | **M. Maïbèche-Coisné**<br>Odorant-degrading enzymes of moths | S1-O3 |
| 13.05-13.20 h | **P. Acín**<br>In the search of differential antennal proteins in males and females of *Sesamia nonagrioides* using proteomic techniques | S1-O4 |

## Symposium 5. Plant-herbivore interactions

**Short Oral Presentations** (Aula 3)

**Chair: M. Hilker**

| | | |
|---|---|---|
| 12.20-12.35 h | **A. Ortiz**<br>EAG and behavioural responses of *Capnodis tenebrionis* L. to host-plant volatiles | S5-O1 |
| 12.35-12.50 h | **E. Conti**<br>Role of the host plant, *Vicia faba*, and induced volatiles in host location and sexual communication of *Lygus rugulipennis* | S5-O2 |
| 12.50-13.05 h | **V. Soroker**<br>Host-plant selection by the broad mite, *Polyphagotarsonemus latus* and how it is affected by plant defenses | S5-O3 |
| 13.05-13.20 h | **J.L. Boevé**<br>The microhabitat hypothesis to explain differing chemically based defence strategies in herbivorous Hymenoptera | S5-O4 |
| 13.20 - 15.05 h | Lunch | |
| 15.05-16.05 h | **Poster Session (Symposia 1 and 5)**. Coffee | |

**Special lecture** (Aula Magna)

**Chair: W. Francke**

| | | |
|---|---|---|
| 16.05-16.35h | **J. Meinwald**<br>Exploring the chemistry of biotic interactions | SL3 |

**Short Oral Presentations** (Aula Magna)

**Chair J. Bohlmann**

| | | |
|---|---|---|
| 16.35-16.50 h | **M. Hilker**<br>Early herbivore alert: Insect eggs induce plant defence | S5-O5 |
| 16.50-17.05 h | **M. Heil**<br>Volatile-mediated priming of an indirect defence in nature | S5-O6 |
| 17.05-17.20 h | **R.C. Schuurink**<br>Intraspecific variation in a herbivorous mite accounts for differential defense of tomato plants | S5-O7 |
| 17.20-17.35 h | **A. Mithöfer**<br>Discriminating bites from wounds: A molecular approach | S5-O8 |
| 17.35-17.50 h | **R. Büchler**<br>Enzymes involved in the homeostasis of N-acyl amino acids in the gut of herbivorous insects | S5-O9 |
| 17.50-18.05 h | **F. Francis**<br>Investigation of plant-aphid interactions using a proteomic approach | S5-O10 |
| 18.05-18.20 h | **T. Winter**<br>Tritrophic interactions in soybean: effects of ambient UV radiation | S5-O11 |
| 18.20-18.35 h | **D. Spiteller**<br>Fluoride ions as elicitors of plant defence reactions | S5-O12 |

**Short Oral Presentations** (Aula 3)

**Chair: B. Moeller**

| | | |
|---|---|---|
| 16.35-16.50 h | **V.C. Clark**<br>The ecology and evolution of chemical defense in poison frogs | S5-O13 |
| 16.50-17.05 h | **S. Dobler**<br>Coping with cardenolides in host plants: How many tricks are there to evolve tolerance or resistance? | S5-O14 |
| 17.05-17.20 h | **S. Discher**<br>Throwing light on sequestration processes in leaf beetle larvae | S5-O15 |
| 17.20-17.35 h | **J.A. Timbilla**<br>A grasshopper and its beneficial drug source: the African story of *Zonocerus variegatus* and the neophyte *Chromolaena odorata* | S5-O16 |
| 17.35-17.50 h | **F. Marion-Poll**<br>Phytoecdysteroids taste bitter | S5-O17 |
| 17.50-18.05 h | **B. Schlumpberger**<br>Variation in composition and attractiveness of floral scent in a highly polymorphic *Andean cactus, Echinopsis ancistrophora* | S5-O18 |
| 18.05-18.20 h | **H.E.M. Dobson**<br>Pollen odors as cues to pollen-foraging bumble bees | S5-O19 |
| 18.20-18.35 h | **J.X. Becerra**<br>The impact of insect-plant coevolution on plant community structure | S5-O20 |

## Monday, July 17

## Symposium 2. Semiochemical practical approaches for pest control

**Invited Contributions** (Aula Magna)

**Chair: T.C. Baker**

| | | |
|---|---|---|
| 8.30 - 9.00 h | **C. Oehlschlager**<br>Development of a monitoring and mass trapping system for coffee berry borer | S2-IC1 |
| 9.00 - 9.30 h | **T.C. Baker**<br>Pros and cons of using high release-rate, low density dispensers for mating disruption | S2-IC2 |
| 9:30 - 10.00 h | **P.J. Landolt**<br>Applications of non-pheromone attractants for management of insect pests | S2-IC3 |
| 10.00 - 10.20 h | Coffee Break | |
| 10.20 - 10.50 h | **C. Löfstedt**<br>Mating disruption of pyralid moths in commercial facilities | S2-IC4 |
| 10.50 - 11:20 h | **J. Miller** and **L. Gut**<br>Competitive attraction as a primary mechanism of moth mating disruption in tree fruit crops | S2-IC5 |

**Short Oral Presentations** (Aula Magna)

**Chair: D. Hall**

| | | |
|---|---|---|
| 11.20-11.35 h | **A. Khrimian**<br>Field responses of invasive brown marmorated stink bug, *Halyomorpha halys*, to geometric isomers of methyl 2,4,6-decatrienoate | S2-O1 |
| 11.35-11.50 h | **A. Svatoš**<br>Probing hydrocarbon mixtures in insects by MALDI-TOF-MS | S2-O2 |
| 11.50-12.05 h | **A. Zhang**<br>Chemical identification of female sex pheromone of pink hibiscus mealybug and chirality recognition among stereoisomers by males | S2-O3 |

**Short Oral Presentations** (Aula 3)

**Chair: J. McNeil**

| | | |
|---|---|---|
| 11.20-11.35 h | **J. Zhu**<br>Semiochemical-based monitoring and control on soybean aphids | S2-O8 |
| 11.35-11.50 h | **S.Y. Dewhirst**<br>The rosy apple aphid sex pheromone: A new component | S2-O9 |

| | | |
|---|---|---|
| 11.50-12.05 h | **P.H.G. Zarbin**<br>Identification and field evaluation of the aggregation pheromone of the Brazilian papaw weevil *Pseudopiazurus obesus* | S2-O10 |
| 12.05 - 13.05 h | **ISCE General Assembly** | |
| 13.05 - 15.00 h | Lunch | |
| 15.00 - 15.15 h | Coffee | |

**Short Oral Presentations** (Aula Magna)

**Chair: J. Meinwald**

| | | |
|---|---|---|
| 15.15-15.30 h | **J.N. McNeil**<br>The effect of wind speed and atmospheric pressure on the response of *Aphidius ervi* males response to pheromones | S2-O4 |
| 15.30-15.45 h | **M. Tóth**<br>Species spectrum of noctuids attracted to female-targeted synthetic attractants in Europe | S2-O5 |
| 15.45-16.00 h | **D.M. Suckling**<br>Mass trapping of apple leaf curling midge | S2-O6 |
| 16.00-16.15 h | **J.G. Logan**<br>The search for natural repellents from human beings against biting insects | S2-O7 |

**Short Oral Presentations** (Aula 3)

**Chair: J. Aldrich**

| | | |
|---|---|---|
| 15.15-15.30 h | **M.G. Lorenzo**<br>Chemical characterization of the aggregation pheromone from faeces in three vectors of Chagas disease | S2-O11 |
| 15.30-15.45 h | **S. Colazza**<br>Chemical analysis of residues left by walking adults *of Nezara viridula* which induce arrestment behavior in the egg parasitoid *Trissolcus basalis* | S2-O12 |
| 15.45-16.00 h | **B.A. Kimball**<br>Minimizing deer browse damage: Practical application of a protein repellent | S2-O13 |
| 16.00-16.15 h | **H.Y. Fadamiro**<br>Pheromone-based attract-and-kill tactic for managing lepidopteran pests of crucifer crops: Is disruption of sexual communication a predictor of efficacy? | S2-O14 |
| 16.30 - 20.30 h | **Excursion. City Tour** | |

# Tuesday, July 18

## Symposium 3. Biosynthesis and pheromone production

**Invited Contributions** (Aula Magna)

**Chair: W.L. Roelofs**

| | | |
|---|---|---|
| 8.30 - 9.00 h | **A. Rafaeli**<br>Regulation of pheromone production in moths | S3-IC1 |
| 9.00 - 9.30 h | **A.P. Rooney**<br>Evolutionary genomics of moth sex pheromone desaturases | S3-IC5 |
| 9.30 - 10.00 h | **C. Löfstedt**<br>Recruitment and loss of pheromone components in the evolution of Lepidoptera | S3-IC2 |
| 10.00 - 10.20 h | Coffee Break | |
| 10.20 - 10.50 h | **C. Tittiger**<br>Pheromone biosynthesis and regulation in bark beetles | S3-IC3 |
| 10.50 - 11.20 h | **G. Fabriàs**<br>Fatty acid desaturases in moth pheromone biosynthesis | S3-IC4 |

**Student Awards presentations** (Aula Magna)

**Chair: J. Millar**

| | | |
|---|---|---|
| 11.20-11.35 h | **C. Merlin**<br>Identification and functional characterization of a circadian clock in moth antennae | S1-O5 |
| 11.35-11.50 h | **B.G. Ambrogi**<br>Behavioural evidence for a male-produced aggregation pheromone in *Sternechus subsignatus* Boheman (Coleoptera: Curculionidae) | S2-O15 |
| 11.50-12.05 h | **S.M. Butler**<br>Is Z-9-tricosene (muscalure) required for house fly mating? | S2-O16 |
| 12.05-12.20 h | **S. Geiselhardt**<br>Allomones as sex pheromones in *Parastizopus* (Coleoptera: Tenebrionidae) | S2-O17 |

**Short Oral Presentations** (Aula Magna)

**Chair: J. Millar**

| | | |
|---|---|---|
| 12.20-12.35 h | **J.A. Millar**<br>Aggressive chemical mimicry of a host's sex pheromone by a phoretic nest parasite | S3-O1 |
| 12.35-12.50 h | **J.R. Aldrich**<br>Methyl 2,4,6-decatrienoates attract stink bugs (Hemiptera: Heteroptera: Pentatomidae) and tachinid parasitoids | S3-O2 |

**Short Oral Presentations** (Aula 3)

**Chair: G. Blomquist**

| | | |
|---|---|---|
| 11.20-11.35 h | **J.A. Byers**<br>Production and predator-induced release of defensive chemicals by plant bug *Lygus hesperus* | S3-O7 |
| 11.35-11.50 h | **J. Ruther**<br>Semiochemicals involved in the sexual communication of the parasitoid *Nasonia vitripennis* | S3-O8 |
| 11.50-12.05 h | **M.L. Lewis**<br>Tracking down aphid alarm pheromone synthase: A botanical approach | S3-O9 |
| 12.05-12.20 h | **S. Matsuyama**<br>Biosynthesis of mandibular gland pheromones in social hymenopterans | S3-O10 |
| 12.20-12.35 h | **M.D. Ginzel**<br>Endocrine regulation of pheromone production in the *pinyon Ips* (Coleoptera: Scolytidae) | S3-O11 |
| 12.35-12.50 h | **M. Strandh**<br>Transcriptional analysis of the pheromone gland of the turnip moth, *Agrotis segetum* (Noctuidae) | S3-O12 |

12.50 - 14.45 h Lunch

14.45 - 15.30 h **Poster session (Symposia 2 and 3)**. Coffee

## Symposium 4. Causes and consequences of variation in Lepidoptera sex pheromones

**Invited Contributions** (Aula Magna)

**Chair: R.T. Cardé**

| | | |
|---|---|---|
| 15.30 - 16.00 h | **J.D. Allison**<br>Patterns of variation in lepidopteran sex pheromones | S4-IC1 |
| 16.00 - 16.30 h | **K.F. Haynes**<br>Genetic mechanisms of variation in lepidopteran sex pheromones | S4-IC2 |
| 16.30 - 17.00 h | **R.T. Cardé**<br>Evolutionary causes and consequences of variation in lepidopteran sex pheromones | S4-IC3 |

**Symposium 3**

**Short Oral Presentations** (Aula Magna)

**Chair: A. Rafaeli**

| | | |
|---|---|---|
| 17.00-17.15 h | **G.J. Blomquist**<br>Regulation of pheromone production in the pine engraver beetle, *Ips pini*: Effect of antennectomy on pheromone production | S3-O3 |
| 17.15-17.30 h | **D.R. Hall**<br>Chemistry of the African coffee stemborer, *Monochamus leuconotus*: But where's the ecology? | S3-O4 |
| 17.30-17.45 h | **S. Schulz**<br>Chemical composition and putative function of lipids occurring on the silk or cuticle of spiders | S3-O5 |
| 17.45-18.00 h | **M.P. Juarez**<br>Triatomine bugs: Cuticular lipids as intraspecific chemical cues | S3-O6 |

**Symposium 4**

**Short Oral Presentations** (Aula 3)

**Chair: K. Haynes**

| | | |
|---|---|---|
| 17.00-17.15 h | **T. Ando**<br>Synthesis and characterization of 3,13- and 2,13-octadecadienyl compounds for identification of the sex pheromone secreted by clearwing moths | S4-O1 |
| 17.15-17.30 h | **L. Pélozuelo**<br>A genetic factor unrelated to female sex pheromone influences the assortative mating of the pheromone races of the European corn borer *Ostrinia nubilalis* | S4-O2 |
| 17.30-17.45 h | **R.D. Girling**<br>Analysis and manipulation of odor-plume structure from a piezo-electric pheromone release system and its effects on upwind flight of male moths to pheromone | S4-O3 |
| 17.45-18.00 h | **J.M. Lassance**<br>Male odour drives female choice in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae) | S4-O4 |
| 20.30 h | **Concert at the Church of Santa Maria del Mar** | |
| 21.30 h | **Banquet**<br>**Social Lecture**<br>**Student Awards**<br>**Presentation of the 23rd ISCE Meeting by W. Boland** | |

## Wednesday, July 19

## Symposium 5. Plant-herbivore interactions

**Invited Contributions** (Aula Magna)

**Chair: Th. Hartmann**

| | | |
|---|---|---|
| 8.30 - 9.00 h | **B. Lindberg Møller**<br>Cyanogenic glucosides in plants and insects and their role in plant-insect interactions | S5-IC1 |
| 9.00 - 9.30 h | **J. Bohlmann**<br>Terpenoid synthases and cytochrome P450 enzymes in the interacting genomes of conifers, bark beetles and fungal pathogens | S5-IC2 |
| 9.30 - 10.00 h | **W. Boland**<br>Sequestration of plant-derived glycosides by leaf beetles: A model system for evolution and adaptation | S5-IC3 |
| 10.00 - 10.20 h | Coffee Break | |
| 10.20 - 10.50 h | **U. Wittstock**<br>Special weapons - exceptional countermeasures: Glucosinolate hydrolysis in plants and their insect herbivores | S5-IC4 |
| 10.50 - 11.20 h | **D. Ober**<br>Plant defenses utilized by insects: Functional and evolutionary aspects of the pyrrolizidine alkaloids | S5-IC5 |

**Plenary Lecture (ISCE Silver Medal Award)**

**Chair: C. Schal**

| | | |
|---|---|---|
| 11.20 - 12.10 h | **C. Oehlschlager**<br>Management of insect pests in tropical crops by mass trapping | SL2 |

**Short Oral Presentations** (Aula Magna)

**Chair: W. Boland**

| | | |
|---|---|---|
| 12.10-12.25 h | **C. Müller**<br>Impact of UV-radiation on a beetle's preference for white mustard | S5-O21 |
| 12.25-12.40 h | **H. van Leur**<br>A heritable glucosinolate polymorphism within natural populations of *Barbarea vulgaris* | S5-O22 |
| 12.40-12.55 h | **C.I. Keeling**<br>Mechanistic approach to understanding the chemical diversity of conifer terpenoids | S5-O23 |
| 12.55-13.10 h | **E. Mateus**<br>Analysis of the chemical composition of *Pinus spp* needles using comprehensive two-dimensional techniques: GCxGC and GCxMS. | S5-O24 |
| 13.10 h | **Closing Ceremony** | |

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# POSTER PRESENTATIONS

## Symposium 1. Biochemistry and molecular biology of olfactory reception



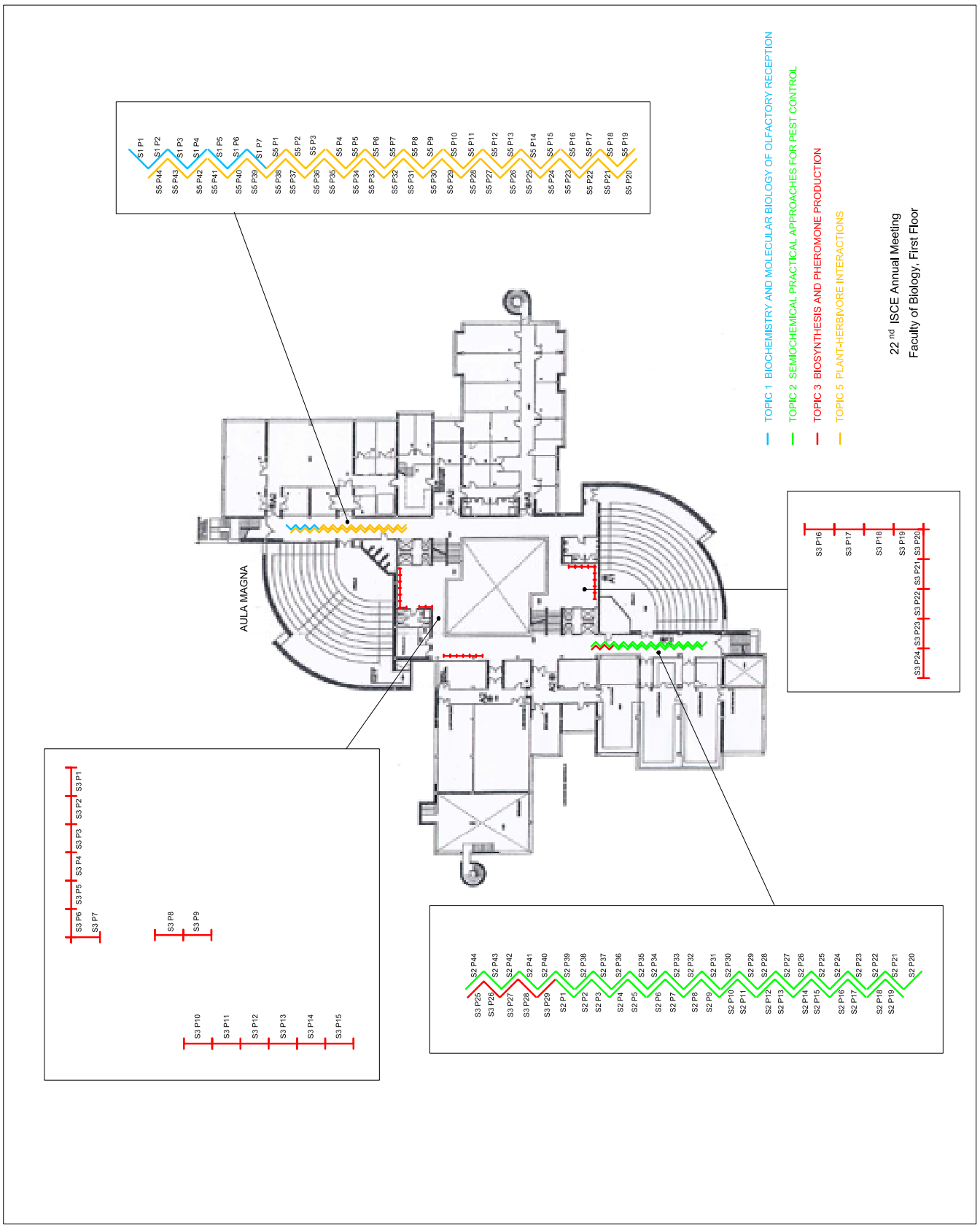
## Symposium 2. Semiochemical practical approaches for pest control

## Symposium 3. Biosynthesis and pheromone production

## Symposium 5. Plant-herbivore interactions

# POSTER LOCATION MAP

# POSTER LOCATION MAP



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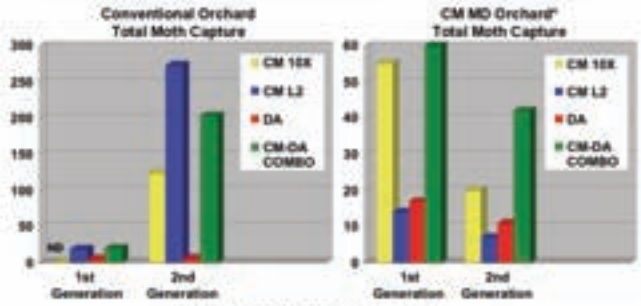
- Highest 2nd Generation Capture Rate
- Precise CM BioFix & Emergence Profile
- Long Lasting: More Than 2 Months
- Use Like CM Pheromone
- Better Than CM Pheromone Alone
- Specific for CM

## BENEFITS

- Highest Capture Rate
- Full Season Monitoring/Consistent Results
- Precision Timing/More Knowledge
- Labor Savings
- Easy to Use
- Cost Effective
- Easy to ID/Count

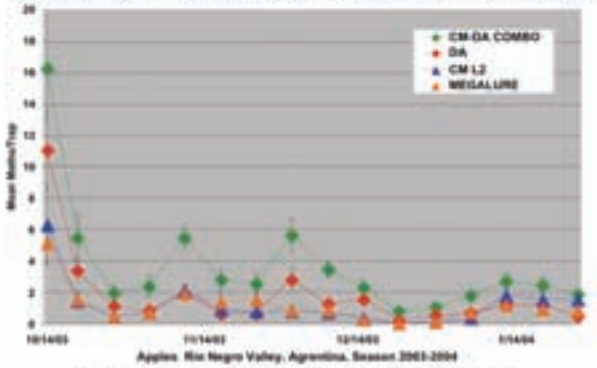
**Comparison of PHEROCON® DA & Pheromone Based Lures for Monitoring CM**




Washington State, Apples, 2003 Season

*400 dispensers per acre
ND=No Data

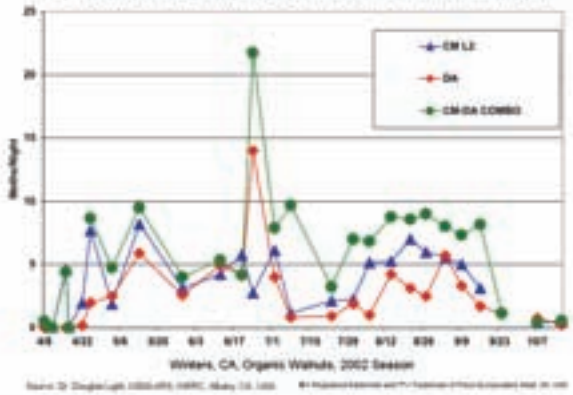
**Comparison of PHEROCON® DA and Pheromone Based Lures for Monitoring CM in Orchards Treated with Four Commercial Codling Moth Mating Disruption Dispensers.**




Apples, Rio Negro Valley, Agrentina, Season 2003-2004

96 Rope, Within # RAK, # ISOMATE, # CHECKMATE and # NOMATE Treated Orchards.

**Comparison of DA2313 and/or CM Pheromone for Monitoring CM**




Winters, CA, Organic Walnuts, 2002 Season

Please Note: PHEROCON® CM-DA COMBO captures both females and males. But it is not necessary to identify the sex of insects captured to follow the exceptional capture levels. Simply count the PHEROCON® CM-DA COMBO trap capture similar to the approach used with PHEROCON® CM pheromone alone.

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# ABSTRACTS

# PLENARY LECTURES

## Silver Medal Lecture

# Chemical ecology by way of the brain: Explorations of moth-host interactions

**J.G. Hildebrand, T.A. Christensen, P.G. Guerenstein, C. Reisenman and J.A. Riffell**

Arizona Research Laboratories Division of Neurobiology, University of Arizona, POB 210077, Tucson AZ 85721-0077, USA
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The olfactory system serves decisive roles controlling specific behaviors of insects, including our research subject -- the giant sphinx moth *Manduca sexta*. A principal goal of our work is to understand neurobiological mechanisms through which information about specific, behaviorally significant olfactory stimuli is encoded, processed, and integrated with inputs of other modalities in the brain and how odor information ultimately initiates and controls the moth's characteristic, natural behavioral responses. Our work is intended to help advance fundamental knowledge about olfaction and at the same time to contribute to understanding of insect biology in the quest for improved methods for controlling pests and fostering beneficial insects.

The studies to be emphasized in this presentation have focused mainly on the antennal lobes (ALs), the paired, primary olfactory centers in the insect's brain. Primary olfactory centers in most vertebrates and invertebrates are characterized by glomeruli – numerous condensed-neuropil structures in which primary-sensory and central neural elements interact synaptically – but the functional significance of glomeruli has been uncertain until recently. In male *Manduca*, certain sexually dimorphic glomeruli, constituting the male-specific macroglomerular complex (MGC) in the AL, are responsible for processing sensory information about individual components of the female's sex pheromone. In addition, several other identified, sexually dimorphic and isomorphic glomeruli are by now well known. We have characterized many individual neurons, and especially uniglomerular projection neurons (PNs), associated with MGC glomeruli in the male AL and other characterized glomeruli in males and females. This lecture will offer an overview of our studies of glomeruli and their interactions, based on single- and multi-unit neurophysiological analysis of the processing of olfactory information in the AL, and will illustrate key conclusions: (1) the AL is remarkably similar to its vertebrate counterpart, the olfactory bulb, and can teach us much about primary neural processing of sensory information about odors; (2) olfactory glomeruli are organized chemotopically and are sites of odor processing using multiple coding mechanisms; and (3) neurons in different glomeruli interact through intra- and inter-glomerular synaptic connections that shape responses and underlie odor-information processing.

We focus on odor-dependent behaviors that are crucial for the survival of the moths: mate-seeking and interactions with hostplants for feeding and oviposition. In some of those studies, olfactory neurobiology has led us to recognize naturally occurring volatiles that may, or clearly do, function in the chemical mediation of such interactions. This lecture will feature examples of such "reverse chemical ecology," or "chemical ecology by way of the brain," and particularly our recent work on floral volatiles and feeding (and pollination) behavior of moths.

## Silver Medal Lecture

# Management of insect pests in tropical crops by mass trapping

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Insecticide-based management insect pests of tropical crops requires multiple applications often on a year round schedule. Attendant problems of environmental contamination, worker health and resistance management are principal forces behind the search for alternative methods of pest control in these high pressure environments. In the early 1990's we and others began a search for pheromones of several of the major coleopteran pests of palms, sugarcane and banana. A few years of intensive identification effort yielded sex and aggregation pheromones as well as some kairomones for the primary target insects. A few additional years of equally intensive effort on the part of numerous private sector and government personnel yielded robust insect pest management techniques utilizing the discovered semiochemicals. This presentation will trace development of mass trapping systems for several important Coleopteran pests of tropical crops.

*Rhynchophorus* palm weevils are world-wide pests of oil, coconut and date palm. Males produce aggregation pheromones that strongly attract both sexes only when presented with food. In Neotropical America *R. palmarum* causes direct larval damage and vectors red-ring nematode. The author's commercial group and collaborators developed a management system of trapping and removal of red-ring nematode infested palms that is the only economically viable technique in the Americas to combat this weevil and its associated nematode. Use of 1 trap / 5 ha reliably lowers weevil associated damage by 80% over a year.

In the Middle East and Southern Europe *R. ferrugineus* (red palm weevil) the most important Coleopteran pest of date palm. The aggregation pheromone for this pest was discovered in the author's former laboratory in Canada. Collaboration of the authors commercial group with governments in the Middle East yielded a trapping system that significantly lowers damage due to this pest at 1 trap / ha.

*Oryctes rhinoceros* is a pest of oil palm in Southeast Asia. The aggregation pheromone for this pest was also identified in the author's former laboratory in Canada. A robust managment system has been developed by the commercial group of the author and Southeast Asian collaborators. At 1 trap / 2 ha pheromone-baited traps are as effective in lowering damage to young palms as the alternative insecticide treatment.

*Cosmopolites sordidus* (banana corm weevil) is a world-wide pest of banana and plantain. The author's commercial group and collaborators from the Costa Rican Ministry of Agriculture developed a pheromone-based trapping system that is effective at 4 traps / ha in lowering corm damage to less than 10% and increasing yields 10-20% over a crop cycle. Each of the above semiochemical-based trapping strategies is cost effective vs insecticide treatment.

It is estimated that approximately 500,000-600,000 Ha are currently under management by mating disruption while 120,000 – 150,000 Ha are under management by mass trapping.

## Special Lecture

# Exploring the chemistry of biotic interactions

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While vision and hearing are particularly important to us in our interactions with one another and with our environment, chemical interactions clearly play a more central role in the lives of most other species. Only in the last half-century, however, have most of the experimental techniques essential for the elucidation of these chemical interactions become available. Some of our current research on insects, arachnids, snakes, and plants will be presented in this lecture. With the increasing power of separation techniques, of establishing molecular structures using ever smaller quantities, and of synthesizing useful quantities of bioactive compounds, chemists of the future will continue to be invaluable partners in the study of biotic interactions. From such collaborative interactions, we can anticipate not only the joys of discovery and understanding, but also the benefits to society of increasing "useful knowledge", particularly in the fields of medicine, agriculture, and forestry. To achieve these desirable goals in the face of the very limited resources currently available for research in chemical ecology will not be easy. In addition, overcoming the difficulties posed by misdirected and counterproductive concerns of biopiracy, by the high rate of species extinction, and by the worldwide problem of preserving our fragile environment in spite of increasing population pressure, constitutes a not inconsiderable challenge for all of us.

# SYMPOSIUM 1

# BIOCHEMISTRY AND MOLECULAR BIOLOGY OF OLFACTORY RECEPTION

# Peripheral networks: The biochemistry of odor presentation

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An odor molecule that encounters an insect may interact with a variety of different proteins, different gene products each designed/evolved to interact specifically with that molecule. Some of these gene products act in concert to transport and transduce the odor molecule, including odorant binding proteins (OBPs) and odor receptors (ORs). But a considerable variety of these gene products, odor degrading enzymes (ODEs) act to inactivate the odor molecule and in so doing maintain a low signal background on the animal. Strikingly, each has one or more binding sites independently designed/evolved to interact with the same or similar ligand(s). Historically, early efforts studying the biochemistry of insect olfaction focused on the enzymatic degradation of pheromone molecules, including studies of Kasang (1971) in *Bombyx mori* and Ferkovich et al., (1973) in *Trichoplusia ni*. The discovery of a Pheromone Binding Protein in 1981 (Vogt and Riddiford) and the proposal that it acts as a pheromone transporter (Vogt, Riddiford and Prestwich, 1985) changed the prevailing paradigm of transport via pore-tubules and dramatically shifted the biochemical focus towards the transport and delivery of pheromone to receptors. And the identification of insect Odor Receptors in 1999 (Clyne et al., Vosshall et al.) refocused these efforts once again, towards odor recognition and coding and the genetic regulation of OR expression. Yet remarkably few efforts have considered these interactions as a whole; the roles of pheromone/odor inactivation have been bizarly ignored except by a few notable exceptions (e.g. Ishida & Leal 2005; Maïbèche-Coisne et al., 2004; Riba et al., 2005; Rogers et al., 1999; Vogt & Riddiford 1986); and little effort has taken advantage of the evolutionary diversity of these proteins to understand common mechanisms underlying their function and interactions. I will discuss these issues as well some recent work on SNMPs, and constructively challenge certain emerging views.

[1] Kasang (1971) in *Gustation and Olfaction* (eds. Ohloff, Thomas) p. 245 Academic Press, NY
[2] Clyne etal (1999) *Neuron* **22:** 327
[3] Ferkovich et al (1973) *Nature* **242**: 53
[4] Ichida & Leal (2005), *PNAS* **102**: 14075
[5] Maïbèche-Coisne et al (2004) *Chem Senses* **29**: 381
[6] Riba et al (2005), *J. Agric. Food Chem.* **53**: 1158
[7] Rogers et al (1999) *J. Exp. Biol.* **202**: 1625
[8] Vogt & Riddiford (1981) *Nature* **293**: 161
[9] Vogt & Riddiford (1986) *J. Chem. Ecol.* **12**: 469
[10] Vogt, Riddiford & Prestwich (1985) *PNAS* **82**: 8827
[11] Vosshall et al (1999) *Cell* **96**: 725
also:http://www.biol.sc.edu/~vogt/pubs/v47.pdf

# Bug's life in the fast lane: Rapid binding, transport, release and inactivation of semiochemicals

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To communicate with pheromones and other semiochemicals, insects evolved a highly sensitive, selective, and dynamic olfactory system. While flying en route towards a calling female or another pheromone source, male moths, for example, can quickly detect pheromone pockets and subsequently reset the sensory system while traveling through the clean air spaces between pockets of chemical signals. Reception of these semiochemicals by specialized structures in the periphery, such as antennae and maxillary palps is mediated by three major classes of olfactory proteins: odorant-binding proteins (OBPs), odorant receptors (ORs), and odorant-degrading enzymes (ODEs). The large number of semiochemicals-detecting sensilla distributed over the surface of the insect antennae and maxillary palps most likely contribute to the sensitivity of the insect olfactory system. ORs are embedded on the dendrites of olfactory receptor neurons that are housed in these sensilla. They are surrounded by a sensillar lymph and OBPs serves as the conduit between the external environment (air) and the ORs. By rapidly capturing molecules reaching the port of entry of these sensilla, the pore tubules, OBPs might contribute to the sensitivity of the system. The delivery of odorants to the receptors, on the other hand, is expedited by a pH-mediated conformational change. The fact that odorant receptors have been expressed and activated in heterologous systems devoid of OBPs seems to contradict the hypothesis that these proteins contribute to the selectivity of the insect olfactory system, but the jury is still out. By contrast, there is recent and growing evidence suggesting that ODEs are essential for sustained odorant-mediated flights. By rapidly degrading stray pheromone molecules (after they activate the receptors), ODEs reset the olfactory system so as to allow the insect to follow an odorant trail by detecting new incoming chemical signals.

# The molecular basis of pheromone and odorant reception in the tortricid moth, *Epiphyas postvittana*

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Olfaction is a critical sense for the Lepidoptera which use smell to find mates and locate host plants. Understanding the molecular basis of olfaction in moths will reveal novel targets for behaviour modification and control of pest moths. The lightbrown apple moth (*Epiphyas postvittana*) is an important tortricid pest of horticultural crops in Australasia, especially because market access can be seriously impacted by its presence in exported shipments of fruit. Moreover, *E. postvittana* can act as a model for other tortricid and horticultural pest moth species, where techniques such as mating disruption are commonly used.

We have conducted an expressed sequence tag (EST) programme on the antennae of *E. postvittana* to isolate genes/proteins involved in the peripheral events of odorant reception. 5,739 ESTs have been sequenced resulting in a non-redundant set of 2,353 sequences, containing 808 contigs and 1,545 singletons. Among the sequences are genes encoding many odorant binding proteins including pheromone binding proteins (3), general odorant binding proteins (2), chemosensory proteins (7), antennal binding proteins (3), and a new family of predicted odorant binding proteins that are similar to juvenile hormone binding proteins (4). There are also many enzymes among the sequences including carboxylesterases (5 full length cDNAs), p450s (13), and GSTs (10), presumably involved in degrading odorants.

Three olfactory receptors (ORs) were found amongst the ESTs. One of the ORs represents an orthologue of the non-canonical OR, OR83b. A second OR is similar to the pheromone receptors of *Bombyx mori*, although it is not sex specific in its expression. Preliminary functional assays in Sf9 cells on this receptor reveal that it responds to the compound citral, but not the major component of the sex pheromone of *E. postvittana*. The third OR is not closely related to any other OR previously described from moths or other insects. However, we have identified orthologues of this receptor that display high levels of sequence similarity in eight other Lepidoptera including *B. mori* (65.6% identical at the amino acid level), suggesting that this receptor may play an important conserved role in moth olfaction.

# Molecular mechanism underlying insect sex-pheromone reception

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Sex pheromones released by adult female moths are detected by narrowly-tuned olfactory neurons in the conspecific male antennae. The silk moth, *Bombyx mori*, is a perfect model for the pheromone study because of the simplest pheromone communication system, wherein a single chemical compound, called bombykol, elicits the full array of sexual behaviors. We discovered two male specific olfactory receptors (ORs), BmOR1 and BmOR3, that were mutually exclusively expressed in a pair of adjacent pheromone sensitive neurons in male antenna and were co-expressed with a highly conserved Or83b family receptor, BmOR2 [1]. The expression of these ORs in the olfactory neurons of a trichodea sensillum was confirmed by double *in situ* hybridization with pheromone binding protein (PBP), which is expressed in the supporting cells that surround pheromone-sensitive neurons in the male moth antenna. Two voltage clamp recordings of *Xenopus* oocyte expressing these ORs demonstrated that BmOR1 was specifically tuned to bombykol whereas BmOR3 was a specific receptor for bombykal, an oxidized form of bombykol. Thus, the BmOR1-BmOR2 combination elicited a response to bombykol with the threshold concentration of ~100 nM, and the BmOR3-BmOR2 combination conferred a response to bombykal with the threshold concentration of ~30 nM. Co-expression with BmOR2 promotes the functional expression of BmOR1 and BmOR3 in the plasmamembrane fraction, and confers pheromone-stimulated cation channel activity. Other members of the Or83b family also had the same effect. Current-voltage relationships with various extracellular ion compositions showed that the bombykol-induced current was carried preferentially by monovalent cations. The current induced by bombykol was substantially inhibited by ruthenium red, a blocker of some TRP channels, implicating the presence of a novel transduction mechanism. The similar ligand-stimulated cation current was also observed when general ORs in *Drosophila* were co-expressed with Or83b or BmOR2. Both odorant and pheromone signaling pathways appear to be mediated by a common mechanism in insects. Supported by PROBRAIN, Japan.

[1] Nakagawa et al. (2005) *Science* **307**: 1638-1642

# Receptors and binding proteins for moths' pheromones

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Mating behavior in many insects is initiated and controlled by pheromones. The remarkable capacity of male moths to recognize and discriminate female released pheromones is based on specialized chemosensory neurons in the sensilla hairs of the antenna. These cells receive the species-specific pheromones with high sensitivity and selectivity and convert the chemical signals into electrical neuronal signals. To reach the dendritic sensory membrane of the olfactory neurons, pheromone molecules enter the antennal sensilla hairs through pores in the cuticle and traverse the sensillum lymph towards the dendritic membrane of the sensory cells; globular soluble proteins in the sensillum lymph, pheromone binding proteins (PBPs), are supposed to ferry the hydrophobic compounds across the aqueous solution. Upon arrival at the sensory cell, pheromones seem to interact with distinct receptor proteins in the dendritic membrane, which are members of the G-protein coupled receptor superfamily, initiating intracellular transduction cascades which generate the electrical responses. Thus, the receptors are not only recognition molecules for the pheromones but also the initiator of the chemo-electrical transduction process. In search for candidate pheromone receptors, we have identified a small family of genes in the tobacco budworm *Heliothis virescens* and the silkmoth *Bombyx mori* which encode receptor proteins with features suitable for sex-pheromone receptors. It was found that several of these genes were selectively expressed in the antennae of male moths and *in situ* hybridization studies revealed that expression of these receptor types was confined to antennal cells, which were located beneath pheromone-responsive sensillar hairs. Furthermore, the receptor-expressing cells were surrounded by cells expressing pheromone binding proteins, a feature characteristic for pheromone-responsive neurons. The notion that cells labeled by hybridization with specific riboprobes indeed generate receptor proteins was confirmed and extended using receptor-specific antibodies. It was found that a relatively high density of the protein was present in sensory dendrites projecting into these sensilla hairs; in addition proximal axonal processes appears to be specifically labeled. Towards a more direct assessment whether the candidate receptors are indeed receptors for pheromones, stable cell lines were generated expressing distinct receptor types as well as an appropriate G-protein. Cells were challenged with defined pheromones as well as odorous compounds and their responses were recorded in calcium-imaging experiments. The results indicated that expression of candidate pheromone receptors rendered HEK cells responsive to low doses of pheromones dissolved in organic solutions, such as DMSO; responsiveness appeared to be selective for pheromones. Subsequent experiments revealed that binding proteins could substitute DMSO to keep the hydrophobic compounds in solution. Moreover, the results suggest that PBPs may significantly contribute to the remarkable ligand specificity of the system. These data suggest an important functional interplay of specific receptor and binding protein subtypes in recognizing and discriminating distinct pheromones.

# Non-linear effects and the function(s) of odorant-binding proteins

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Insects have several odorant-binding proteins, some of which bind general odorants and some of which bind pheromones. The latter are also known as pheromone-binding proteins (PBPs). We have studied the two PBPs from the gypsy moth (*Lymantria dispar*) and have noticed several non-linear effects. For example, early in our studies we noticed that there are non-linear effects in ligand affinities as determined by equilibrium binding assays. This is especially noticeable when blends of odorants are studied. Recently, we have also noticed unusual orders in the binding kinetics. We have applied fluorescent tagging and other physical techniques, to better understand the unusual thermodynamic and kinetic effects. The picture that is beginning to emerge from these studies is that PBPs are very dynamic entities, with multiple functions.

# Identification of olfactory genes in a crop pest, the cotton leafworm *Spodoptera littoralis* (Lepidoptera, Noctuidae), by an expressed sequence tag approach

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Olfaction in insects includes different peripheral steps: interactions with binding proteins and olfactory receptors, signal transduction process, ligand deactivation… The recognition and selection of specific odorants by the antennae relay on the combinatorial expression and intervention of a multitude of different actors, most of which remain to be discovered, such as olfactory receptors or ion channels. Indeed, only few olfactory receptors are known to date in insects and they appear as new G-protein coupled receptor families of extremely divergent genes.

We then set up an expressed sequenced tag (EST) strategy for the discovery of new olfactory genes in our model, the crop pest moth *Spodoptera littoralis*. This strategy consisted in the elaboration of a cDNA library constructed from RNA extracted from 12000 *S. littoralis* male antennae. A normalization step was introduced in the library synthesis (Invitrogen, CA, USA). This step is particularly suitable to enrich the library in rare transcripts, favouring the discovery of novel genes. 2000 clones from the library were randomly sequenced from their 5' end (Tag). Sequences were analysed and compared to public databases (GenBank, trEMBL) using bioinformatics. This led to the identification of a panel of molecular elements potentially involved in the peripheral olfactory steps in *S. littoralis*. Indeed, a repertoire of olfactory genes was established, including odorant-binding proteins, olfactory receptors, degrading enzymes, ion channels. Through their diversity in the same species, they are supposed to act in a combinatorial/sequential way to ensure dynamic discrimination of several thousand of possible ligands. Their functional studies will address their respective contribution to the olfactory response. In addition, numerous modulating elements (hormone receptors, clock genes…) were discovered, suggesting that the olfactory response may be modulated by endogenous/exogenous factors at the peripheral level (hormonal statute, circadian clock…).

Since many insect behaviours are mediated by olfactory cues (sexual recognition, animal/plant host interaction), the discovery of new olfactory molecular targets, together with their possible regulation, is a particular challenge in agriculture (crop pests) and human health (insect disease vectors).

## Odorant-degrading enzymes of moths

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Perireceptor events in insect antennae take place in the sensillar lymph, where sensory neurons are exposed to odorants but also to potential xenobiotics. The olfactory system has thus evolved mechanisms for inactivating odours to minimize signal saturation, as well as mechanisms for detoxifying a variety of substances. Various enzymes, such as esterases or aldehyde-oxidases, have been shown to degrade pheromones in Lepidoptera antennae, suggesting that they could participate in odour degradation after interaction with receptors, and thus playing an important role in signal termination. Other kind of enzymes, well known for their role in detoxification processes, such as cytochrome P450, have been also found in the antennae, where they could be involved in both odorant and/or xenobiotic metabolism.
Using noctuid moths as models, we have investigated the molecular mechanisms of odorant deactivation. In this regard, we have identified potential genes involved in odorant/xenobiotic metabolism in different species. We have also isolated several esterase cDNAs expressed in the antennae of moths, which used acetates as major pheromone compounds: one gene in the cabbage armyworm *Mamestra brassicae* [1], one in the corn borer *Sesamia nonagrioides* and six different genes in the cotton leaf worm *S. littoralis,* through the analysis of an antennal EST library. The comparison of the deduced protein sequences of these putative esterases revealed a diversity that could reflect distinct substrate specificities, in agreement with the different chemical structures of the pheromone components. Esterase activities from antennae and other tissues have been compared, revealing complex expression patterns. Functional studies from *S. littoralis* esterases are in progress in order to precise their catalytic properties.
In addition, we have isolated in *M. brassicae* four cytochrome P450 cDNAs expressed in the olfactory sensilla tuned to sex pheromone and plant's volatile detection [2, 3] and seven putative antennal P450s were identified recently in *S. littoralis* antennae. These results demonstrate the diversity of this kind of biotransformation enzymes in the olfactory tissues and suggest the occurrence of a P450-dependant oxidative metabolism involved in odour degradation in insects, as known in vertebrates.

[1] Maïbèche-Coisne M. *et al.* (2004) *Chem. Senses*, **29**: 381-390
[2] Maïbèche-Coisne M. *et al*. (2002) *Insect Mol. Biol.* **11**: 273-281
[3] Maïbèche-Coisne M. *et al.* (2005) *Gene* **346**: 195-203.

# In the search of differential antennal proteins in males and females of *Sesamia nonagrioides* using proteomic techniques

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Olfaction plays a major role in insect behaviour, particularly in the reproduction process. In this regard and among the antennal proteins, the odorant-binding proteins (OBPs) are capable of interacting with hydrophobic odorant molecules, including pheromones, and transport them through the aqueous lumen to the dendritic olfactory receptors [1]. After this interaction and to avoid the continuous stimulation of the receptors, the odorant molecules are degraded by the action of the odorant-degrading enzymes (ODEs). A better understanding of these antennal proteins may result in the development of an effective tool for pest control, since the inhibition of the elements implicated in the reception, recognition and degradation of odorant molecules would imply modification of the insect behaviour.
In the last few years a series of technological developments in the protein analysis area have made possible the study of an organism proteome that is the total protein expressed in a given cell or tissue. In this context, proteomics is an emergent technology that allows identification of proteins and protein complexes in biological samples. In this work we present for the first time the identification of differential proteins in the antennae of both sexes of the Mediterranean corn borer, *Sesamia nonagrioides*. In this context, we have obtained a protein map by means of two-dimensional gel electrophoresis where proteins are separated by isoelectric point and molecular mass. After determining different protein expression in antennae of both sexes using the appropriate software (Platinum Image Master), the proteins have been excised and digested *in gel* with trypsin. The peptides obtained have been analysed by mass spectrometry (MALDI-TOF and nESI) allowing the identification of several proteins associated with the olfaction. This study is an approach to obtain a comprehensive and integrated view of the antennal proteome of the Mediterranean corn borer.

[1] Vogt, R., Callahan, F., Rogers, M., Dickens, J. (1999) *Chem. Senses* **24**: 481-495

# Identification and functional characterization of a circadian clock in moth antennae

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Circadian clocks, organized in central and peripheral clocks, control circadian rhythms in physiology and behaviour in a wide array of organisms. In particular, an antennal peripheral clock has been demonstrated to be necessary and sufficient to generate *Drosophila* olfactory rhythms in response to food odours. Nocturnal insects, such as moths, mainly use olfactory cues in various aspects of their life and then appear as good models for olfactory rhythm studies. The moth pheromonal communication has been demonstrated to undergo daily rhythms, like the female pheromone emission, used to attract conspecific males for mating, as well as the corresponding male behavioural response.
We thus investigated the occurrence of a putative antennal clock in the olfactory organs of noctuid moths, which could participate in the pheromonal communication rhythms *in vivo*. We focused our work on the characterization in the antennae of two circadian genes that appear as key components of circadian clocks, *period* (*per*) and *cryptochrome* (*cry*). *Per* is a core component of the circadian clock. *Cry* encodes a photoreceptor flavoprotein present in both brain and peripheral tissues. In other tissues than brain, *cry* has been shown to be also an integral component of the endogenous clock mechanism, in particular in *Drosophila* antennae. From moth antennae, we isolated two full-length cDNAs encoding clock genes, *period* and *cryptochrome*, which appeared to be expressed throughout the body. In the antennae, expression of both transcripts revealed by *in situ* hybridization was restricted to cells that likely represent olfactory sensory neurones [1]. In addition, we demonstrated by real-time PCR that *per* and *cry* mRNAs cycled circadianly in the brain as well as in antennae. These results provide evidence that a circadian peripheral oscillator occurs in moth antennae.
In order to decipher the molecular basis of olfactory rhythms, we are now investigating the possible existence of clock-controlled olfactory genes, by quantifying olfactory genes expression rhythms.

[1] Merlin C. *et al*, (2006) *Insect Mol. Biol.* **15**: 137-145.

# Molecular cloning and expression pattern of two new pheromone-binding proteins in the corn stemborer *Sesamia nonagrioides* (Lepidoptera: Noctuidae)

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The stemborer *Sesamia nonagrioides* is an important pest of maize in the Mediterranean Basin. Integrated pest management strategies are being developed against this species, including olfactory-mediated behavioral modification centered on the sex pheromone, in which the main component is (Z)-11-hexadecenyle acetate. To better understand the molecular mechanisms of pheromone reception in this species, we have undertaken the identification and characterization of its antennal Pheromone-Binding Proteins (PBPs). ºAlthough these proteins are supposed to play an important role in the first steps of odor recognition by carrying pheromone components to their receptors, no PBPs have been described to date in *S. nonagrioides.*

Two antennal cDNAs encoding PBPs were newly identified in *S. nonagrioides* antennae by reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA end (RACE-PCR) strategies, and named SnonPBP1 and SnonPBP2 [1]. The deduced proteins showed all the characteristics of PBPs: they are small secreted proteins indicated by the presence of a leader sequence, they present acidic pI (5–6) and contain six cysteines in positions conserved across OBPs. The alignment of the two mature *S. nonagrioides* PBPs with lepidopteran PBPs showed that maximum conservation was observed with noctuid PBPs. Sequence similarities of noctuid PBPs were summarized in a neighbor-joining tree that revealed clusters of PBPs from various species.

Furthermore, expression pattern studies, established by *in situ* hybridization to RNA in both male and female antennae, revealed that SnonPBP1 and SnonPBP2 expressions were associated with olfactory sensilla, in both males and females (1). These results suggest that *S. nonagrioides* females may detect their own pheromone, as already proposed in other Lepidoptera from biochemical and electrophysiological studies.

Behavioral studies are in progress to elucidate the physiological meaning of female pheromone perception.

[1] De Santis et al (2006) *J. Chem. Ecol.* in press.

# Ligands for *Drosophila* olfactory receptor neurons

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Thanks to powerful genetic and behavioral tools *Drosophila* is an excellent experimental model for olfactory studies. In order to find novel, natural odor ligands for *Drosophila's* olfactory receptor neurons, head space odor collections were made from both ripe and rotten fruits, which are natural biological sources of attractive odors. Wild type flies were tested by single receptor neuron recording techniques. Initial recordings were made from receptors on the maxillary palps, as odor ligands for this olfactory organ are not well characterized. All three types of olfactory sensilla on the palp, each with two types of receptor neurons, were investigated. PB1 sensilla responded to ripe banana and to ripe and rotten apple head space collections (neuron A- large spikes). PB2 sensilla responded to ripe and rotten strawberries (neuron A- small spikes). PB3 sensilla responded to ripe mango and orange, and to rotten mango and orange (neuron A- large spikes). Additionally, PB3 sensilla responded to ripe apple, banana, strawberries and to rotten apple, banana, strawberries neuron B (small spikes). Chemical identification of active components from these fruit odor blends is under way. Additionally, dose response curves were generated for all receptor neuron types using single active synthetic compounds in order to define the best possible ligands. 3-methylphenol (3MP) and 4-ethylphenol (4EP) seem to be very effective ligands for the PB1 sensillum as they give high responses already at low stimulus doses.

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# An overview of current US regulations for genetically engineered organisms and a look at traits on the horizon

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The USDA APHIS Biotechnology Regulatory Services (BRS) is responsible for regulating the importation, interstate movement and field release of genetically engineered organisms. There are currently two processes available to applicants: 1. Notifications – an expedited process available for plants that meet specific qualifications and applicants must follow pre-established performance standards; and 2. Permits – available for all plants and organisms whereby applicants submit design protocols for review and work with a biotechnologist to establish the conditions for movement or release. I will provide an overview of the USDA APHIS BRS regulations and procedures.

The primary focus of research regarding the environmental impact of genetically engineered organisms has been on crop plants expressing insect resistance (ie Cry toxins from *Bacillus thuringiensis*) and herbicide resistance (ie glyphosate resistance through insertion of non-glyphosate responsive EPSPS). Future products, however, may include genes that protect the plant against abiotic stresses such as drought, salinity and low nutrient availability. As part of a cascade of effects, these stress tolerance genes that alter plant physiology to abiotic factors may also alter plant responses to biotic stresses, such as insect feeding. We look to the research community to help elucidate the potential environmental impact of these new traits and help inform our risk assessments.

# Comparison of autofluorescence and immunostaining for the study of antennal lobe morphology in moths

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The first centre of olfactory information processing of the insect brain is the antennal lobe (AL). It consists of neuropilar aggregations, the glomeruli, which morphology can be species and sex specific. Three-dimensional AL morphology combined with individual neuron staining is essential to unravel the processing of olfactory information and the behavioural responses of insects to odours. Two common techniques used in the study of AL morphology are glutaraldehyde-enhanced autofluorescence and immunostaining. As part of a study on olfactory processing in moths we have applied both techniques to five species. In three of them (*Anarsia lineatella*, *Cydia molesta* and *Sesamia nonagrioides*) AL morphology is described for the first time. For the other two species, immunostaining (*Cydia pomonella*), or both immunostaining and autofluorescence (*Lobesia botrana*) have been used before.
Autofluorescence was enhanced with 2% glutaraldehyde and immunostaining was done with a synapsis-specific antibody (Synapsin). ALs were inspected in a confocal microscope and fluorescence was ranked as high or low. There was significant species, but not sex, effect in autofluorescence. Highest and lowest percentages of individuals with autofluorescence were observed in *A. lineatella* and *L. botrana,* respectively. With immunostaining there were no differences between species or sex, all preparations being equally visible in the confocal microscope. Enhanced autofluorescence is cheaper and simpler than immunostaining, but its usefulness depends on the species and sex used.

# Functional mechanisms of anti-predator behaviors induced by predator's chemical cues on tadpoles

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In freshwater systems, many prey species show behavioral response like reduction in activity level to predator's chemical cues. Such chemically induced behavior has been thought to be related to avoid being detected and attacked by predators. However, there are little empirical tests of the effects of behavioral response to predator's chemical cues for predator avoidance or escape. In this study, we experimentally investigated how prey's behavioral responses to predator's chemical cues decrease vulnerability during encounters with predator. We used as prey an anuran species tadpole *Hyla japonica*, and as predator a dragonfly nymph *Anax parthenope julius*. All trial was conducted as follow; tadpoles were exposed to either the presence or absence of dragonfly nymph's chemical cues and dragonfly nymph can have detected tadpole via visual cues but no direct interaction. When compared to absence treatment of chemical cues, the time up to the first attack of dragonfly nymph increased for tadpoles in the presence treatment. The frequency of attack of dragonfly nymph decreased during trials in the presence treatment in comparison with the absence treatment. Most of attacks by dragonfly nymph occurred when the tadpoles were moving. Tadpoles exposed to chemical cues increased the burst swimming speeds and swimming distances, respectively. In addition, dragonfly nymph's approach to the swimming tadpoles was less frequent in the presence treatment than in the absence treatment. Our study exhibited that prey's behavioral responses to predator's chemical cues decrease probability of being attacked by predator. Prey have not only reduced activity but also other anti-predator behavior, and may vary their behavior according to the distance of predator. We are characterizing the nature of chemicals that induces anti-predator behavior by using SPE and HPLC.

# Reproductive isolation mechanisms between two sympatric simultaneous hermaphroditic shrimp, *Lysmata wurdemanni* and *L. boggessi*

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We investigated prezygotic and postzygotic isolation between two *Lysmata* shrimp by observing intra- and inter-specific crossing. *Lysmata wurdemanni* and a closely related species *Lysmata boggessi* are similar in morphology, life history, reproductive cycle, and courtship behavior. They co-occur in southern Florida. Experiments reveal that female-role (simplified as female thereafter) *L. wurdemanni* accepted only conspecific male-role shrimp (simplified as male thereafter), whereas *L. boggessi* females mated with males from both species. However, when males of both species were present, *L. boggessi* females always mated with the conspecific males. Male *L. boggessi* failed to mate with *L. wurdemanni* females. Observations demonstrated that male *L. boggessi* did not respond to the sex pheromones (distance and contact pheromones) secreted by female *L. wurdemanni*. Male *L. boggessi* did not display any pre-copulatory behavior to the newly molted female *L. wurdemanni*. Although mating was successful between *L. wurdemanni* males and *L. boggessi* females, the resulting embryos lived only for up to 10 days and failed to hatch. The results in this study indicate that the two shrimp species are prezygotically and especially postzygotically isolated. Behavioral observation suggests that chemical and visual cues cause prezygotic isolation.

# Aquatic ecotoxicity of a pheromonal antagonist in comparison to the parent major component of the attractant

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(*Z*)-11-hexadecenyl trifluoromethyl ketone (*Z*11-16:TFMK), an analogue of (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac), the major component of the sex pheromones of the Mediterranean corn borer *Sesamia nonagrioides* and the cabbage armyworm *Mamestra brassicae*, has been shown to be a successful antagonist of the pheromone in electrophysiological and behavioral tests as well as in the field. The antagonist has displayed low toxicity to mice in comparison to *Z*11-16:Ac but ecotoxicity studies of these compounds over other non-target species is still lacking. Mating disruption experiments using formulations of *Z*11-16:TFMK on maize fields may affect other organisms living in aquatic habitats via spray drift or indirectly throughout runoff inputs. In this paper we present acute and sublethal toxicity tests of *Z*11-16:TFMK and *Z*11-16:Ac against the cladoceran *Daphnia magna* and the chlorophyte *Desmodesmus subspicatus*, species routinely used to screen the toxicity of new or known compounds to aquatic organisms (Directive 2000/60/EC). Toxicities of both chemicals were moderate with $EC_{50}$ values ranging from 3.11 to 103.74 mg $l^{-1}$ in algae growth, from 0.07 to 1.20 mg $l^{-1}$ in *Daphnia* survival, and from 0.10 to 0.53 mg $l^{-1}$ in *Daphnia* feeding rate. In all cases *Z*11-16:TFMK was more toxic than the naturally occurring pheromone component. In addition, studies on the effect on B-esterases, including cholinesterase (AChE) and carboxylesterase (CbE), in *Daphnia* showed that neither compound elicited any effect on AChE activity but CbEs were dramatically inhibited at concentrations of inhibitor 5 fold lower than those impairing survival or feeding. This suggests that toxicity of the TFMK is likely to occur through inhibition of other serine esterases, whereas the toxic effects of the pheromone component are likely to be caused by non specific non polar narcosis.

# SYMPOSIUM 2

# SEMIOCHEMICAL PRACTICAL APPROACHES FOR PEST CONTROL

# Development of a monitoring and mass trapping system for coffee berry borer

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Coffee berry borer (CBB) *Hypothenemus hampei* is the world's most important insect pest of coffee. Each year CBB causes over $1,000,000,000 in damage through larval consumption of maturing berries. At the roasting stage weight of CBB infested berries is reduced. Typical treatment to lower damage involves one or two applications of endosulfan to which resistance is well established. CBB is known to be attracted to mixtures of methanol and ethanol emitted in ratios favoring methanol (~3:1). We have determined the ideal release rate for this mixture is in the range of 200 mg/day and developed a system to maintain an attractive ratio of release of the two attractive components for about 60 days. Monitoring of CBB is optimal using small red multifunnel traps baited with methanol:ethanol lures. When trapping of CBB is conducted using 20 traps / ha it is found that increases in yield are the same as for endosulfan treatment. Costs of trapping are approximately the same as for endosulfan treatment. Year round trapping shows that the best time to remove CBB by this technique is after harvest and before the next crop of berries reaches the stage at which they are attractive. Leaf alcohol and relatives are highly repellent to CBB in trap shutdown experiments but are not sufficiently robust repellents to protect coffee against CBB in field experiments.

# Pros and cons of using high release-rate, low density dispensers for mating disruption

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Widely spaced, high-emission-rate pheromone dispensers are continuing to be explored as a possible effective way to deploy pheromone mating disruptant blends on large contiguous hectares of crops such as corn, cranberries, and tree fruits. The emission rate of the dispensers is from 5 to 10 times higher than other currently available hand-placed dispensers. Fewer dispensers are therefore needed for effective disruption. In one currently available formulation consisting of polyethylene bags, only ca. 12 dispensers are needed per treated hectare of cranberries or tree fruits, and they are retrievable. These dispensers have been commercially available on cranberries against the blackheaded fireworm, *Rhopobota naevana*, and the sparganothis fruitworm, *Sparganothis sulfureana*, as well as in corn against the European corn borer, *Ostrinia nubilalis*. They have also been shown to be effective against the oriental fruit moth on peaches, almonds, and apples, and against phycitine moth pests in grain stores. Against the first flight of the European corn borer, the percentage of feral females mating in grassy aggregation areas was shown to be reduced by an average of 50%, and the dispensers significantly reduced the frequency of mating throughout both the first and second flights by 30%. Damage in research plots and in commercial fields during the first flight was consistently reduced by 50%. The density of bags needed to be deployed was only 2.5 dispensers per hectare of corn. These data revealed that the reduced damage is a result of forcing females to take longer to attain their first and second matings and not by preventing them from mating. This is the only type of mating disruptant formulation whose per-ha. dispenser density (and amount of A.I. per ha.) diminishes significantly as the hectarage treated increases, even while maintaining the same dispenser grid-spacing. There is also a second geometric advantage of such systems that is provided by the stronger pheromone strands that are behaviorally active farther downwind than in lower-emission-rate dispensers. The longer-distance behavioral activity results in a greater ratio of hectarage covered relative to angular change in wind direction than for lower-emission-rate dispensers. However, dispenser density is more adversely affected by odd-shaped fields than in coger-emission rate systems having closely spaced dispensers. Other potential weaknesses in high-emission-rate systems include their plumes being more subject to being attenuated by foliage in tree fruit crops due to vegetation-produced turbulence and adsorption by leaves. In above-canopy deployments such as in cranberries or in grassy areas near corn, long-distance plumes are more vulnerable to rising or falling currents than are lower-emission-rate, closely spaced dispensers.

# Applications of non-pheromone attractants for management of insect pests

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Feeding attractants provide opportunities for monitoring and management of pest insects by trapping or baiting of males and females. It is suggested that the killing of females versus males may be more effective for reducing populations. Flowers and fermented sugar solutions (sweet baits) are sources of food for adults of many insects. Investigation of these sources yielded several chemical attractants, such as acetic acid with isobutanol for many vespid wasps, acetic acid with 3-methyl-1-butanol (AA/MB) for many moths, and phenylacetaldehyde with beta myrcene (PAA/BM)for other moths, including alfalfa looper, cabbage looper and soybean looper. Unlike moth sex pheromones which are formulated in milligrams to be released in sub-micrograms per hr, these feeding attractants require 2 to 3 orders of magnitude more, up to several milligrams per hr released and multiple gram load amounts. We developed a polypropylene bottle dispenser with release controlled with a hole in the lid, providing zero order controlled release for at least 30 days. This dispenser was combined with a flower-mimic target (badminton birdie) coated with a pesticide formulation (Permethrin in Teflon® grease) to make an attract-and-kill station. In 3-minute flight tunnel assays, 80% of starved female alfalfa looper and cabbage looper moths were attracted to a PAA/BM lure and 90% of those contacting the station were killed. In commercial fields of alfalfa, alfalfa looper moths in monitoring traps were reduced ca 75% with the presence of 50 attract-and -kill stations per hectare. In screen house tests, oviposition by alfalfa looper moths on lettuce plants was reduced 98% by placement of 2 attract-and-kill stations per screen house. Similar results were obtained with the same station design with an AAMB lure targeting *Lacanobia subjuncta* in apple orchards. Monitoring traps (AAMB and blacklight) showed a <75% reduction in numbers of female *L. subjuncta* moths with 50 attract-and-kill stations per hectare.

# Mating disruption of pyralid moths in commercial facilities

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Three pyralid moths, the Mediterranean flour moth (*Ephestia kuehniella*), the almond moth (*Ephestia cautella*) and the Indian meal moth (*Plodia interpunctella*), infest food products all over the world and cause severe problems in factories, shops and households.
The pheromone mating disruption technique (MD) has been attempted previously for control of these moth species, but ~~it~~ has not yet become a commercial alternative. We employed MD by releasing the moths' common major pheromone component (*Z*9,*E*12)-tetradecenyl acetate (approximately 2 mg/100 $m^3$/day) in four mills, a chocolate factory, a pet shop and a pet food warehouse during 7-21 months. New MD dispensers were usually added after 2-3 months.
Catches in pheromone monitoring traps decreased and air concentrations of the main pheromone component released from the MD-dispensers, measured by a portable EAG recorder in two mills, increased directly after the application of the treatment. During the treatment fewer moths were observed in the localities and the number of complaints from customers of mill products decreased. EAG recordings verified high atmospheric concentrations of the pheromone during the whole treatment period and the pheromone concentration did not decrease to pre-treatment levels until 12 months after removal of the MD dispensers. The pest populations gradually recovered after termination of the treatments.
A decrease in attraction to pheromone-baited monitoring traps after MD application may be due to disruption of trap catches rather than an actual decrease in insect numbers. Thus, a decrease in pheromone-trap catches after application of MD compared to before treatment, does not necessarily show a reduction in population size, but pheromone-baited traps can still be used to monitor changes in insect numbers *during* periods of treatment. In the chocolate factory, it was possible to use traps baited with water to obtain an additional and independent measurement of the female as well as male *E. cautella* population levels (Ryne et al. 2002). Pheromone traps showed a 94% catch reduction and monitoring with water traps showed a significant decrease in total catch (5.0 and 1.6 individuals/trap/week before and during treatment respectively). We tested the significance of the results by fitting the observed data to a first order autoregressive model. This enabled us to test the data with a 95% confidence interval comparing trapping numbers before mating disruption treatment with trapping data during the experiment. We suggest that this statistical approach may be used more frequently in mating disruption experiments where it is extremely difficult to control external factors and therefore equally difficult to use a comparable control plot to evaluate the treatment.
We conclude that MD has a large potential for controlling all three moth species.

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# Competitive attraction as a primary mechanism of moth mating disruption in tree fruit crops

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Over the past few years we have been exploring ways of achieving moth mating disruption exceeding 95 % efficacy even under high densities with limited insecticide inputs. The foundation for this work has been a series of studies examining the mechanisms underlying pheromone-based mating disruption in tortricid moth pests of fruit. Collectively, our results support competitive attraction or false-plume-following as an important, if not essential, component of communicational disruption of tortricid moths. Four main lines of evidence have led to this conclusion. Disruption of tortricids is density-dependent. Under high population densities, disruption increases as a function of increasing density of pheromone release sites. Effective mating disruption using high-dosage dispensers occurs in the field despite overall atmospheric concentrations not reaching levels high enough to desensitize moths by adaptation or habituation. Males are attracted to high-dosage dispensers in the field. Finally, the theoretical properties of competitive-attraction phenomena have proved consistent with the majority of results obtained in the field. Key traits of competitive attraction are: concave profiles on untransformed axes, with an asymptotic approach to zero catch; a straight line with positive slope when 1/catch is plotted against dispenser density; and a straight line with negative slope when catch is plotted against dispenser density $*$ catch. Key traits of non-competitive disruption profiles include: an initial linear disruption profile on untransformed axes; a concave inverse plot; and a re-curving complex plot. Of 13 published disruption profiles the authors were able to locate, 11 were a better fit to the predictions of competitive attraction than a non-competitive disruption mechanism. If competitive attraction plays a major role in achieving mating disruption, the following practical implications should guide us in developing high-performance approaches and formulations: 1) distribution should be uniform rather than clumped, 2) dispenser density should be high, and 3) attractiveness should be competitive with females.

# Field responses of invasive brown marmorated stink bug, *Halyomorpha halys*, to geometric isomers of methyl 2,4,6-decatrienoate

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The brown marmorated stink bug, *Halyomorpha halys*, is a polyphagous pest indigenous to northeast Asia where it damages various trees, vegetables, and leguminous crops. *H. halys* is also a serious nuisance pest as the adults hibernate en masse in buildings. In 2001, the brown marmorated stink bug was reported for the first time in the U.S., in Allentown, PA. Surveys conducted to determine host-plant preference and potential feeding damage in North America found adults and nymphs *H. halys* on over 60 different plants. *H. halys* adults can fly, and thereby extend their range, but dissemination is also accomplished by hitchhiking on vehicles. Typical methods for detection and monitoring stink bugs include the use of pheromone-baited traps, but the pheromone for *H. halys* has yet to be identified. However, the brown marmorated stink bug was reportedly attracted to the aggregation pheromone of the brown-winged green bug, *Plautia stali*, methyl (2*E*,4*E*,6*Z*)-decatrienoate. We expediently synthesized all geometric isomers of methyl 2,4,6-decatrienoate and, in 2003-2005, conducted field studies with selected compounds. We now report that *H. halys* is attracted not only to the *E,E,Z*-isomer, but also to at least two other isomers, most notably methyl (2*E*,4*Z*,6*Z*)-decatrienoate, a compound known to be part of pheromones of pentatomids in genus *Thyanta*. The *Z,E,Z*-isomer, heretofore not known to be attractive to *H. halys*, did evoke an electrophysiological response in the antennae of *H. halys* males (as expectedly did the *E,E,Z*-isomer), and was moderately attractive in the field. Analyses of volatiles collected from dispensers used in field trials showed that all three compounds rapidly isomerized under daylight to form complex mixtures, some of which were more attractive to *H. halys* than the individual isomers. Almost equal numbers of both sexes, as well as nymphs, were found in traps baited with methyl decatrienoates, yet repeated efforts to detect these compounds from the bugs themselves have failed. One explanation for this phenomenon is that *H. halys* uses pheromone(s) of other bugs as kairomone(s) to aggregate and/or to find food.

# Probing hydrocarbon mixtures in insects by MALDI-TOF-MS

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Hydrocarbons (HCs) are widely used in the industry and ubiquitously occur in nature. They form surface waxy protection layers on plants and insects and participate in a variety of insect-insect and plant-insect interactions. HCs are routinely analyzed by GC/MS. Both GC and EI MS have, however, limitations for high molecular weight compounds (sample decomposition, absence of molecular ions in EI spectra etc.). GC/MS enables one to detect only part of HCs and thus does not provide complete information about HCs in complex mixtures.
To study medium and high molecular weight HCs, matrix-assisted laser desorption/ionization mass spectrometry was used. Lithium 2,5-dihydroxybenzoate, LiDHB [1], provided the best ionization efficiency, minimum of the background cluster ions, and it was sufficiently soluble in organic solvents. Simple isotope pattern greatly simplify spectra interpretation. MALDI was used to study mixtures of cuticular HCs of various plants and insects [1][2]. Different HC profiles were recorded showing saturated and unsaturated HCs up to 70 carbons. Accurate mass measurements using TOF analyzer enabled determination of their elemental composition. A number of high molecular weight HCs beyond the analytical capabilities of GC/MS were newly described. The ability of MALDI to discriminate among species varying in the degree of their relatedness was found to be similar to GC/MS. However, neither MALDI-MS nor GC/MS data were able to describe the phylogenetic relationships.
MALDI with LiDHB matrix is a complementary method to GC/MS for hydrocarbons analysis.

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# Chemical identification of female sex pheromone of pink hibiscus mealybug and chirality recognition among stereoisomers by males

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The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), is an invasive species. It is a serious new threat to U.S. agriculture, forestry, and the nursery industry, involving more than 200 different plants. Potential cost to the United States at $750 million per annum has been estimated if this exotic pest cannot bee controlled. Two new compounds that together constitute the female sex pheromone of the *M. hirsutus* were isolated, identified, and synthesized. They are (*R*)-2-isopropenyl-5-methyl-4-hexenyl (*S*)-2-methylbutanoate [common name is (*R*)-lavandulyl (*S*)-2-methylbutanoate)] and [(*R*)-2,2-dimethyl-3-(1-methylethylidene)cyclobutyl]methyl (*S*)-2-methylbutanoate (which we refer as (*R*)-maconelliyl (*S*)-2-methylbutanoate). Maconelliol is a novel cyclobutanoid monoterpene, and its structure has been established by enantioselective synthesis from precursors of known structure and configuration. Therefore, the pheromone component, maconelliyl 2-methylbutanoate, represents a heretofore undescribed natural product. A 1:5 synthetic mixture of the two *RS* esters (1 μg/rubber septum) proved to be a potent attractant in field bioassays.
However, our field studies also suggested that the two chiral centers in the sex pheromone of *M. hirsutus* could elicit different male responses. The chiral center in the acid moiety of the pheromone seemed to be more critical than the alcohol portion of the pheromone molecule for attractiveness. The objective of the current study was to test this hypothesis by deploying stereoisomeric blends in pheromone traps. Captures of male *M. hirsutus* showed that pheromone with the naturally occurring *R*-*S* blend was most attractive and that pheromone with the unnatural *S*-*S* configuration was less attractive. In addition, the *RS*-*R* blend (containing *R*-*R* and *S*-*R* stereoisomers) yielded captures of male *M. hirsutus* that were comparable to blank controls and an inhibitory effect was observed when *R*-*R* and *S*-*R* were combined with naturally occurring *R*-*S* blend. These results suggest a unique chirality recognition mechanism; olfactory discrimination among different pheromone stereoisomers depends upon both asymmetric centers. The *S*-configuration on the acid moiety elicits attraction, whereas the *R*-configuration induces inhibition. However, the attractive activity shows some degree of tolerance towards chirality change in the alcohol portion of the pheromone molecules, which is unusual in insect semiochemical communication systems.

# The effect of wind speed and atmospheric pressure on the response of *Aphidius ervi* males response to pheromones

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The effect of wind speed and distance from the source on the male response of the aphid parasitoid, *Aphidius ervi* (Hymenoptera: Aphidiidae) to a pheromone source was studied in a wind tunnel. The number of males taking flight, entering the plume and successfully reaching the source, decreased at wind speeds >50 cm/s wind speed. Furthermore, the proportion of those attempting upwind flight that fell to the ground increased with increasing wind speed. In contrast, distance from the source had no significant effect on any of the parameters examined. While males flight behavior was significantly reduced at 70 cm/s some males walked to source when there was a bridge connecting the pheromone source and the release platform. This suggests that ambulatory behavior could be a significant component of male mate searching of *A. ervi* when wind conditions are too strong for upwind flight.
The possible effects of variation in atmospheric pressure on male flight behaviour to the long distance pheromone, as well as to the short distance one, were also investigated. The only significant effect observed was that the higher the atmospheric pressure at the time the assay was conducted, the higher the greater number of males reaching the source. These finding differ significantly for those previously reported for another aphid parasitoid, *A. nigripes*, and the reasons for such differences are discussed.

# Species spectrum of noctuids attracted to female-targeted synthetic attractants in Europe

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Female-targeted synthetic attractants containing iso-amyl alcohol, iso-butyl alcohol and acetic acid have been described in the literature for some noctuid spp. in North America. The present study was undertaken to investigate the species spectrum of European noctuids attracted to iso-amyl alcohol based synthetic baits, and to study the potential of such baits for practical application.

Traps baited with a blend of iso-amyl alcohol, iso-butyl alcohol and acetic acid captured in sizeable numbers and significantly more than in unbaited traps from the noctuids (Lepidoptera: Noctuidae) *Agrotis segetum* Schiff., *A. exclamationis* L., *Amathes c-nigrum* L., *Apatele rumicis* L., *Amphipyra pyramidea* L., *Dipterygia scabriuscula* L., *Discestra trifolii* Hfn., *Euxoa aquilina* Schiff., *Euclidia glyphica* L., *Mamestra brassicae* L., *M. oleracea* L., *M. suasa* Schiff., *Mythimna albipuncta* Den. & Schiff., *Noctua pronuba* L. and *Trachea atriplicis* L. The ratio of females in the catch was generally high in all species captured. Among these spp. several important pest noctuids are included.

The addition of 3-methyl-1-pentanol to the ternary mixture did not influence captures. The presence of iso-butyl alcohol in the mixture was important for catching *A. rumicis*, *D. trifolii* and *E. glyphica*, but showed no significant effect for the other spp.

When compared with the performance of the respective pheromonal baits in *A. segetum*, *M. brassicae* and *M. oleracea*, the iso-amyl alcohol based baits caught ca. 5-10% of the catch in pheromone traps. The addition of a respective pheromone inhibitor to the iso-amyl alcohol based baits did not influence catches of males in *M. brassicae* and *M. oleracea*.

When comparing the species spectrum attracted to the iso-amyl alcohol based bait and the long-known phenylacetaldehyde noctuid lure, phenylacetaldehyde attracted predominantly Plusiinae and Melicleptriinae spp. (i.e. *Autographa gamma* L., *MacDunnoughia confusa* Steph. and *Helicoverpa armigera* Hb.), while iso-amyl alcohol based lures attracted spp. from other subfamilies. *E. glyphica* was atttracted equally well to the two types of baits. Presenting the two types of female-targeted baits in one trap was usually not advantageous for most spp.

# Mass trapping apple leaf curling midge (Diptera: Cecidomyiidae)

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Apple leaf curling midge (*Dasineura mali*) is a significant pest of young apple trees and can be a non-damaging quarantine species on mature apples. The recent identification of the female sex pheromone is enabling new direct control tactics such as mass trapping to be considered. Any direct control tactic using male suppression will require knowledge of the frequency of multiple mating, dispersal and colonisation rates, and the efficiency of male removal. Males were able to mate up to five times, when presented in a 10 female to one male group, which was designed to simulate male suppression. Male catch in response to the pheromone loading was curvi-linear over four orders of magnitude, with limited signs of catch saturation at 30 mg loaded on rubber septa. Mass trapping was investigated using a high dose (3 mg) pheromone lure combined with oil-based traps based on the New Zealand "Lynfield trap" for tephritid fruit fly surveillance, applied to young orchard blocks, at 500 traps per ha. Monitoring traps indicated that male catch exceeded 95% suppression in the treated plots compared to the control plots, over a four month period. There was a lack of shoot tip infestation, indicating limited female dispersal. Replicated transects of frequency of infested shoots from a mature orchard across the adjacent young block confirmed that colonisation by females was very limited beyond 30m from the orchard boundary. Differences in dispersal between sexes may be present, which could be due in part to the greater wing loading in gravid females, estimated at 1.8 fold higher than that of males. Thus for mass trapping this species using pheromone appears to be technically feasible at this stage of investigation.

# The search for natural repellents from human beings against biting insects

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Haematophagous insects are differentially attracted to vertebrate hosts. This is expected to be caused by differences in semiochemical profiles of individual human hosts, although the responsible semiochemicals have not been identified previously. Behavioural studies using a Y-tube olfactometer confirmed the differential attractiveness of human volunteers to the yellow fever mosquito, *Aedes aegypti*. Volatile chemicals were collected from all volunteers using air entrainment and the extracts were analysed behaviourally with *Ae. aegypti* and the Scottish biting midge, *Culicoides impunctatus*. The behavioural responses to the extracts were similar to those previously observed to the hands of the volunteers. Electrophysiological responses of *Ae. aegypti* and *C. impunctatus*, to volatiles from the air entrainment extracts, were recorded using coupled gas chromatography-electroantennography (GC-EAG). EAG-active compounds were tentatively identified using GC-mass spectrometry (GC-MS) and were confirmed by peak enhancement. A quantitative and qualitative analysis of compounds within the extracts revealed significant differences in chemical profiles. The amounts of several chemicals were significantly greater in the unattractive individuals than the attractive individuals. Five such compounds caused significant reductions in flight activity and attraction of *Ae. aegypti* and *C. impunctatus* when presented alongside an attractive human hand in the Y-tube olfactometer, thus accounting for the lack of attraction towards the hands of the unattractive volunteers. A field trial was conducted against *C. impunctatus* and revealed that certain compounds and mixtures were more effective than the commercial repellent DEET. The identification of such behaviourally active compounds could lead to improved control technologies, whereby such compounds could potentially be incorporated into new, safe and natural repellents against biting insects.

# Semiochemical-based monitoring and control on soybean aphids

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The soybean aphid is a newly invasive insect species that seriously threatens U.S. soybean production. It is the only aphid species to develop large colonies on soybeans in North America. Its infestation has spread to over 20 US states and three provinces in Canada, which whittles soybean growers' profits and causes hundreds of million dollar losses. In 2003, the total acreages with soybean aphid infestation from three biggest soybean growing states, Iowa, Illinois and Minnesota, reached over 10 million acres with yield loss ranged from 32%-45%. In 2005, over 2 million acres of soybean were treated with insecticide for soybean aphid management just in Iowa alone. Across the Midwest states a significant amount of soybean acreage was treated with insecticide highlighted by the experience in Michigan were over 80% of the total acreage was treated once and an additional 10% treated 2 to 3 times during the 2005 growing season. Although the current recommendation for soybean aphid management (scout and insecticide application) has been effective for reducing yield losses, it has been laborious and inaccurate, and also kills beneficial insect predators and parasitoids, which can lead to new aphid outbreaks as well as to an increase in the populations of other soybean pests. Furthermore, insecticides may not be entirely effective, and populations of soybean aphids can rebound quickly after the application.
In the last three years, we have studied infochemical interactions among soybean aphids, soybean plants and their natural enemies. We have successfully identified sex pheromone structures of soybean aphids, as well as several soybean plant associate volatiles that are attractive to their spring migrant alate. We have also demonstrated that the aphid induced soybean plant defensive compound, methyl salicylate, acts as not only a deterrent to soybean aphid colonization, but also be used as chemical cues for food searching of some soybean aphids' beneficial insects. Furthermore, several volatile compounds emitted from early growth stage soybean plants could be used for monitoring their spring migration and to establish an early warning system for further action. In this presentation, I will report our newest development on how we use this information for the integrated soybean aphid management, and will also introduce some potentially commercial products that come along with this research for practical use in aphid or other insect pest control.

# The rosy apple aphid sex pheromone: A new component

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The rosy apple aphid (*Dysaphis plantaginea* (Passerini)) is the most serious pest of apple in Europe [1]. Currently control of *D. plantaginea* is achieved by the use of insecticides, but the future of insecticide usage is compromised. Thus, new approaches for controlling *D. plantaginea* are required.
The life cycle of *D. plantaginea* includes many female parthenogenetic (asexual) generations on the secondary host (*Plantago spp*) followed by one sexual generation on the primary host (*Malus silvestris,* apple). Oviparae (true sexual females) release a sex pheromone which attracts males. The sex pheromone potentially provides an alternative approach for controlling *D. plantaginea,* hence studies were initiated to fully characterise the sex pheromone components.
Volatile samples were collected from *D. plantaginea* oviparae and putative pheromone components were located by coupled gas chromatography – electroantennography (GC-EAG) using male antennae. Active peaks were then identified tentatively by GC coupled with mass-spectrometry (GC-MS) and confirmed by peak enhancement with authentic compounds on GC columns of different polarities. The two major components were shown to be (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and *(*4a*S*,7*S*,7a*R*)-nepetalactone. The full determination of the enantiomeric composition of these two components was achieved by chiral GC (nepetalactone) and microscale NMR (nepetalactol).
A third EAG-active component was identified as dolichodial. In behavioural bioassays, dolichodial elicits a strong behavioural response by male *D. plantaginea* and the generalist aphid parasitoid, *Aphidius ervi*. This is the first time this chemical has been identified in a volatile collection from oviparae and a behavioural response recorded.

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# Identification and field evaluation of the aggregation pheromone of the Brazilian papaw weevil *Pseudopiazurus obesus*

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The papaya weevil *Pseudopiazurus obesus* Marshall, 1922 (Coleoptera: Curculionidae) is a pest of economic importance for the producing areas of the northeast region of Brazil. Laboratory bioassays employing host plant alone, volatiles released from male and female conspecifics alone, and a combination among the insect volatiles and host plant suggested the presence of a male-produced aggregation pheromone. GC analysis of airborne volatiles released by males and females showed the existence of three male-specific compounds, in a ratio of 83:15:10, which were identified by GC-MS and micro-derivatization as grandisal, grandisol, and a new derivative of grandisol that we named papayanol. The biological activities of synthetic racemates were tested in the laboratory /Y-olfactometer) and in the field (traps). Results showed that males and females were significantly more attracted to grandisal alone and to a 1:1:1 ratio of the ternary mixture, both in combination with host plant odors. Enantioselective gas chromatography using a modified cyclodextrin as the stationary phase, proved the natural grandisal and grandisol to be the enantiomerically (almost) pure (1*R*,2*S*)-stereoisomers. Work is now in progress to test pure enantiomers of grandisal and grandisol and to establish the absolute configuration of papayanol.

# Chemical characterization of the aggregation pheromone from faeces in three vectors of Chagas disease

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Triatomine bugs use their excrement as a chemical landmark to recognize refuges and this signal induces the assembling of individuals inside the most appropriate shelters. To identify and characterize the volatile compounds responsible for this attraction, we analyzed separately faeces samples from *Triatoma infestans, Triatoma brasiliensis* and *Panstrongylus megistus.* Thus, the volatiles of each sample were captured using solid phase micro-extraction (SPME) from its deposition until 6 days after, to reveal the emission profile of the substances. After GC-MS analysis of the desorbed compounds, the chromatographic profiles from the faeces of the three species were compared. The compounds appearing in samples of all species were then tested in behavioural experiments. Dose-response curves were determined with each of the three species for acetic, isovaleric and hexanoic acids, 2,3-butanediol and acetamide,. All substances modified the behaviour of insects of the three species at specific concentrations. Moreover, a blend of 10 ng of each of these substances attracted the insects in the same bioassay. Finally, shelters baited with this blend were tested in overnight assays offering a control and a baited refuge for simultaneous choice. The results show that baited artificial refuges are more attractive, recruiting more insects into them than control clean shelters. The attractive effect of the mixture was similar to that of natural faeces of triatomine bugs. Further field tests with slow release formulations of this mixture are being done to allow evaluating their use as baits for the detection and control of natural populations of Chagas disease vectors.

# Chemical analysis of residues left by walking adults of *Nezara viridula* which induce arrestment behavior in the egg parasitoid *Trissolcus basalis*

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Chemical residues deposited by walking adults of the southern green stink bug (SGSB), *Nezara viridula* (L.) (Heteroptera: Pentatomidae), which play a role as a contact kairomones inducing arrestment behavior in the egg parasitoid *Trissolcus basalis* (Wollaston), were investigated in laboratory experiments. Wasp females encountering an area contaminated by host adult chemical residues show an arrestment response characterized by a prolonged periods of walking and to systematic return to the stimulus after encountering the treatment borders. When SGSB adults were dissected into separate body parts, legs and dorsal laminar pronotum of adult females elicited equal responses, while legs and dorsal laminar pronotum of adult males elicited a lower responses. These results are in agreement with wasp preference for female chemical residues as showed by previous experiments, and they suggest that contact kairomones are not confined in the legs, but they are compounds present in the adult cuticula. Hexane extracts of laminar pronotum and legs of SGSB adult induced stronger responses then methanol extracts. Silica gel chromatography for SGSB cuticula extracts indicated that the activity was associated with the fraction of saturated and unsaturated hydrocarbons. The potential significance of these results to the host location behavior of *T. basalis* in the field is discussed.

# Minimizing deer browse damage: Practical application of a protein repellent

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Damage to agricultural, forest, and horticultural resources by deer has been recognized as a substantial economic problem for some time. Browsing may result in complete plant loss or reduce future value of commodities via decreased yields and plant deformities. Fear of browse damage may also result in reduced tree and shrub purchases by homeowners. A number of commercially-available repellents have been employed to deter browsing of trees and shrubs by deer. I propose that two mechanisms describe mammalian responses to herbivore repellents: altered palatability and neophobia. Under this view, the protein fraction of animal-based repellents negatively impacts palatability of the treated forage item. At the same time, odors produced via protein and lipid degradation serve as cues signaling palatability via associative learning. While novel stimuli may deter herbivory, the availability of alternative forage and the repellent's mode of action influence the effectiveness of herbivore repellents. Thus, habituation to repellents that rely solely on neophobia to reduce browsing is typically rapid.

Model mammalian herbivores and captive deer were employed to study foraging behavior and responses to animal proteins. These bioassays demonstrated that technical-grade hydrolyzed casein significantly reduced browse damage by captive deer to evergreen shrubs and conifers versus the control ($p < 0.0001$). Commercial sources of hydrolyzed casein (baby formulas) also reduced browsing of edible plants. However, deer avoided plants treated with baby formulas only when control plants were available. The importance of alternative foods on the persistence of avoidance behavior was clearly demonstrated in a bioassay with lambs. Persistence of a flavor aversion was not only influenced by the presence of alternatives, but also the nutritional quality of the alternative ($p < 0.0001$). Taken together, these studies indicate that hydrolyzed casein is a safe and effective deer repellent to minimize browsing and that the availability of alternative foods will greatly impact persistence of the avoidance behavior. Thus, a simple repellent formulated with hydrolyzed casein-containing products, such as baby formula, may offer considerable browse protection when alternative forage is available.

# Pheromone-based attract-and-kill tactic for managing lepidopteran pests of crucifer crops: Is disruption of sexual communication a predictor of efficacy?

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Attract-and-kill (attracticide or lure-and-kill) techniques incorporating an attractant (e.g., pheromone) and a contact insecticide (e.g., permethrin) have shown significant promise for control of several lepidopteran pest species including *Pectinophora gossypiella*, *Epiphyas postvittana*, *Cydia pomonella*, and *Grapholita molesta*. Disruption of sexual communication (measured by trap shutdown) has been proposed as a key operative mechanism of this tactic. The diamondback moth (DBM), *Plutella xylostella*, cabbage looper (CL), *Trichoplusia ni*, and imported cabbageworm (ICW), *Pieris rapae* are the key pests of cabbage and other cruciferous plants (*Brassica spp.)* in North America. The larvae of the three species feed on the foliage and cause direct damage to the marketable leaves of cole crops, and are usually managed together as a single caterpillar complex, commonly referred to as the cabbage caterpillar complex. The sex pheromones of two of the species (*P. xylostella* and *T. ni*) have been characterized. However, a sex pheromone has not been characterized for the third species (*P. rapae*). The main objective of this study was to evaluate the potential of attract-and-kill as an alternative management tactic against the cabbage caterpillar complex.
We evaluated two pheromone-based experimental attracticide (Last Call$^{TM}$) formulations (IPM Tech. Inc, Portland, OR): Last Call$^{TM}$ DBM for *P. xylostella* and Last Call$^{TM}$ CL for *T. ni*. No Last Call$^{TM}$ formulation was available against the third species, *P. rapae*. Laboratory toxicity experiments confirmed the effectiveness of each LastCall$^{TM}$ formulations in killing conspecific males that made contact. In replicated small plots of cabbage and collards in central Alabama over four growing seasons (fall 2003, spring 2004, fall 2004, and spring 2005), an attracticide treatment receiving the two Last Call$^{TM}$ formulations, each applied multiple times at the rate of 1600 droplets per acre was compared against *Bacillus thuringiensis* subspecies *kursatki* (*Bt*) spray at action threshold, and a negative untreated control. Efficacy was measured by comparing among the three treatments male capture in pheromone-baited traps, larval counts in plots, and crop damage rating at harvest. LastCall$^{TM}$ provided acceptable pest control comparable to *Bt* in three of the four seasons. Efficacy of LastCall$^{TM}$ was dependent upon lepidopteran population densities, which fluctuated from season to season. In general, LastCall$^{TM}$ was effective at low to moderate population densities of the three species, such as typically occurs in the fall in central Alabama, but not in the spring when high *P. rapae* population pressure typically occurs in central Alabama. Significant reductions in pheromone trap captures did not occur in LastCall$^{TM}$ plots suggesting that elimination of males by the toxicant (permethrin), rather than disruption of sexual communication, was the main mechanism of effect.

# Behavioural evidence for a male-produced aggregation pheromone in *Sternechus subsignatus* Boheman (Coleoptera: Curculionidae)

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The Brazilian soybean stalk weevil, *Sternechus subsignatus* (Coleoptera: Curculionidae), is a pest of economic importance for the soybean producing areas in many regions of Brazil. The control of *S. subsignatus* is difficult, because their eggs and larvae are protected inside the host plant, predominantly within the main stems. These behavioural characteristics allow them to escape and survive from insecticide applications. Therefore, complementary strategies of pest management remain to be evaluated, including the use of semiochemicals. The objective of this study was to evaluate the presence of pheromone in the chemical comunication of *S. subsignatus*. Volatiles from both sexes of *S. subsignatus* were collected by aeration on Super Q, extracted with hexane, and concentrated using a stream of argon for one insect-equivalent (1IE). The behavioural response of males and females was evaluated using Y olfactometer. The treatments applied were host plant alone and a combination of volatiles released from males and females with the host plant, in different dosages; 0.3IE, 1IE, and 3IE. Pure air (to host plant) and hexane plus pieces of stem from the host plant (to extract) were used as control. The results obtained demonstrate that the communication among *S. subsignatus* conspecifics is mediated by the presence of semiochemicals, more specifically by aggregation pheromones, since there was significant attraction of both sexes to male insect volatiles when added to the host plant. Six male-specific compounds were detected in the chromatographic analyses, wich are the most important pheromone candidates in the species. Studies are underway to fully elucidate the structures of these compounds, in order to evaluate their biological activity on *S. subsignatus* conspecifics, under laboratory and field conditions.

# Is *Z*-9-tricosene (muscalure) required for house fly mating?

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The cuticular hydrocarbon *Z*-9-tricosene (trade name Muscalure), produced by the adult female, is regarded as a key sex pheromone component of the house fly (*Musca domestica*). *Z*-9-tricosene has been an additive to many toxic fly baits since its isolation and identification by Carlson et al. (1971) [1]. Darbro et al. (2005) [2] surveyed mostly F1-F3 progeny from ten wild house fly populations in southern California, and mean amounts of *Z*-9-tricosene on females (72-96 hours old) ranged from undetectable to 1113 ng /fly, or up to 5% of the total cuticular hydrocarbons. For this study house flies in the act of mating were collected from six dairies located in three USA states: southern California (hot and dry), Georgia (hot and humid) and Minnesota (cool and humid). The cuticular hydrocarbon profile of 500 mating females was determined by gas chromatography. All populations had some muscalure, but it was undetectable (<4 ng/fly) on 49.9% of the females overall. Females that did produce *Z*-9-tricosene had population means ranging from 15.55 ng/fly in Georgia to 268.9 ng/fly in southern California. Female and male age was determined by pterin analysis of fly heads. Mating females tended to be younger than males. Although muscalure accumulates with age in lab populations, it was significantly correlated with age in only one Minnesota population. The role of muscalure in the mating system of house flies will be discussed.

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# Allomones as sex pheromones in *Parastizopus* (Coleoptera: Tenebrionidae)

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Males of the genus *Parastizopus* exhibit a special pheromone emitting behavior. They do a headstand, expose the aedeagus and remain in this posture for a few seconds. During this behavior, the males use their aedeagus as evaporation surface for the pheromone. We collected the pheromones of *P. armaticeps* and *P. transgariepinus* by holding a SPME fiber (100 µm PDMS) close to the aedeagus and analyzed the composition by GC/MS. *P. armaticeps* emits a ternary mixture of *m*-cresol (52%), ethyl-1,4-benzoquinone (46%) and 3-ethylphenol (2%), whereas *P. transgariepinus* emits pure 1-tridecene. These substances are known to be defensive agents in tenebrionids, and at least ethyl-1,4-benzoquinone and 1-tridecene are compounds of the pygidial defensive secretion of both species. Nevertheless, as none of the other pygidial defensive substances were detected during calling, we could rule the pygidial gland out as pheromone source. In fact, the pheromone is produced in the aedeagal glands, a special feature in *Parastizopus*. This implies that in both species biosynthetic pathways of defensive secretion compounds are transferred from the pygidial gland to the newly evolved aedeagal glands. Associated with this spatial shift is a functional shift from allomones to sex pheromones.
Chemo-orientation experiments using a servosphere showed that the ternary pheromone mixture (1µg) in *P. armaticeps* and 1-tridecene (1µg) in *P. transgariepinus*, respectively, elicited upwind walking towards the pheromone source in females.

# Chemical ecology for the biological control of *Delia radicum* with *Aleochara bilineata* and *Aleochara bipustulata*: Characterization of the attractiveness of a single widespread molecule with original field bioassays

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The cabbage root fly (*Delia radicum*), whose larvae develop in Brassicaceae roots, is a major pest of cole crops in Brittany (France). The only chemical insecticide currently used with success against this pest will be banned in 2007 and other control solutions are urgently needed. Biological control with predators and parasitoids of the cabbage root fly is one of the methods considered. Two naturally occurring staphylinid species, *Aleochara bilineata* and *A. bipustulata*, are of major interest, being both predators of the eggs and larvae and parasitoids of the pupae of *D. radicum*.
In order to find ways to improve the presence and predatory efficiency of these ground dwelling coleopterans, we designed two original set ups allowing to test the effects of different volatiles directly in the field. The first one consisted of a matrix of "attractive mini-pitfalls", each containing a different test volatile. It yielded information on the attractiveness of up to seven volatiles tested simultaneously. The second was a matrix pattern of potted cabbage plants prepared in the lab and placed in a cabbage field. Each potted plant contained a different test volatile and allowed the insects to enter and leave. At each observation time point, this set up gave a measure of the density of staphylinids around each plant/volatile combination, taking into account both the attractiveness and the residence time of the predators in the pots.
Individuals from the field populations of the two staphylinids studied were strongly attracted by heavily infested brassica roots. Among the volatiles tested, one compound, found in abundance in the mixture of rotten tissues and *D. radicum* larvae, appeared to be especially attractive to both species. The discovery of the strong activity of this compound is a new step for the understanding of the chemicals interactions existing between the cabbage root fly, its host plants and its natural enemies. It opens new perspectives for the biological control of this major pest. It also offers an interesting case study of the use of a single widespread molecule as a key foraging information.

# Mating behavior and role of Brindley's and metasternal glands of the blood-sucking bug *Triatoma infestans* (Heteroptera: Reduviidae) vector of Chagas disease

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The possible existence of chemical signals involved in sexual behavior of triatomines has been studied. In the blood-sucking bug *Triatoma infestans* an assembling behavior of males around a mating pair has been described, probably induced by chemical volatiles released by the couple. Adult triatomines posses two pairs of main exocrine glands: Brindley's and metasternal glands. Evidences supported a role as an alarm pheromone for volatiles from Brindley's glands, although some reports also associated these glands to sexual behavior of triatomines. The blend produced by Brindley's glands is a complex mixture of volatiles, including a ketone (2-butanone), several aliphatic alcohols, esters, fatty acids and an aromatic alcohol, being isobutyric acid its main component. A less complex blend composed of ketones and alcohols, being 3-pentanone the main constituent, was recently identified from *T. infestans* metasternal glands. It has also been shown that both exocrine glands discharged their volatiles when adults are mechanically disturbed. In addition, it has been occasionally detected volatiles emitted by metasternal glands during copula. Here we tested if both kinds of glands are involved in different features related to sexual behavior. We performed a selective occlusion of both types of glands of the female, separately and together, and analyzed the behavior of males. An experimental arena, under controlled environmental conditions and a video equipment for the registration of the insect behavior and data storage was used. On every assay, four males and one female were released in the arena. We quantitatively recorded: a) the mating frequency, b) males' copulatory attempts frequency, and c) the spatial distribution of males in presence of a mating couple. Our results show that metasternal glands are involved in the sexual behavior of *T. infestans*. The volatile compounds eventually emitted by the metasternal glands of the female promote mating occurrence, while males' mating attempts are not affected. Besides, these glands also promote the assembling behavior of males around a mating pair previously described for this species. On the other hand, Brindley's glands of the female do not affect mating frequency or assembling behavior of males. These evidences will allow a potential chemical manipulation of the behavior of *T. infestans* using sex pheromones. We discuss the possible role of these glands in the biology of this vector of Chagas disease.

# Oviposition deterrence of *Minthostachys* species (Lamiaceae) against the potato tuber moth

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The potato tuber moth is a noxious pest of potato under storage conditions. The use of repellent plants is an environmentally sound alternative to the application of chemical pesticides in stores. First, we evaluated the protective effect of native *Minthostachys* species (Lamiaceae) against tuber infestation by the potato tuber moth in a rustic store in Cusco, Perú. We covered potato tubers with dried shoots of *M. spicata* and *M. glabrescens* and compared tuber damage with a control treatment of corn stubble. Second, we conducted a no-choice oviposition bioassay in the laboratory, testing the oviposition deterrence of essential oils of *M. spicata*, *M. glabrescens* and *M. mollis* at natural concentrations. We recorded the number of eggs laid by mated moths on filter paper treated with essential oils of each of the three species and on two control treatments: filter paper treated with solvent (hexane) and untreated oviposition substrate. Third, replicating the former bioassay, we tested for differences in oviposition deterrence among 5 full-sib families of potato tuber moth raised under identical conditions. Thus, we estimated whether the response towards repellent essential oils from *Minthostachys* species exhibited genetic differences within an experimental population of the insect pest. We found that dried, chopped leaves and flowers of *Minthostachys* species reduced the percentage of tuber damage in stores in comparison with the control (5% vs. 12%), but no difference in protection was found between species. Essential oils at natural concentrations deterred moth oviposition, reducing the number of laid eggs approximately in 80% in comparison with control treatments; again, there were no significant differences among *Minthostachys* species. Finally, whereas we detected among-family variation in oviposition on filter papers treated with essential oils, no difference was found in the number of eggs laid on control substrates. Therefore, there was genetic variation for oviposition deterrence in the potato tuber moth and then resistance to repellent plants might evolve.

# Drugs from bugs – Ecological functions of antimicrobial active compounds from insect bodily fluids and their possible applications in plant protection

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The antimicrobial activities of several insect bodily fluids (e.g. hemolymph, regurgitate, or exocrine glandular secretions) were detected in recent years. One of their ecological functions is the protection from infections by entomopathogenous fungi and bacteria, what is known for low mass chemicals from exocrine glandular secretions (e.g. salicylaldehyde, iridoid monoterpenes, aliphatic acids and alcohols) or regurgitate of leaf beetle larvae (Coleoptera: Chrysomelidae) [1, 2], as well as sawfly larvae (Hymenoptera: Tenthredinidae) (data presented here), and antimicrobial peptides (cecropins, attacins, defensins) in hemolymph [3, 4].
Phytopathogens are responsible for enormous crop losses worldwide and therefore threaten human nutrition. In order to explore new applications to fight phytopathogens, we embark on two strategies: (1) Testing of low molecular mass compounds from insect exocrine secretions for external application on plants infested by phytopathogens. Here we present the first results of our investigations: Whole insect bodily fluids (hemolymph, regurgitate) as well as several synthetic components from leaf beetle and sawfly larval secretions (e.g. hexanal, trans, trans-2,4-decadienal, salicylaldehyde) were tested in agar diffusion assays against a phytopathogenous bacterium (*Erwinia amylovora*) and fungus (*Venturia inaequalis*), revealing significant reductions in bacterial growth and fungal germination. (2) The transfer of defence weapons of insects into plants for engineering of disease resistant crops. Transgenic expression of the antifungal insect defensin gallerimycin under control of the mannopin-synthetase promoter is demonstrated to confer resistance against powdery mildew (*Erysiphe cichoracaerum*) in tobacco.

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# Field evaluation of the synthetic sex pheromone from the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Brazil

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The citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a major pest of *Citrus* spp. throughout the world. Traps baited with a binary mixture in the ratio of 3:1 of two EAG-active compounds, (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal, attracted significantly more moths of the citrus leafminer *P. citrella* compared with traps baited with five virgin females and unbaited traps in Brazilian citrus grove. The addition of a third EAG-active compound, (Z)-7-hexadecenal, did not increase trap catch. We did not observe a significant difference in catches by traps loaded with low (50 μg) and high (500 μg) doses of synthetic sex pheromone. These results suggest that a long-lasting, low-releasing pheromone dispenser can be designed to monitor populations of the citrus leafminer.

# New method to determinate pheromone release rate based on thermal desorption and gas chromatography-mass spectrometry

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Many fight methods against pests are based on lures or mating disruption. In both of them, are very important to know the real active compound or pheromone release rates in order to assure that the dispenser are working adequately.
Our laboratory has developed a method to measure the release rate of several compounds during a short time period based on thermal desorption and GC/MS.
The dispensers are submitted to controlled air flow and temperature conditions and the released compound is adsorbed on a Tenax® trap as solid porous adsorbent. Once collected, the sample is thermally desorbed and analyzed by GC/MS system.
An assay was carried out for two Trimedlure (TML) commercial dispensers. Release rates were determined for each dispenser's type at several aging times.
Preliminary assays showed marked differences in the amount of TML released between different types of commercial dispensers and at different aging times.
Second assay was carried out for SELIBATE CS®, mating disruption dispenser of *Chilo suppressalis* Walker.
This method is related with residual amount of TML in the dispensers quantified by solvent extraction and GC analysis.
The variation in release performance of commercial dispensers demonstrates the convenience of routine evaluation of them. This non-destructive method allows to evaluate quick and accurately the current behaviour of dispensers along their useful life.

# Studies on different pheromone dispenser densities to control *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) by mating disruption

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*Chilo suppressalis* Walker is one of the most important rice pests world wide. Rice is frequently grown in an intensive production system in areas adjacent to environmentally sensitive coastal areas, estuaries or deltas. Water run-off contains harmful rice pesticide residues which can cause widespread damage to food chains of the wildlife inhabiting these areas. Therefore, the use of insecticides is problematic.
In Valencia, the control of *C. suppressalis* is subsidized by the government and treatments are supervised by the local office of Ministry of Agriculture. In the last years, they have tested other dispenser densities such as 69 and 51 dispensers $ha^{-1}$ (12 and 14 meters respectively), depending on the infestation history in each area. These densities are lower than the density commercially recommended (100 dispensers $ha^{-1}$). By decreasing dispenser density, the mating disruption efficacy approached 100%. In 2003, a mating disruption control was conducted on 68.2% of the rice growing area. In half of the area 69 dispensers $ha^{-1}$ we used and in the other half 51 dispensers $ha^{-1}$. In the remaining surface a chemical control was carried out with tebufenozide 24% (0.6 L $ha^{-1}$), a low toxicity insect growth regulator (IGR).
It is not feasible to treat with pheromones all the rice growing area due to their high cost. Hence, the optimisation of the technique is essential to expand its use into the total rice area and to eliminate chemical treatments, which cause severe injuries to the environment.
In this way, we proposed a wide-area trial to test several dispenser densities, 39, 31, 25 and 16 d $ha^{-1}$, i.e. 16, 18, 20 and 25 meters between dispensers. Our objective was to determinate the minimal necessary dosage and the optimal dispenser distribution for the mating disruption treatment to be effective. We have carried out a first field trial with three dispenser densities during three years, from 2003 to 2005, in a 100 ha-plot. Subsequently, a wide-area trial was conducted in almost 15.000 ha to check the efficacy of 39 d $ha^{-1}$ to control the rice stem borer. We have also studied the chemical properties of SELIBATE CS® (Aragonesas Agro, Madrid, Spain) dispensers to check if their pheromone release rate is enough to control the three generations of *C. suppressalis* which are normally present in Spain.

# Cuticular hydrocarbons of the woodwasp *Sirex noctilio* (Hymenoptera: Siricidae)

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Our major goal was to characterize the cuticular components of *Sirex noctilio*, a worldwide pest of pines. Despite detailed reports on oviposition, egg-hatching, and larval behavior, little is known about its mating behavior and the semiochemical compounds involved in courtship activity. We identified components of the hexane body-wash by gas chromatography (GC) coupled with mass spectrometry (GC-MS) and found a significant qualitative difference between the two sexes. Specific for the female cuticle are mono- and diunsaturated hydrocarbons with a chain length of C23-C33. The most abundant unsaturated female compounds are 7- and 9-heptacosene, and 7- and 9-nonacosene. Position of the double bonds was determined by the addition of dimethyl disulfide followed by GC-MS analysis. Male body-washes on the other hand contain mono- and dimethyl-branched saturated hydrocarbons in higher concentrations. Moreover, saturated straight-chain hydrocarbons are present in both sexes. Cuticular hydrocarbons are well known as recognition cues in Hymenoptera and likely play an important role in mating behavior.

# Potential sex pheromone components of the plant bug *Macrolophus caliginosus*

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The zoophytophagous plant bug *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) occurs naturally in the Mediterranean region and it is currently commercialized in Europe mainly for the biological control of whiteflies and other pests of greenhouse tomato crops. Conservation and management of their natural populations is another control strategy currently applied in outdoor tomato crops in the Mediterranean region. For its proper use in biological control programs, monitoring of their populations becomes necessary. The family Miridae is atypical among the Heteroptera in that females, not males, release volatile sex pheromones. Attraction has been demonstrated in about 15 species and the sex pheromones of a dozen species have been identified so far. The purpose of our study is to identify a sex pheromone that could potentially be used to monitor *M. caliginosus* populations.

Whole body hexane extracts from virgin males and females were analyzed by gas chromatography-mass spectrometry. A few isolated peaks appeared in extracts from both sexes. Two female-specific compounds (1 and 2) had mass spectra similar to hexyl (3R)-hydroxybutanoate, a pheromone component of two cocoa mirids (*Distantiella theobroma* and *Sahlbergella singularis*), with which *M. caliginous* is taxonomically related. Acetylation of this compound resulted in a molecule whose mass spectrum (EI and CI) and chromatographic behaviour were identical to those of compound 1. Compounds 1 and 2 were identified as hexyl 3-acetoxybutanoate and octyl 3-acetoxybutanoate, respectively. Both compounds were synthesized from the corresponding acid to determine the stereochemistry of the natural products.

The structural similarity of the female-specific compounds with the field tested sex pheromone of the cocoa mirids suggests that they are part of the sex pheromone of *M. caliginosus*. Behavioral assays will test this possibility.

# Calling behavior of *Lonomia obliqua* (Lepidoptera: Saturniidae): Identification and synthesis of the sex pheromone

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The behavior of *Lonomia obliqua* Walker, 1855 (Lepidoptera: Saturniidae) mediated by sex pheromone was studied through the calling behavior of females in laboratory. It was observed that all callings occurred during scotophase, in which 70.6% of the active females called for the first time at 24h of life, 27.8% from 24 to 48h and 1.6% from 48 to 72h. The females started calling at 360min (± 12.8min) and at 290min (±23.9min) on the first and sixth day respectively, delaying the calling with the age. The mean of Total Calling Female Time (TCFT) was 212min (±99min). The average duration of calling was 191min (±97min) on the first day, increasing to 262min (±127min) with six days old females.
The sex pheromone gland was extracted from virgin females from one to three days old, during the calling peak, and the electrophysiological analyses (GC-EAD) indicated the existence of three EAD-active components on the gland extracts. GC-MS (EI and CI) analysis of the major EAD-active peak gave identifying features for a monounsaturated C16-acetate. Chemical derivatization (DMDS reaction) indicated a Δ11 unsaturation on the molecule. Synthetic samples of (*E*)-11 and (*Z*)-11-hexadecenyl acetate were easily obtained by coupling 1-bromo-10-decanol and 1-hexyne, utilizing lithium chemistry. Further GC-analyses on the retention time led to full characterization of the major component as (*E*)-11-hexadecenyl acetate. The minor constituents were identified as the related alcohol, (*E*)-11-hexadecenol, and aldehyde, (*E*)-11-hexadecenal. The ratio for the three components was 1:0.35:0.02 (*E*11-16:Ac : *E*11-16:OH : *E*11-16:Ald). Preliminary indoor bioassays with a mixture of synthetic samples were not significantly different from the response of the males to the gland extract.

# (*Z,E*)-11,13-Hexadecadienyl acetate - Sex pheromone of the grass webworm *Herpetogramma licarsisalis*: Identification, synthesis and field bioassays

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The grass webworm *Herpetogramma licarsisalis* (Walker 1859) (Lepidoptera: Crambidae), an important pest of many tropical and subtropical grasses used for pasture and amenity turf, has recently established in kikuyu dairying pasture in Northland, New Zealand. We undertook the identification and synthesis of the sex pheromone of *H. licarsisalis*, to provide a tool for routine monitoring in the infested area. Two compounds, (*Z*)-11-hexadecenyl acetate (*Z*11-16Ac) and (*Z,E*)-11,13-hexadecadienyl acetate (*Z*11,*E*13-16Ac), were identified from gland extracts of female grass webworm moths, using gas chromatography (GC), GC-electroantennographic detection (EAD) and GC-mass spectrometric (MS) analyses, in conjunction with microchemical techniques (dimethyldisulfide and 4-methyl-1,2,4-triazoline-3,5-dione derivatizations). Field bioassays in Northland, New Zealand (2005), and in Brisbane, Australia (2005, 2006) confirmed that *Z*11,*E*13-16Ac was necessary and sufficient for capture of male grass webworm moths. The pheromone is presently being employed for monitoring of male moth flight activity in New Zealand, and has also been used successfully to trap the species in Hawaii.

# Structure-activity relationships of phenyl propanoids 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) and in the bark of lodgepole pine *Pinus contorta* have been shown to evoke antifeedant effects on *H. abietis*, which is a serious pest of planted conifer seedlings in Europe. In tíos study we evaluated phenyl propanoids as antifeedants for *H. abietis*. Structure-activity relationships were identified by bioassaying related compounds obtained by rational synthesis of functional group analogs and structural isomers. Small variations in the structures of the analogs led to big changes in the antifeedant activity. The Topliss scheme was evaluated as a tool for finding structure-activity relationships and a substance with optimal antifeedant effect. A number of physical parameters (Hansch parameters) were compared aiming at finding the properties of the compounds most important for antifeedant effects. The overall aim was to find commercial antifeedants to protect conifer seedlings against pine weevil damage in forest regenerations. Several potential candidates for practical use as antifeedants have been identified.

# Evaluation of certain plant extracts for their larvicidal activity on the 3rd instar larvae of *Hyblaea puera* Cramer (Hyblaeidae), the defoliator pest of teak (*Tectona grandis*)

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The problems caused by pesticides and their residues have increased the need for effective, biodegradable pesticides with greater selectivity. Alternative strategies have included the search for new types of insecticides and the re-evaluation and use of traditional botanical pest control agents. In the present study, different extracts of three plants namely, *Pterocarpus marsupium* Roxb. (Fabaceae), *Acacia concinna* Lour. (Mimosaceae) and *Lobelia nicotianaefolia* Heyne (Lobeliaceae) have been evaluated for their larvicidal activity on *Hyblaea puera* Cramer (Hyblaeidae), the most important defoliator pest of teak (*Tectona grandis*). Extracts of the heartwood of *P. marsupium*, pods of *A. concinna* and leaf of *L. nicotianaefolia* prepared in seven solvents namely petroleum ether, methanol, chloroform, acetone, ethyl alcohol, ethyl acetate and water were tested for their contact toxicity on the 3rd instar larvae of *H. puera* at a concentration of 2 mg/4 ml. Based on the result these extracts were again tested with 4 concentrations each serially diluted from the stock of 2 mg/4 ml. Among the extracts tested ethyl alcohol and methanol extract of *P. marsupium* brought about 70% mortality. The water extract of *P. marsupium* was the most effective causing 90% mortality of the larvae. The ethyl alcohol, ethyl acetate, methanol and water extract of *A. concinna* showed 100% mortality and the entire extracts of *L. nicitianiaefolia* showed 100% mortality. The data was subjected to probit analysis to calculate $LC_{50}$. The statistical analysis showed that all the three plants had significant insecticidal property and among them *L. nicotianifolia* possess most potent insecticidal property.

# Study of control of mosquito *Aedes aegypti* with plant extract

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The mosquito *Aedes aegypti* L. (Diptera: Culicideae) is the main vector of yellow fever and dengue diseases in tropical regions. The insecticide-driven control of mosquitoes has gradually lost its efficiency due to their high genetic plasticity.
Great attention has been paid to the search of natural insecticides for the control of this vector in the last years. In a program of randomic collection of plants and in the search for new drugs with both larvicidal and adulticidal actions, we have investigated plant extracts. Over 120 extracts have been tested and the ones with higher larvicidal activity have been submitted to guided fractionation by assay using L4-stage larvae. Identification has been carried out by Infrared, Ultraviolet , Hydrogen and Carbon 13 Nuclear Magnetic Resonance and Mass Spectra. In this study, Annonas have shown to be a very active genera, having acetogenins as active principles, while in the Leguminoseae family, the plant *Pterodon polygalaeflorus* presented vouacapan-type diterpenes as the main active principle.

# Mating disruption experiments of the Mediterranean corn borer *Sesamia nonagrioides* with a trifluoromethyl ketone analogue of the pheromone

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The Mediterranean corn borer *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae) is one of the most important pests of maize in the Mediterranean region and North Africa countries. The sex pheromone of this species has been identified as a blend of (Z)-11-hexadecenyl acetate, (Z)-11-hexadecenol, (Z)-11-hexadecenal and dodecyl acetate in a 77:8:10:5 ratio [1].
Trifluoromethyl ketones (TFMKs) are compounds that can inhibit a variety of esterases and proteases, particularly the esterases present in olfactory tissues of insect antennae. These are key enzymes for a rapid degradation of pheromone esters, thus maintaining a low stimulus noise level in sensory hairs, and their inhibition could lead to a disruption of the chemical communication between sexes. In this work we present the results of the mating disruption experiments carried out in 2004 and 2005 to control *S. nonagrioides* using (Z)-11-hexadecenyl trifluoromethyl ketone (Z11-16:TFMK), an analogue of the main component of the pheromone. The tests were conducted in maize fields of the Lleida province and the efficiency of the mating disruption was evaluated in terms of the number of infested plants and number of attacking larvae per plant in treated and control plots. The results show that, for low infestation levels, Z11-16:TFMK is a good pheromone antagonist able to induce a 71-97% reduction of the number of plants attacked in 2004 and 71-86% in 2005, and 72-97% reduction of number of larvae per plant in 2004 and 57-67% in 2005. In addition and surprisingly, the antagonist also reduces the damage induced by the European corn borer *Ostrinia nubilalis* in the treated plots. Although more tests should be done to confirm these results, they suggest that Z11-16:TFMK may be considered as a promising control agent for the pest in future IPM strategies.

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# Antagonism of the pheromone response of males of the leopard moth, *Zeuzera pyrina* L.

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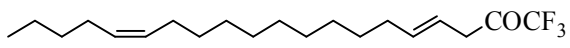
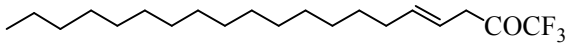
The leopard moth *Zeuzera pyrina* L. (Lepidoptera: Cossidae) is a wood borer of wide distribution, particularly in the Southern regions of Europe, Asia and North Africa. The larvae inflict serious damage on pear, apple, hazel, cherry and olive trees by excavating galleries in shoots and branches, which could eventually cause the death of the tree. The pheromone composition of the moth was identified as a mixture of (*E*,*Z*)-2,13-octadecadienyl, (*E*,*Z*)-3,13-octadecadienyl and (*Z*)-13-octadecenyl acetates in a 78-86:4:18-10 ratio [1][2].
In the course of our work directed to develop pheromone perception inhibitors of insect pests, we present herein the antagonistic activity of the trifluoromethyl ketones **1**-**4**, analogues of the pheromone components of the leopard moth, in electrophysiology, wind tunnel and in the field.




**1**

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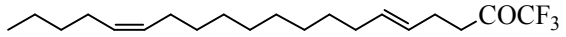
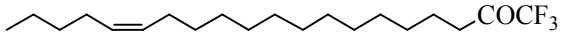



**3**




**4**

Our results show that compound **3** induced males to track erratic flights towards a female source, and, consequently, a remarkable decrease of the number of males contacting with the source. In the EAG and in the field, the most effective antagonist was compound **1**, the analogue of the major component of the pheromone, which significantly reduced the number of male catches when mixed with the pheromone in 5:1 ratio in comparison with the pheromone alone.

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# Comparative biological activity of two pheromone antagonists of the European corn borer *Ostrinia nubilalis*

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The European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) is a major pest of maize and other crops, such as potato, green pepper and winter wheat, in Europe, North America, North Africa, Philippines and Japan. The insect displays polymorphism in its pheromone communication system, and thus whereas the *Z* strain uses a blend of (*Z*) and (*E*)-11-tetradecenyl acetates in 97:3 ratio, the *E*-strain utilizes the same compounds in blends from 1:99 to 4:96 ratio.

In our search for new antagonists of the pheromone action of insect pests, we have found that (Z)-11-tetradecenyl methyl ketone, an analogue of the major component of the Z strain pheromone, induced similar type of effect in laboratory bioassays and in the field than the corresponding fluorinated analogue (Z)-11-tetradecenyl trifluoromethyl ketone. Both compounds induced in the field a remarkable decrease in catches when mixed with the pheromone compound in 10:1 and 5:1 ratios, and in wind tunnel a notorious decline of the number of males contacting with the source. In addition, both compounds display some electrophysiological activity in EAG. To explain these effects, we hypothesize that both compounds inhibit the pheromone action through competitive interaction with the parent pheromone for the same olfactory receptor neurons. However, in esterase inhibition assays the methyl ketone did not elicit any inhibitory action (IC50 > 100 μM) while the fluorinated analogue displayed excellent activity (IC50 0.07 μM). Therefore, the fluorinated ketone exerts its action also by inhibiting the antennal esterases of the insect in contrast to the non-fluorinated ketone.

# Development of monitoring traps for the orange wheat blossom midge, *Sitodiplosis mosellana* in the UK

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The orange wheat blossom midge, *Sitodiplosis mosellana*, is a common and increasingly important pest of wheat in the Northern Hemisphere, causing severe yield losses in years of high infestation. Initial infestations are difficult to detect and wheat growers in the UK have become increasingly concerned with this pest. A pheromone trap, which is now commercially available, has been developed to meet their demand for a more reliable monitoring system. Field trapping experiments with synthetic 2,7-nonadiyl dibutyrate [1], the female produced sex pheromone of *S. mosellana*, demonstrated that pheromone traps were highly attractive to males, caught very few non-target organisms and gave a good indication of peak midge emergence and abundance of midges throughout the season. A reasonable correlation between maximum trap catch and subsequent midge infestation was found.
Investigations into variation of trap catches at the field level and farm level have indicated that there can be very large variations in trap catch even between fields on the same farm. However, grids of traps set up within individual fields indicated that there was less variation in trap catch at the field level. The very patchy distribution at the farm level may be related to differences in soil moisture level in different fields which could affect midge emergence. Comparisons of male catches in pheromone traps with catches of females on adjacent yellow sticky traps showed that in certain situations the movement of females from emergence sites could influence subsequent infestation levels in the crop. Males mate with females at emergence sites and are less mobile. As the pheromone traps only catch males the movement of egg-laying females from emergence sites is a factor which complicates the assessment of risk to crops when using pheromone trap data. However pheromone traps were much more selective and caught higher numbers of insects than yellow sticky traps. It would be advantageous to develop a trap which selectively caught female *S. mosellana*. Although we have identified a blend of kairomones which is attractive to female midges under laboratory conditions [2] we have yet to develop a field formulation of these compounds that maintains ratio integrity which is a crucial part of the signal [3].

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# Pheromones and kairomones for control of the sorghum chafer, *Pachnoda interrupta*, through mass trapping

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The sorghum chafer, *Pachnoda interrupta* (Coleoptera: Scarabidae), is a major pest on sorghum that is an important Ethiopian staple crop. High population densities and recurring invasions from surrounding shrub- and bushland make it a difficult pest to control. In addition, application of insecticides is labour-intensive, and their use also has detrimental effects on human health and environment. Our objective is to improve a locally developed "lure and kill" trapping method by replacing the current bait (fruit) with kairomones from host plants combined with aggregation and/or sex pheromones. Field trapping with single synthetic odour compounds – identified from sorghum and alternative hosts using GC-EAD followed by GC-MS – show promising results, with a trapping efficiency comparable to that of a complex natural odour source, mashed banana. Trap catch with live beetles as bait indicate the presence of a female-emitted sex pheromone. Traps baited with virgin females caught significantly more males than traps baited with mated females. There are also indications of an aggregation pheromone active outside of the mating season, where traps baited with beetles and food caught more beetles than either food or beetles alone.

# The suitability of sex pheromone traps for implementing IPM strategies against *Agriotes sordidus* illiger (Coleoptera: Elateridae)

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The total catches and seasonal abundance of *A. sordidus* was examined in La Rioja (Spain) during the last 4 years. They were used sex pheromone traps (YATLOR funnel, Furlan et al, 2001) with specific pheromone, made at Plant Protection Institute of Budapest. Monitoring covered the period from late March until late September. The pheromones were replaced every 40 days. There was only one trap/field, and grows were mainly potato, pea, wheat and sugar beet, There were 1 field in 2002 and 2003, 10 in 2004 and 2005, and 5 in 2006.
In each field were placed 10 bait-traps, made and used according to the description given by Chabert & Blot, 1.992. This consisted of a plastic pot 10 cm in ? with holes drilled in the bottom; these pots were filled with vermiculite, 30 ml. of wheat seeds and another of corn seeds. The pots were wetted before being placed into the soil just below the surface and covered with an 18 cm ? plastic lid placed a few cm above the rim of the pot. Traps were checked by hand-sorting the contents after 10 days and wireworm extracted were counted.
The total catches / pheromone trap and year, seasonal catches, catches /grow are presented.
The relationship between pheromone trap catches of adult click beetles and wireworm catches in bait-traps during the first 40 days after sowing time for any year is very clear, and for every years is also significative..
The relationship with beetles catches of the year before and the number of larvae in the bait traps on the next year could be in the same way.
The use of this sex pheromone traps can help us to improve the wireworm control in potato grows.
If we use them the year before we can avoid fields with high populations, and if we use them after sowing time during 40 days, we will know the risk of damage and if we need improve the control in our crop one year.

# Biological solution for control of *Ceratitis capitata* by mass trapping

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The Mediterranean fruit fly *Ceratitis capitata* is one of the most destructive agricultural pests worldwide. It is native of sub-Saharan Africa and has a long history of invasion success.
Cera Trap® is an attractant formulation free of pesticides based on a liquid protein from pig intestinal mucosa obtained by an exclusive method of enzymatic hydrolysis.
The med fly, strongly attracted, enters into the traps (McPhail model or similar) baited with 200-400 mL of Cera Trap® and, not being able to escape, drowns and dies in the liquid.
Field trials conducted in San Pedro Pinatar, Murcia (Spain) with sensitive mandarin trees (cv. Marisol and Beatriz) have shown that the capture levels of this system were as good as those obtained with the standard product (ammonium acetate, trimethylamine and putrescine) with the advantage that in our case the use of a DDVP strip or other insecticide is not necessary as it is in the standard.
Volatiles release rate determination studies were conducted in a thermal chamber Dycometal CETM-25/81 and have been done both: in isothermal conditions and as a function of temperature.
These assays show that in conditions of fixed temperature the rate emission is linear in time and that the kinetic is strongly dependent on temperature.
Volatile and semi-volatile compounds were isolated by headspace trapping and identified using coupled gas chromatography-mass spectrometry (GC-MS) followed by comparison of mass spectra with those in the Wiley 7$^{th}$ Edition spectral library. The main volatile compounds identified in Cera Trap® were piperazindiones with different substituents (pyrrolo(1,2-a)pyrazine-1,4-dione, 3-Isobutylhexahydropyrrolo(1,2-a)pyrazine-1,4-dione and 3-benzyl-1,4-diaza-2,5-dioxobicyclo(4,3,0)nonane).
A more exhaustive identification analysis of these semiochemicals and further studies of their role as fly attractants are currently being done.

# Sex attractant pheromone from the rice stalk stink bug, T*ibraca limbativentris* Stal (Hemiptera: Pentatomidae)

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The male-produced sex pheromone from the Brazilian rice stalk stink bug is reported. Olfactometer bioassays with sexually mature males and females showed that males attracted females, suggesting that males release a sex pheromone for this species. Males were not attracted to either sex, nor were females attractive to conspecific females. Attraction of the females to males was significantly higher at night time. The headspace volatiles collected from male and female bugs, *Tibraca limbativentris* were analyzed by GC and GC-MS. Two male-specific compounds were identified as isomers of 1'*S*-zingiberenol in extracts from males, whereas a series of defensive compounds were identified in extracts from both sexes. Synthesis was undertaken for the isomers of zingiberenol that contains three chiral centers in the structure. The synthetic procedure produced two groups of material, zingiberenol-I containing 4 isomers, namely (1RS,4RS,1'R)-4-(1',5'-dimethylhex-4'-enyl)-1-methylcyclohex-2-en-1-ol, and zingiberenol-II containing 4 isomers, namely (1RS,4RS,1'S)-4-(1',5'-dimethylhex-4'-enyl)-1-methylcyclohex-2-en-1-ol. Bioassays carried out with the two groups of stereoisomers revealed that the group of zingiberenol-II, was as attractive to females as the crude extract of male volatiles. The absolute configuration of the two pheromonal components remains to be fully elucidated but the 1'S stereochemistry is established for both isomers.

# Pheromone baited traps as a monitoring technique for the neotropical brown stink bug, E*uschistus heros* (Fabricius) (Heteropera: Pentatomidae)

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Stink bugs are the most important pests of soybean in Brazil and other countries of South America. These insects form a complex, with species composition and abundance varying between regions. In the last years the neotropical brown stink bug, *Euschistus heros*, has increased its importance becoming the major pest in some regions of Brazil. Nowadays, more than four million liters of chemical insecticides are used annually to control stink bugs in Brazil. New and alternatives techniques to control these pests might contribute to a more ecological approach to their management. This work presents the results of the studies with the principal component of sexual pheromone of brown stink bug in order to develop a population monitoring technique for management decisions. Synthetic methyl 2,6,10-trimethyldecanoate formulated in lure device (Fuji Flavor-Japan) was used in traps assembled with two liters transparent plastic (PET) bottles of soft drinks. Experiments conducted during two consecutives years in laboratory and in field conditions showed that: a) traps baited with 1 mg of pheromone attracted efficiently females of brown stink bugs, b) the lure device maintained their attraction efficiency for more than 30 days and their liberation rate followed an inverse sigmoid curve, c) baited traps attracted stinkbugs for distances over 60 m, in field conditions, d) When the lures were replaced each 30 days reached an efficiency similar to shake cloth sample technique, normally used to monitor stink bug populations, e) traps were more efficient than cloth sample technique in low population densities (< 3 individuals per linear sampling unit (1 m long), f) pheromone traps may be distributed only in the borders of the soybean fields in the density of 1 trap/ha. Results from laboratory and field tests showed that this technique can be an efficient alternative to population monitoring of the neotropical brown stink bug for the Brazilian conditions.

# In vitro evaluation of chemicals from two exocrine glands of *Atta* species against human pathogenic microorganisms

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Leaf-cutting ants secrete chemicals from their exocrine glands, specially from the mandibular and metapleural glands. These substances are regarded as alarm pheromone and proteting barrier for their mutualistic fungus, respectively [1][2][3]. Considering these informations, antimicrobial assays "in vitro" were conducted using hexane and aqueous solutions of alkanols, terpenoids and carboxylic acids, and wild and resistant strains of the following pathogens *Escherichia coli, Staphylococcus aureus* and *Candida albicans* to find out if these solutions would exert any inhibitory effect on these microrganisms. The activity of the testing solutions was measured by means of the technique described by Bauer et al. (1966) [4], which measures the inhibition of the microorganism growth in the vicinities of an area where the solutions were applied. The results demonstrated that most of the solutions tested were very effective to inhibit bacteria and fungi, including those that are resistant to antibiotics commonly used to fight human infections. Among these compounds, the monoterpenoids, citral and geraniol, the alcohol, 4-methyl-3-heptanol and the acids hexanoic and octanoic were very effective against strains of *Candida albicans*. These findings suggest that these chemicals may be used to inhibit the growth of pathogenic strains.

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# Is there any similarity between the volatile compounds released by *Anastrepha obliqua* calling males and their preferential and alternative host fruits?

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Males of fruit flies (DIPTERA: TEPHRITIDAE) release a complex blend of volatiles compounds to attract co-specific males and females (aggregation pheromone). Males fruit fly from the genus *Bactrocera* use chemicals acquired from their host by feeding as part of their aggregation pheromone [1]. Considering this information, studies were performed aiming to find out if the chemical constituents released by preferential, *Averrhoa carambola* L. (starfruit) and alternative, *Carica papaya* L. (papaya) fruit hosts whose fruit fly larvae fed on, exert any influence in the chemical composition of the odours released by calling males of *Anastrepha obliqua*. The volatile compounds from *A. obliqua* calling males and those released by their mature host fruits were extracted by means of headspace technique using Tenax® and activated charcoal as adsorbents. Desorptions were carried out by use of HPLC grade *n*-hexane and the resulting extracts were analysed by GC-MS. The results demonstrated that the chemicals found solely in papaya fruits were also present in extracts of *A. obliqua* calling males, including linalool which was not found amongst the volatiles released by starfruits and also in extracts of fruit flies whose larvae fed on this fruit, showing that the volatile ingested by these insects at the larvae stage may be used by adults as part of their sex pheromone bouquet.

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# Chemical mimicry by the ectoparasitic mite *Varroa* destructor infesting *Apis cerana* and *A. mellifera* broods

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*Varroa destructor* is an ectoparasitic mite of the honeybee. Its original host is *Apis cerana* (*Ac*) in Asia but it has become a severe threat for *A. mellifera* (*Am*) in Europe and in the rest of the world. The mite feeds and reproduces on the honey bee brood. Contact between the founding *Varroa* female and *Apis* fifth instar larvae prior to cell capping is a key factor triggering mite reproduction.

In Asia, *Ac* and *Am* are found in the same area and even in the same apiaries. When *Ac* tolerates the mite, chemical control is necessary to protect *Am* from the mite. Chemical communication in involved in the resistance of the bee to the mite. Chemical mimicking by the mite probably plays an important adaptive role in the infestation process since the original host *Ac* has a highly developed capacity for detection, whereas the detection capacities of the new host *Am* are extremely limited. In this framework we studied the chemical signature and camouflage used by *Varroa* infesting *Ac* and *Am* broods.

To study factors underlying this chemical mimicking, we performed tests involving transfer of mites from an *Ac* brood to either another *Ac* brood or an *Am* brood, or of mites from an *Am* brood to either another *Am* brood or to an *Ac* brood. The chemical signatures of both *Apis* nymphs and their *Varroa* parasites were studied for all 4 transfer situations.

Analysis of mitochondrial polymorphism has indicated that *Varroa* parasitizing *Ac* colonies presented a different haplotype from *Varroa* parasitizing *Am* colonies, even if the colonies of the two bee species are located in the same place.

We found that the mites are first mimicking the cuticular components of the host on which they are placed, whatever their haplotype. But the camouflage of the Varroa mite from *Ac* is more efficient. The two haplotypes develop an astonishing ability for mimicry depending of the evolutive pressure of the host.

# Host-detection and host-recognition variability in *Cotesia plutellae*: A possible origin of control failure in *Plutella xylostella* management?

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Brassicaceae crops make up a key vegetable production on a world scale. One of its most destructive pests is the diamondback moth (DBM), *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera, Plutellidae), whose caterpillars attack leaves and can cause almost 90% crop loss. Resistance toward many insecticides leads to more difficulties for DBM management. Among the numerous alternative control techniques, parasitoids are the most used, *Cotesia plutellae* (Kurdjumov, 1912) (Hymenoptera, Braconidae) being a prime example. However, numerous attempts to introduce it into various areas of the world have often yielded mediocre results. Explanations of these results often involve notions of competition, maladaptation to the new environment and the use of non-selective insecticides. However, the host recognition capacities of the parasitoid have been never questioned.

Hymenopteran parasitoids commonly find their hosts using various chemical stimuli produced by the host or by the plant. These stimuli lead the wasps to the host habitat, to the host plant and finally to the host itself. The females of *C. plutellae* are primarily attracted by green leaf volatiles produced by wounds left by herbivores. After landing on a damaged cabbage leaf, females have the ability to recognize a plant infested by its host through antennal contacts with host kairomones. In contrast, encounters with non-host kairomones put an end to the searching behaviour of females. Despite these mechanisms of host location, females are likely to encounter non-host insects on cabbage. To ensure that eggs are laid in a suitable host, cues perceptible at very short range are necessary. We have recently shown that the females of *C. plutellae* detect their hosts through a short and unique antennal contact, and we hypothesize that gustatory stimuli are elicitors of oviposition behaviour.

Here, we investigate the role of cuticular lipids as gustatory stimuli for host acceptance. We characterized the cuticular lipid composition of DBM caterpillars and assessed their biological activity on the oviposition behaviour of *C. plutellae* females through behavioural tests.

A total cuticular lipid extract of host caterpillars was fractionated into a hydrocarbon fraction and a non-hydrocarbon fraction. Neither fraction alone had any effect on oviposition behaviour in *C. plutellae* but the hydrocarbon fraction alone did seem to have a positive effect on the rate of antennal contact by the females. To induce oviposition behaviour, both fractions were necessary and reflect a synergism between at least one compound in each fraction. Identification of cuticular lipids shows that hydrocarbons were dominant (77%). Non-hydrocarbon compounds were mainly represented by 15-nonacosanone (18% of the total lipid extract). This ketone is rare in insect cuticle lipids and is thought to originate from the cabbage epicuticle where it is dominant with *n*-$C_{29}$ and 14- and 15-nonacosanol also found among the cuticular lipids of the host caterpillar. As no single compound was found to be able to elicit oviposition, synergy between several compounds present on the host cuticle must be required to elicit a specific response in *C. plutellae* females. Implications for biological control and ecology will be discussed.

# The use of propheromones for the monitoring of the spiny bollworm, *Earias insulana,* population in organic cotton

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The spiny bollworm, *Earias insulana*, is a serious pest of organic cotton. The pheromone components of this pest were identified earlier in the 80's as *E*10,*E*12-16Ald and *Z*11-16Ald. Since then both components have been synthesized and tested in Israel. However, the use of this pheromone was found to be very problematic. The main component, *E*10,*E*12-16Ald is unstable and forms trimmers readily. Attempts to stabilize it by adding different stabilizers have failed. Therefore, we decided to test the possibility of using propheromones [1]. The idea was to protect the unstable aldehyde group from trimmerization by derivatization with a protecting group which may decompose under field conditions and release the pheromone.
Four propheromones have been synthesized by reacting *E*10,*E*12-16Ald with four protecting groups. Three different trialkylsilylenolethers and a hydrazide derivative were prepared and their solutions were impregnated into four types of dispensers. The hydrazide derivative was impregnated on a filter paper and the silanes were impregnated into polyethylene and rubber dispensers. Traps with each treatment were exposed in an organic cotton field in Kibbutz Kefar-Baruch, Israel Valey.
It is known that the type of dispenser has a significant influence on the trap efficiency. We have tested in this research four dispensers, i.e. polyethylene, two commercial rubber septa and a rubber septum manufactured specially for this trial. The new rubber dispenser was found to be the superior to all the other. In the second stage of our work we compared the activity of the four propheromones with that of *E*10,*E*12-16Ald. The trimethylsilylenolether (TMSE) pheromone derivative was superior to all the other propheromones. The difference in trap-catch between this propheromone and the pheromone was minor. In the final test we added the second pheromone component (20% *Z*11-16Ald) to the bait and compared the activity of the propheromones with that of the *E*10,*E*12-16Ald. During the first 4 weeks, the efficacy of the pheromone lures was larger than that of the best propheromone (TMSE derivative). However, the activity of the propheromone increased gradually and became comparable with the pheromone.
Unstable pheromones, such as *E*10,*E*12-16Ald, can be derivatized with an appropriate protecting group. The TMSE propheromone of the spiny bollworm was found to be the most active propheromone. It seems that this propheromone is more stable than the pheromone and can replace it for field use by using the optimal dosage.

[1] Streinz L., Horak A., Vrkoc J., Hrdy I. (1993) *J. Chem. Ecol.* **19**: 1-9.

# Antennal pH receptive sensilla in searching for favourable habitats, refugia and overwintering sites in ground beetles (Coleoptera, Carabidae)

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Ground beetles are abundant in agricultural landscapes all over the world and could be important natural enemies of a number of insect pests in conditions of sustainable agriculture and integrated pest management. Very little is known about the role of external chemical stimuli in the searching for favourable habitats, refugia and overwintering sites of these useful beetles, however. Our results suggest that pH receptive cells innervating numerous taste sensilla on the antennae of these beetles may be involved. In single sensillum tip recording technique, the responses of antennal taste sensilla of the ground beetle *Pterostichus aethiops* to 100 mM $Na^+$-salts and their mixtures with 1 and 10 mM NaOH were compared. Two cells, the salt cell and pH cell phasic-tonically responded to the electrolytes tested. An increase in pH by 0.3-0.6 units in 100 mM $Na^+$-salt solutions, caused by the content of 1 mM NaOH, was too small, except for alkaline $Na_2HPO_4$, to influence the firing rate of the cation cell and pH cell significantly. However, different sensitivity of the two cells to increased pH was clearly demonstrated when the concentration of NaOH in 100 mM stimulating salt solutions was increased to 10 mM. Increasing pH by 1.2-2 units caused the 1st s firing rate to increase by 140-1050% and 0-26% in the pH cell and cation cell, respectively. Compared to the acetate and phosphate buffer series method used for identification of the pH receptors in ground beetles earlier, considerably stronger responses of the pH cell to a similar increase in pH were observed when the NaOH method was used for testing. At the same time, undesirable changes in salt ions concentration that occur when stimulating solutions differing by 1-2 pH units are prepared were much smaller using the NaOH method. In *P. aethiops*, is demonstrated that in its preferred acid forest habitats and overwintering sites in brown-rotted wood at pH 3 to 5 the antennal pH sensitive cell does not discharge or discharges at very low frequency with the first s firing rate close to 1 imp/s or lower. Areas with a higher pH seem to be unfavourable to this insect and when contacted the pH cell signals with a stronger response. These results are in a good correlation with behavioural laboratory and ecological field experiments on pH perception in ground beetles.

# Electroantennogram and behavioral responses of *Anopheles albimanus* Wiedemann (Diptera: Culicidae) to human sweat collections

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*Anopheles albimanus* Wiedemann (Diptera: Culicidae) is the main vector of malaria in the coastal area of southern Chiapas, Mexico. This species is believed to be zoophagic; however, a portion of the population enters houses and feeds on humans, causing malaria transmission to continue. In the Chiapas coastal area, differences in mosquito abundance entering houses have been identified, with an increased risk where abundance is higher. A study to evaluate the chemical-ecological factors that determine mosquitoes entering certain houses in search for a human host is ongoing. Presented herein are the results on the electrophysiological response of *An. albimanus* to sweat collected from different parts of the body, as well as the electrophysiological and behavioral responses to the sweat's body part that showed the highest electrophysiological response. The study was conducted in the Nueva Independencia village (14°37´30"N 92°16¨14" W), municipality of Suchiate, Chiapas. We explained the purpose of the study and invited villagers to participate, after which, ten persons out of those willing to take part, were randomly selected from five houses with high (580 ± 15 mosquitoes/trap/night) and five houses with low (18 ± 2 mosquitoes/trap/night) mosquito abundance. High and low abundance categories were established after 10 indoor collections (using CDC up-draft UV light-traps) were conducted in every house during the rainy season. Sweat was collected from the face, chest, arms, legs and feet from the 10 individuals (labeled A-J) using the technique of Meijerink *et al.* (2000), one hour after their daily activities were finished. The sweat volatiles were extracted using dynamic headspace and a Super Q absorbent cartridge. The volatiles extracted with hexane (HPLC grade) were exposed to the antennae of 5-8 days old females to observe the electrophysiological response. One-way ANOVA was used to determine differences in EAG amplitudes responses between body parts. For behavioral experiments, hexane was used as control and a paired *t*-student test was used to determine differences between treatment and control.
The amplitudes of EAG responses to face sweat (-0.2 mV ± 0.02, N=25) were higher than responses to arms (-0.13 ± 0.02,), chest (-0.14 ± 0.03,), legs (-0.11 ± 0.01,) and feet (-0.11 ± 0.02,), respectively ($P<0.05$). Filter papers impregnated with sweat from the face were exposed to 25 females (five replicates) in a one dual port olfactometer. A filter paper impregnated with hexane was placed as control in the other port. Significant attraction response to sweat from the face of seven of the ten people tested was obtained (A $P=0.0001$; B $P= 0.0022$; D $P= 0.0107$; F $P= 0.0267$¸G $P= 0.0385$; H $P= 0.0001$; J $P= 0.0008$), while three showed no significant response (C $P=0.5536$; E $P= 1.0000$; I $P= 0.6914$). The data presented here indicates that sweat from the face caused higher attraction and could be a preferred site for *An. albimanus* to bite. The sweat is being analyzed by GC-MS. These results, along with other chemical-ecological factors currently under evaluation in the study area, will contribute to elucidate the differences in mosquito abundance from different houses.

# Electrophysiological responses of sorghum midge, *Contarinia sorghicola*, to candidate sex pheromone compounds

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The sorghum midge, *Contarinia sorghicola* (Coquillett) (Diptera, Cecidomyiidae), is one of the most destructive pests on grain sorghum, *Sorghum bicolor* L., in Asia, Africa, Australia, Europe and USA. At high population densities the midge can cause 100% crop losses. One reason for the high damage levels caused by *C. sorghicola* is the lack of a good monitoring method for determining the time of emergence of the adult midges, the life stage against which chemical control is most effective. Currently, time–consuming scouting and visual estimates of ovipositing females are required to determine economic thresholds. The objectives of this project is to identify the sex pheromone of the sorghum midge with the purpose of using it in a pheromone-based monitoring system. Coupled gas chromatographic electronantennographic detection (GC-EAD) analyses of ovipositor extracts of virgin *C. sorghicola* females revealed two compounds that elicited responses in male antennae. Synthetic candidate pheromone compounds show identical retention times and also elicit antennal responses.

# Electrophysiological responses of antennal taste sensilla to sugars in the predatory ground beetle *Pterostichus aethiops*

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Most electrophysiologists studying insect contact chemoreception have been focused on taste sensilla of herbivores. Very little is known on functioning of taste sensilla in predatory insects such as ground beetles agriculturally important as biological control agents. In the future, a good knowledge on external stimuli crucial in ground beetles' searching behaviour could allow to manipulate with these predatory beetles in agricultural lands more effectively. In Platynini, Pterostichini and Bembidiini, approximately 70 taste bristles are located on the antennae of adults. Using single sensillum tip recording technique, in addition to the salt and pH cells found in ground beetles earlier, the third chemosensory cell of four innervating large antennal taste bristles was electrophysiologically identified as a sugar cell in the ground beetle *Pterostichus aethiops*. This cell generated action potentials of considerably smaller amplitude than these of the salt and pH cells, and phasic-tonically responded to sucrose and glucose over the range of 1-1000 mM tested. Significantly higher rates of firing were recorded in response to sucrose compared to that evoked by glucose. During the first second of the response, maximum rates of firing of the sugar cell reached up to 19 and 37 imp/s when stimulated with glucose and sucrose, respectively. Both sugars are important in plant carbohydrate metabolism. These ground dwelling insects may come into contact with live and decayed plant material everywhere in its habitat including its preferred overwintering sites in brown-rot decayed wood which does not contain soluble sugars and cellulose, and which is composed of pure lignin. In conclusion it can be we hypothesize that low content of soluble sugars in their overwintering sites and refugia is unfavourable for these ground beetles, most probably to avoid contact with dangerous fungi.

# Sex pheromone compounds mediating host specificity in the egg parasitoid *Telenomus busseolae*

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Several studies showed that egg parasitoids are able detect host sex pheromones produced by adult hosts as a host cue. In this way female wasps are directed toward an area where host mating is in progress and where an oviposition has probably taken place or is soon to occur. *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) is a solitary egg parasitoid of various noctuids (Lepidoptera, Noctuidae) belonging to the genera *Sesamia*. In field observations, has been demonstrated that *T. busseolae* reacts to the pheromone emitted by females of the Mediterranean stem borer, *Sesamia nonagrioides* (Lefebvre) and the pink stem borer, *Sesamia. calamistis* (Hampson). It is known that the sex pheromone produced by calling lepidopteran females is usually a mixture of compounds. In laboratory experiments, it was shown that three components of the synthetic sex pheromone of the Mediterranean stem borer, *e.g.* (*Z*)-11-16:Ac (the main component), (*Z*)-11-16:Ald and 12:Ac, attract wasp females, while a fourth compound, (Z)-11-16:OH, did not. Interestingly, these active compounds are also present in the sex pheromone blends of other host *Sesamia* species, and in the sex pheromone blends of other non-host noctuids species. To evaluate the role played by some components of noctuids sex pheromone in the host specificity of *T. busseolae*, we carried a series of laboratory experiments with *Y*-tube olfactometer coupled a video tracking and motion analysis system. In particular we analyzed the behavioural response of *T. busseolae* females to the main compounds of the synthetic sex pheromones of the corn earworm, *Helicoverpa armigera* (Hübner). Bioassays were carried out with the whole corn earworm sex pheromone blend [(*Z*)-9-16:Ald : (*Z*)-11-16:Ald at ratio of 3 : 97], and the following single compounds: (*Z*)-9-16:Ald present only in the corn earworm sex pheromone, (*Z*)-11-16:Ac the main component of the Mediterranean stem borer sex pheromone, and (*Z*)-11-16:Ald common to both noctuids. All the treatments have been bioassayed at two different doses (0.1 mg and 1.0 mg). The results showed that *T. busseolae* females were **1-** attracted by (*Z*)-11-16:Ac at 0.1 mg and by (*Z*)-11-16:Ald at 1.0 mg; **2-** not attracted by the whole sex pheromone of the corn earn borer at both concentrations; and **3-** slight deterred by (*Z*)-9-16:Ald at both concentrations. These results suggest that (*Z*)-9-16:Ald is a key compound which prevents *T. busseolae* females responding to the corn earworm sex pheromone.

# Pheromonal communication in house-dust mites

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Faeces of the house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* are a major source for house dust allergens. To develop potential control methods, the pheromonal communication of *D. pteronyssinus* was studied in a static two-chamber olfactometer with living mites. When males were offered as odour source, female mites as well as males where attracted to the respective odour field. Mite nymphs did not react to the males. When females were offered, males and nymphs but not females were attracted. This indicates that males and females release odours that differ in chemistry and function. Future studies will focus on the identification of the chemicals that are released by the mites and on their biological activity.

# Flight activation and sexual attraction in the haematophagous bugs *Triatoma infestans* and *Rhodnius prolixus* (Heteroptera: Reduviidae) vectors of Chagas disease

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In the last decades evidence suggests the possible utilization of chemical cues for sexual communication in several triatomine species. In addition, several factors affecting flight dispersal as nutritional state, sex, female reproductive state and others, were described. Here we explored the relation between flight activation and the presence of conspecifics of the same or opposite sex in two triatomine species. We studied the chemical modality as a possible cue involved in the flight activation of *Triatoma infestans* and *Rhodnius prolixus*. Assays consisted in exposing a group of adult males or females to the presence of another group of adult bugs of the same species. Experimental series conducted were: a) males stimulated with females, b) females stimulated with males, c) males stimulated with males. To measure the spontaneous flight initiation control assays were performed in absence of stimulus. Each experimental group was located on a hanging platform in the centre of a cubic cloth jail and the stimulus source was located 1 mt. away from it. In this way, flying bugs could not contact the stimulus source. We quantify, in one-night assays, under controlled environmental conditions and in complete darkness the number of insects that initiated flight from the platform (activation). *T. infestans* males showed a larger proportion of flight initiation when confronted to conspecific females (stimulus) than control assays. Similar results were obtained from *T. infestans* females. Nevertheless, in male presence, *T. infestans* males show a lower activation flight proportion than control assays. Males of *R. prolixus* almost doubled their flight initiation proportion in female presence, compared to their performance in absence of stimulus. However, *R. prolixus* females exhibited no difference in presence or absence of conspecific males. Nonetheless, *R. prolixus* males initiate flight in a lower proportion in presence of males than in absence of stimulus. In absence of visual (functional darkness) and mechanical (hanging platform) stimuli, these results suggest that males of both species activate initiating flight by means of chemical cues in presence of conspecific females, probably mediated by volatile compounds that would allow the encounter of sexes. The behavior of these species could be modified if the source of the chemical signal that promotes flight activation is identified. This could be a useful potential tool for the vectorial control of Chagas disease.

# Temporal modulation of odour perception: does olfactory sensitivity change globally or according to the behavioural context?

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Many biological processes in animals change rhythmically, even in the absence of environmental cycles. The daily variation in odour sensitivity in insects has been demonstrated both, at the sensory level (i.e., receptor cells), and at the behavioural level. It is worthwhile to note that, along the day, a specific modulation (i.e., affecting each odour independently) would be more advantageous than a general change in olfactory responsiveness. The reason is that different odours are associated to behavioural contexts allocated at different temporal windows. As a consequence, maximal responsiveness should be expected to occur 1) during periods of activity and 2) when a given odour becomes biologically relevant. Previous evidence, obtained in *Drosophila* and *Leucophaea* does not support however these assumptions. Paradoxically, in both cases maximal sensitivity seems to occur during resting periods, whereas general changes in olfactory sensitivity would occur. The reasons for this paradox are not understood, deserving to be analysed in other species. We chose *Rhodnius prolixus*, who exhibits a bimodal activity pattern, for testing whether or not olfactory sensitivity to signals relevant at different times of the day, varies in a related way. In nature, bugs normally leave their refuges seeking for food guided by host-emitted cues (e.g. $CO_2$) at the beginning of the night, and they return to shelters following assembling pheromones before sunrise. We studied the daily dynamics of responsiveness to carbon dioxide and to aggregation cues. Our results show that the responsiveness to these two odours does not remain constant along the day, but is restricted to precise temporal windows instead. As predicted for an adaptive response, responsiveness to $CO_2$ only occurs at dusk while assembling pheromones were attractive exclusively at dawn. Thus, our results support a specific modulation of the responsiveness to odours, rather than a general one, as maximal chemosensitivity matches the behavioural context to which each odour is associated with.

# The KLP+ (’hat’) trap, a non-sticky, attractant baited trap of unusual design for catching selected beetle pests

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In the course of research aimed at the development of non-sticky, easy-to-use alternative trap designs for the capture of selected beetle pests, a newly designed "hat" trap, codenamed CSALOMON® KLP+, was compared with conventional trap designs. Baited with an attractant, the new trap design was constructed to exploit the preference of most beetles to crawl upwards along vertical surfaces.
In the case of the western corn rootworm (WCR) *Diabrotica v. virgifera* (Coleoptera, Chrysomelidae) the new KLP+ traps baited with pheromonal or floral baits were equally sensitive as the former PAL or PALs sticky "cloak" designs, but the KLP+ traps’ catch capacity and selectivity was much higher. When baited with the floral WCR bait, the KLP+ trap proved to be more sensitive in capturing female WCR, than the former yellow sticky PALs trap design.
In tests aimed at capturing cabbage flea beetles (*Phyllotreta* spp., Coleoptera, Chrysomelidae), the new KLP+ trap design was baited with allyl isothiocyanate, which is a well known attractant for flea beetles. In all four spp. caught in the test (*Phyllotreta cruciferae* Goeze, *Ph. vittula* Redtenbacher, *Ph. nigripes* Fabr., and the closely related *Psylliodes chrysocephalus* L.), the KLP+ trap design performed better than the previously used CSALOMON® VARL+ funnel traps in all respects studied.
In conclusion, the new KLP+ trap design, baited with the respective attractants, appears to be advantageous to use for the trapping of both WCR and cabbage flea beetles, and can be recommended for use as a trapping tool in plant protection practice in the detection and monitoring of these pest Coleoptera.

# Better dispensers for semiochemicals: Application to Mediterranean fruit fly para-pheromone

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A new generation of agricultural methods are based on the release of a lure for insect attraction or confusion, and success of these methods depends on the efficacy of the dispenser emission of the attractant. Currently the most used semiochemical dispensers are based on polymeric matrix or rubber septa. In these cases, the semiochemical release depends on weather conditions and their life is too short to be effective during the whole season. In this work new dispensers with mesoporous materials were studied to obtain more efficient kinetics of emission and more long-lasting dispensers. For this study, the selected pest was Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and the lure used was trimedlure (TML). A field study comparing attractant release values was made. Furthermore, a high dependency of attractant release rate on temperature with polymeric dispensers when compared to mesoporous dispensers has been demonstrated.

# New biodegradable controlled-release pheromone dispenser for mating disruption of the European grapevine moth *Lobesia botrana* Denis and Schiffermüller (Lepidoptera: Tortricidae). Evaluation of field efficacy

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A new type of eco-friendly hand applied pheromone dispenser for mating disruption of the European grapevine moth, based on sepiolite, has been developed. In order to evaluate its efficacy under field conditions, a mating disruption trial was conducted in 9,52 ha (plot A)of commercial vineyards in Requena, Valencia, Spain. Shortly before the beginning of the first flight period, pheromone dispensers were set up in two adjacent plots in which vines from Tempranillo (plot A1; 3,91 ha) and Bobal (plot A2; 5,61 ha) were cultivated. A reference plot next to the trial (plot C; 1,97 ha) where insecticide treatment is only applied when justified. by the level of the pest. Results were compared with those obtained in a field trial with Isonet-L dispensers (Shin-Etsu, Japan), in a 13,5 ha vineyard from Garnacha (plot B). This assay was carried out by the "Conselleria d´Agricultura, Pesca i Alimentació" from the Valencian Government.

The efficacy of the mating disruption technique (MDT) was evaluated through periodic observation of damage caused by larvae and adult captures in monitoring traps, either in the mating disruption areas as in the reference plot.

In addition, a comparative study of pheromone release patterns from new dispensers and commercial Isonet-L, was followed. All dispensers were aged in laboratory in a thermostatic camera (30ºC and 0,35 m/s wind speed) and in field trial conditions. Residual pheromone remaining in dispensers after different time intervals was evaluated by gas-liquid chromatography and release rates were determined.

New dispensers were highly efficient as pheromone dispensers for the MDT against *L. botrana* under our conditions. The success of this technique against the first generation of the pest was found to vary with the treated plot and a complementary insecticide treatment was required in plot A2, where pest pressure was historically higher. The efficacy against the second and third generations was highly satisfactory. Adult captures of males were highly inhibited (>99,25%) according to those registered in the reference plot. The MDT was more effective in plots A1 and A2 than in plot B, being 98,9% and 87,5% more effective than the chemical control in plot C, respectively.

Release patterns of new dispensers were better than Isonet-L´s, with more adequate first order kinetics than commercial, both in laboratory and in field conditions. Isonet-L half-lives in laboratory and field were 15,80 y 29,75 days, respectively, while new dispensers were less sensitive to exposed conditions (around 46 days, in both cases). Thus, new dispensers could be an eco-friendly alternative for the control of *L. botrana* as their release patterns were adequate for the MDT, minimizing the waste of pheromone and because they are biodegradable.

# Inter and intra specific functions of sex pheromones in the shore crab, *Carcinus maenas*

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The reproductive behaviour of the common shore crab, *Carcinus maenas* is controlled by sex pheromones that are emitted by both sexes. We used the synthetic female sex pheromone to induce the attraction of male crabs towards the partner, as well as the induction of typical mate guarding behaviour. Chemical signals emitted into the environment do not exclusively reach the 'intended' receiver but will be detectable for predators, competitors as well as other related crustacean species occurring in the same ecosystem. As we hypothesize that the female produced sex pheromone is directly linked to the female's moulting process, we also examined the species specificity of the compound. We exposed known predators, closely related species, as well as other decapod crabs (soft-shell as well as hard-shell maters) to the female pheromone. We tested the synthetic female shore crab pheromone on swimming crabs *Necora puber*, snow crabs *Chionoecetes opilio*, spider crabs, mud crabs *Panopeus herbstii*, edible crabs *Cancer pagurus*, blue crabs *Callinectes sapidus*, arrow crabs *Stenorhynchus seticornis*, shrimps *Lysmata boggessi* and *Lysmata wurdemanni*. In some of the above tested species we found positive responses to the compound, suggesting that it is not species specific. We conclude that the compound, or very similar related chemicals, may function as sex pheromones in many other Crustacean species.

## Chemical signals in *Carcinus maenas*

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We studied the control of the reproductive behaviour of the common shore crab, *Carcinus maenas* and examined the function of sex pheromones in the control of pair formation and mating. Here we show how sex pheromones are emitted by both sexes and control the attraction towards the partner as well as mate guarding behaviour. We found that 20-hydroxyecdysone (Crustecdysone), the moulting hormone in shore crabs, has sex specific feeding deterrent properties deterring males from prey (*Mytilus edulis*) and from synthetic feeding stimulants (glycine, taurine). In contrast, intermoult female crabs (known to engage in cannibalism) were less affected and rarely deterred from feeding. We conclude that the moulting hormone, in combination with the attractive and mating inducing properties of the female produced sex pheromone ensures that male crabs do not engage in cannibalism and form reproductive pairs to mate with 'soft' shelled mature female crabs. This so far overlooked sex specificity of a known behavioural function of crustecdysone may explain why this compound has been erroneously hypothesized as potential sex pheromone in earlier studies.

# Does experience matter in mate choice? Female ragworms *Neanthes arenaceodentata* make an informed choice

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Selective mate preference is a common occurrence in nature, and is associated with maximising reproductive success. In marine polychaetes the majority of species exhibit broadcast spawning reproduction strategies where mate choice is seen as unlikely. In contrast to this *Nereis acuminata* (Ehlers, 1868) also known as *Neanthes arenaceodentata* (Moore, 1903) exhibits monogamous pairing. *Nereis acuminata* shows excessive levels of intra-sexual aggression, but little fighting between the sexes. The reproductive process also includes the unusual element of male parental care of offspring after the monotelic female dies within hours of releasing the eggs into the burrow. Since a female's reproductive success entirely depends upon the performance of the male in caring for the young, we hypothesize that females are deemed to be choosy in mate selection. Here we show that the females actively choose their mates based on two social factors- 'dominance' as expressed through winning male-male fights and 'experience in fatherhood'. We also propose that this recognition of a male's 'quality' as a potential father is based on chemical signals. This provides new evidence to the evolution of sexual selection based on recognition of potential 'quality' of male partners by odour cues in the drive to maximise success in reproduction.

# Chemical communication by signal crayfish (*Pacifastacus leniusculus*) in agonistic and mating interactions

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Urine-borne chemical signals are considered to play an important role in regulating social interactions of freshwater crayfish. Previous studies have shown that male crayfish release status-specific urinary signals during fights that effect the behaviour of the receiver (Breithaupt & Eger, J. Exp. Biol. 205, 2002). Despite interest in developing a pheromone trap to control invasive species there has been limited research into crayfish sex pheromones. Stebbings et al. (J. Chem. Ecol. 29, 2003) recorded behavioural responses (increased activity, touching) in males when water or urine from receptive females was released from an airstone suggesting that receptive females release urinary pheromones. We aim to clarify the role of chemical communication during mating by assessing the behavioural context of urine release and identifying any sex-specific differences.

Our study used the dye fluorescein to visualise the pattern of urine release by male and female crayfish (*Pacifastacus leniusculus*) in staged fights and mating interactions. Behaviours were scored during social interactions and the timing of urine release recorded.

Urine was generally released more frequently during social interactions than in isolation (Wilcoxon signed rank; $p<0001$). Both female and male fights followed a characteristic behavioural pattern peaking at unrestrained use of claws against an opponent. There was no difference in the relative duration of urine release (% of total interaction time) by male and female crayfish in staged fights (t-test; $p>0.05$). However, we found sex specific differences in urine release during courtship interactions. In contrast to females, males spent less time releasing urine in courtship interactions than in fights. Females released significantly more urine than males during mating (Two-way ANOVA; $F_{3,54} = 6.39$, $P< 0.05$). The pattern of female urine release differed significantly over defined stages of courtship interactions with the highest percentage of urine release occurring 3 minutes prior to mating onset (Kruskal Wallis; $p= 0.01$). This pattern was not evident for male crayfish ($p>0.05$), and males released significantly less urine than females in the 3 minutes prior to mating (Wilcoxon signed rank; $p<0.01$).

Our results suggest that males change their behaviour from fighting to courtship as a result of receiving female urine-borne pheromones early in an interaction. Males also reduce aggressive urine signalling. The results emphasize the importance of urinary pheromones in crayfish allowing males to assess sex and receptivity of the female. Future experiments will focus on the identification of the sex pheromone using HPLC and bioassay driven purification techniques. The resultant compound can then be employed in field trapping studies in order to develop a species-specific trap which may become a useful pest control tool in efforts to control signal crayfish populations.

# Mating behaviour and sex pheromone in Malaysian banana weevil *Cosmopolites sordidus*

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The mating behaviour and studies on sex pheromone was conducted on the pest of banana *Cosmopolites sordidus* (Coleoptera: Curculionidae) in a banana plantation at Johore, Malaysia. Female *C. sordidus* was observed to attract the male in the mating sequence. Male *C. sordidus* recognizes his mate after 0.4±2.922 min. Duration of copulation was 8.2±2.353 min. The male was able to recognize his mate from 5 cm to 15 cm. Male weevils which had their antenna removed were not able to detect the location of the female. Male showed positive response towards female crude extract, extract of female thorax, crude eluted with 70% hexane and 30% diethyl ether. The spot with Rf=0.36 resulted from thin layer chromatography analysis also positive response from males. Analysis using gas chromatography mass-spectrometry gave cyclohexadecane as one of the peaks in the chromatogram. The paper discusses the function of cyclohexadecane in the banana weevil.

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# SYMPOSIUM 3

# BIOSYNTHESIS AND PHEROMONE PRODUCTION

# Regulation of pheromone production in moths

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In several moth species pheromone production is under neuroendocrine control due to the circadian-regulated release of PBAN, a neurohormone widespread throughout insects, and whose role has been well elucidated in many lepidopterans. Research in our laboratories on *Helicoverpa spp*. showed that PBAN activity causes the influx of extracellular calcium that promotes the production of cyclic-AMP in isolated pheromone glands.The pheromonotropic action of PBAN involves its specific binding to a G-protein coupled receptor (GPCR) present in membranes of specialized cells found in the intersegment between the ultimate and penultimate segments of the female's ovipositor. Subsequent research led to the identification of genes encoding GPCRs (*H. zea*: AY319852 and *H. armigera*: AY792036), which are homologous to PRX-amide-GPCRs from *Drosophila, Aedes* and neuromedin U-receptors in vertebrates. Cloning and expression in Sf9 insect cells of AY319852 demonstrated functional activity by mobilization of calcium in response to PBAN in a dose-dependent manner and specific binding of a photoaffinity-biotinylated PBAN-analog. PBAN is present in the hemolymph during the night (scotophase) in virgin females but is drastically reduced after mating. At this time, mating leads to a loss of sexual receptivity as a result of an inhibition of sex-pheromone production. This switch in behavior is attributed to male accessory gland (MAG)-derived factors, transferred by males to females at the time of mating. Our previous studies showed that the injection of synthetic *Drosophila* sex-peptide (*Drm*SP) causes a significant inhibition of pheromone production by virgin females of the moth *H. armigera.* To establish the possible role of *Drm*SP-like peptides in female post-mating events, the effect of mating on female pheromonostasis was studied and the spatial and temporal distribution of *Drm*SP-like peptides in mated males and females of *H. armigera* was analysed.
I will review recent developments concerning the neurohormonal control of sex-pheromone production, the changes that occur during development of the adult, the regulation by Juvenile Hormone and the inhibitory effects of male–derived sex-peptides transferred to females after mating.

# Recruitment and loss of pheromone components in the evolution of Lepidoptera

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Constraints on the evolution of novel receptors and new biosynthetic pathways may partly explain the conservatism in the use of pheromone components within moth families. In the evolution of the Lepidoptera novel types of pheromone components have evolved on a few occasions only. Long-chain fatty acid derivatives, including acetates and alcohols, are the most common pheromone components among moths. This type of pheromone components evolved in the early evolution of the heteroneuran lineages (Löfstedt *et al.,* 2004) and dominates among the higher Lepidoptera. The biosynthesis of these components typically involves desaturases with certain regio- and stereo-selectivity. Ongoing research aims at tracing the recruitment of the desaturase genes for pheromone production by studies of pheromone production in selected primitive moth families, including Eriocraniidae, Prodoxidae, and Yponomeutidae.
Saltational events including recruitment or loss of genes coding for pheromone production may in some cases have given rise to new pheromones and new moth species (Baker 2002 and references therein). Roelofs *et al.* (2002) found that the evolution of the Asian corn borer *Ostrinia furnacalis* from its ancestor involved the recruitment of a *pseudogene* to function in female pheromone production (Roelofs et al., 2002). We discovered that in this case the functional integrity of the pseudogene during millions of years may have been maintained by its involvement in the production of the male courtship pheromone and functionalization/nonfunctionalization is rather a case of sex-differential translation (Lassance and Löfstedt, unpublished).

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# Pheromone biosynthesis and regulation in bark beetles

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Bark beetles (Coleoptera: Scolytidae) rely on aggregation pheromones to coordinate a "mass attack" to overcome a host tree's defenses. Monoterpenoid pheromone biosynthesis is thought to have evolved from detoxification reactions since pheromone components are often hydroxylated derivatives of host tree resin components. For example, male *Ips pini* produce copious amounts of (-)-ipsdienol as a pheromone component, but non-pheromone biosynthetic *I. pini* also produce racemic ipsdienol as a probable detoxification product of host tree myrcene. These processes are related but distinct since pheromone components are synthesized *de novo* in the midgut of male *I. pini*. Although not yet fully characterized, the *de novo* ipsdienol biosynthetic pathway requires carbon to be shunted from the mevalonate pathway at geranyl diphosphate, and involves myrcene and ipsdienone as likely intermediates. This pathway is stimulated by juvenile hormone III (JH III) and is also influenced by developmental and antennal effects. An EST survey of pheromone-biosynthetic *I. pini* midguts revealed several genes implicated in pheromone production, including the first example of a geranyl diphosphate synthase (GPPS) from an animal. Microarray analyses revealed coordinate regulation of several genes, including a novel cytochrome P450 and almost all represented mevalonate pathway genes, in a putative "pheromone biosynthetic cluster." When expressed in a baculoviral system, the P450 in the cluster hydroxylates myrcene to make ipsdienol. These analyses provide clues about the evolution of bark beetle pheromone biosynthesis and also help clarify the probable steps in the conversion of geranyl diphosphate to (-)-ipsdienol.

An EST survey of midguts from the mountain pine beetle (*Dendroctonus ponderosae*) is now underway. Analysis of ~2800 preliminary ESTs suggests some closely related P450s may also hydroxylate monoterpenes, likely functioning as detoxification or pheromone biosynthetic enzymes.

# Fatty acid desaturases in moth pheromone biosynthesis

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Desaturation of acyl coenzyme-A esters is a key reaction in the biosynthetic pathways of moth sex pheromones. This reaction is catalyzed by membrane-bound acyl-CoA desaturases that are biochemically and structurally similar to the ubiquitous $\Delta^9$ acyl-CoA desaturase of animals and fungi. Many moth desaturases have now been cloned and their function has been characterized. In contrast to most desaturases of eucariotic cells, the moth enzymes afford unique unsaturated fatty acyl-CoA esters of variable chain lengths, different number and locations of unsaturations and either the ordinary Z or the unusual E double bond geometry.
Mechanistic studies have been conducted and all examples so far investigated comply to a general rule according to which desaturation occurs in two steps: an initial abstraction of the hydrogen atom located nearest the carboxyl end to give an extremely short lived intermediate, followed by the fast elimination of the hydrogen atom attached to the neighboring position. The absolute configuration of the hydrogens removed to give the double bond has also been investigated in some cases. In general, *Z* double bonds arise from loss of both pro-(*R*) hydrogens, whereas *E* double bonds are formed upon loss of the pro-(*R*) hydrogen nearest the substrate carboxyl moiety and the pro-(*S*) hydrogen of the vicinal position.
A newly identified feature of moth desaturases is their bifunctionality, which adds interest to these thrilling enzymes. Specifically, bifunctional enzymes with both delta-11 and delta-10,12 desaturase activities have been found in *Bombyx mori* and *Spodoptera littoralis*. Furthermore, a trifunctional desaturase has been recently cloned from pheromone glands of *Thaumetopoea pityocampa*. This desaturase suffices to produce the characteristic conjugated enyne scaffold of this moth sex pheromone, (*Z*)-13-hexadecen-11-ynyl acetate. The cryptoregiochemistry and stereospecificity of the different desaturation reactions catalyzed by this unique enzyme correlate nicely with its trifunctionality.
Overall, moth desaturases are excellent models to study several aspects of enzymatic desaturation and are invaluable tools to unveil the secrets of one of the most exciting reaction for chemists: the regio and stereospecific formation of double bonds from extremelly unreactive starting materials.

# Evolutionary genomics of moth sex pheromone desaturases

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The sex pheromone blends of female moths are produced by a complex interplay of pheromone production genes, most of which are members of multigene families. The acyl-CoA desaturases, whose members include key players in the biosynthesis of female sex pheromones, form one such family. A considerable amount is known regarding the function and expression patterns of some of these genes in moths, but substantially less is known regarding how the gene family as a whole evolves. Therefore, in order to better understand the evolution of the desaturase family in moths, we have been studying the genomic organization and evolutionary patterns displayed by these genes in the genomes of representative species: the European and Asian cornborers (*Ostrinia nubilalis* and *furnacalis*, respectively) using a combination of genomic, biochemical and computational approaches. We have found that the desaturase multigene family consists of a diverse array of members, some of whose function is unknown or even doubtful. Apparently, much of this diversity is the result of the proliferation of a previously unknown family of RTE-1 retroposons that we have found in the cornborer genomes, as well as a related subfamily in the silkworm moth (*Bombyx mori*) genome. Thus, our results indicate that retroposition has played an important role in generating both functional and non-functional diversity of sex pheromone desaturase genes in the genomes of moths.

# Aggressive chemical mimicry of a host's sex pheromone by a phoretic nest parasite

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Cooperative behaviors are common among social insects such as bees, wasps, ants, and termites, but they have not been reported from insect species that use aggressive mimicry to manipulate and exploit prey or hosts. We report here that larval aggregations of the blister beetle, *Meloe franciscanus,* that parasitize nests of the solitary bee, *Habropoda pallida,* cooperate to exploit the sexual communication system of their hosts. The first instar meloid beetle larvae eclose simultaneously, and move as a group to the tops of grass stems and branches of shrubs. The resulting aggregations visually mimic a female bee, and more importantly, produce a chemical cue that mimics the sex pheromone of the female bee. Male bees are lured to these larval aggregations, and upon contact (pseudocopulation), the beetle larvae rapidly and efficiently transfer en masse to the male bees. The larvae transfer to female bees during mating, and subsequently are transported to the nests of their hosts, where they develop on the nest provisions and the host egg. To mimic the chemical and visual signals of female bees effectively, the parasite larvae must cooperate, emphasizing the adaptive value of cooperation between larvae. The aggressive chemical mimicry by the beetle larvae, and their subsequent transport to their hosts' nests by the hosts themselves, provides an efficient solution to the problem of locating a critical but scarce resource in a harsh environment. We report here the identification of the sex pheromone of the bee, the identification of the chemical cues produced by the beetle larvae that mimic the bee's pheromone, and the successful reconstruction and testing of the attractant blends.

# Methyl 2,4,6-decatrienoates attract stink bugs (Hemiptera: Heteroptera: Pentatomidae) and tachinid parasitoids

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*Halyomorpha halys* (Stål) (Pentatomidae), called the brown marmorated stink bug (BMSB), is a newly invasive species in the eastern U. S. that is rapidly spreading from the original point of establishment in Allentown, Pennsylvania. In its native range, the BMSB is reportedly attracted to methyl *(E,E,Z)*-2,4,6-decatrienoate, the male-produced pheromone of another pentatomid common in eastern Asia, *Plautia stali* Scott. The methyl 2,4,6-decatrienoate pheromone theme also occurs in North American pentatomid species in the genus *Thyanta*, which produce methyl *(E,Z,Z)*-2,4,6-decatrienoate as part of their pheromones. We became interested in field-testing methyl 2,4,6-decatrienoates 1) in an effort to monitor the spread of the BMSB, *H. halys* and, 2) to explore the possibility that *Thyanta* spp. are an alternate host for parasitic tachinid flies that use stink bug pheromones as host-finding kairomones. Here we report the first capture of adult and nymph BMSBs in traps baited with methyl *(E,E,Z)*-2,4,6-decatrienoate in central Maryland, and present data verifying that the tachinid, *Euclytia flava* (Townsend), uses methyl *(E,Z,Z)*-2,4,6-decatrienoate as a kairomone. We also report the unexpected finding that various isomers of methyl 2,4,6-decatrienoate are potent attractants for *Acrosternum hilare* (Say), and other stink bugs and tachinids native to North America, even though efforts to isolate these compounds from the bugs themselves have thus far failed. These data indicate that there are other yet to be discovered Heteroptera in North America that use the methyl 2,4,6-decatrienoates as pheromones, and provides additional evidence that some pentatomids exploit the pheromones of other true bugs as kairomones to find food.

# Regulation of pheromone production in the pine engraver beetle, *Ips pini*: effect of antennectomy on pheromone production

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Bark beetles use monoterpenoid aggregation pheromones to coordinate host colonization and mating. These chemical signals are produced *de novo* in the midgut via the mevalonate pathway, and pheromone production may be regulated by a negative feedback system mediated through the antennae. In this study, we examined the effect of antennectomy on pheromone production and transcription of key mevalonate pathway genes in male pine engraver beetles, *Ips pini* (Say). Antennectomized males produced significantly greater amounts of the pheromone component ipsdienol than podectomized males and those with intact antennae. Likewise, mRNA levels of three mevalonate pathway genes important in pheromone biosynthesis, HMG-CoA synthase, HMG-CoA reductase and geranyl diphosphate synthase, were measured by quantitative real-time PCR and found to be induced to a greater extent with antennectomy; suggesting a transcriptional regulation of pheromone production. Juvenile hormone and feeding have been shown to be important factors in regulating pheromone production in bark beetles. A number of key genes in the pheromone biosynthetic pathway *in I. pini* were identified using genomic techniques as being up-regulated by feeding or treatment with juvenile hormone. Several of these are being cloned and functionally expressed, and an update of this work will be presented.

# Chemistry of the African coffee stemborer, *Monochamus leuconotus*: but where's the ecology?

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The African coffee stemborer, *Monochamus leuconotus* (Coleoptera: Cerambycidae) is an important pest of coffee in many African countries. Gas chromatographic (GC) analysis of volatiles collected from male or female beetles showed the presence of a single, male-specific component. This was identified by interpretation of mass spectra and GC retention data as 2-(4-heptyloxybutyloxy)ethanol. The synthetic compound elicited electroantennographic responses from the antennae of both male and female beetles. In GC analyses of cuticular hydrocarbons collected from male or female beetles by solvent extraction or solid-phase microextraction, single male-specific and female-specific components were detected in the corresponding blends. Identification of these is not yet complete, but there is good evidence they are homologous polyethers. These polyether compounds seem to be characteristic of the Laminae sub-family of the Cerambycidae, the only other example having been found in the Asian long-horn beetle, *Anoplophora glabripennis*, which also belongs to this sub-family. The polyethers presumably have a different biosynthetic origin from that of the hydroxyketones and dihydroxy compounds which are typically produced by males of the main sister group, the Cerambycinae. However, despite the presence of these novel, sex-specific compounds in *M. leuconotus*, no evidence has so far been found for any behavioural effects associated with them. Extensive windtunnel bioassay and field trapping experiments have failed to demonstrate any long-range attraction between the sexes or any behavioural effect of the above male-specific volatile compound. In detailed observations of close-range mating behaviour and experiments with extracts of the cuticular hydrocarbons there was no obvious sexual recognition by antennal contact, as has been observed in many other species of Cerambycidae. The biosynthetic origins and status of these unusual polyether compounds will be discussed.

# Chemical composition and putative function of lipids occurring on the silk or cuticle of spiders

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In contrast to the cuticular components of insects, the chemical ecology of spider lipids present on the silk and/or the body are less well investigated. We explored their chemistry by analysing lipidic extracts of silk and body from several spiders belonging to different families. A major class of compounds are hydrocarbons as has been found in insects. Nevertheless, often alkanes with an even number of carbon atoms in the chain containing a 2-methyl-branch are dominating members of this class. A linkage to amino acid starter units in their biosynthesis is obvious, probably related to the carnivorous nature of the spiders, resulting in a protein-rich diet. The second major group of lipid components typical to spiders are 1-methoxyalkanes (methyl alkyl ethers), often methyl branched. In the Linyphiidae several species contain very similar composition of hydrocarbons, while the ether composition varies. This may be an explanation for the observed species specificity in the pheromone response of different linyphiid species with overlapping habitats and activity windows, which use the same pheromone, 3-(3-hydroxybutyryloxy)-butyric acid. Recently we discovered on the cuticle of other linyphiids 2-methoxyalkanes. They co-occur with 1-methoxyalkanes which show the same chain length and location of methyl branches. The biosynthesis of these compounds must be closely related. In *Latrodectus* (black widow), unbranched 1-methoxyalkanes induced prolonged resting behavior in preliminary behavioral tests. A third group of compounds are esters of unbranched long chain alcohols with medium chain fatty acids which regularly contain multiple methyl branches. They occur often as mixtures of only a few components, strikingly different from the mixtures formed by hydrocarbons or methoxyalkanes. In *Argyrodes elevatus*, male and female lipids consist of just a few different esters and maybe involved in the pheromone system of this species.

# Triatomine bugs: cuticular lipids as intraspecific chemical cues

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The triatomine bugs are vectors of the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease in Latin America. These insects spend daylight hours assembled in shaded places, in narrow contact with other conspecifics. Aggregation behaviour plays an important role in their survival by facilitating the location of refuges, cohesion of aggregates, and also reinforcing their nocturnal habits. During daylight, it helps keeping insects in their refuges, a defensive mechanism against potential predators (chickens, dogs, rodents) or human beings that turn into their blood-feeding hosts at night. There are evidences that aggregation is mediated by thigmotaxis, by volatile substances from their dry faeces, and by chemotactile signals of lipid nature present in their cuticle. Cuticular lipids are known to play a role in sexual behaviour in many insects, although no reports are available in triatomines. The cuticular lipids of *Triatoma infestans* include a complex mixture of hydrocarbons, free and sterified fatty acids, alcohols, and sterols. We analyzed the response of *T. infestans* fifth instar nymphs to cuticular lipid extracts. Insects were exposed to different amounts either of the total epicuticular lipids, or their individual fractions. Assays were performed in a circular arena, employing a binary choice test with a filter paper impregnated with a chemical, and another with hexane. Insects were attracted and assembled around papers impregnated with the epicuticular lipids. Among the lipid fractions tested, only the FFA fraction promoted bug aggregation. We also investigated the response to different amounts of selected fatty acid components, receptiveness varied with the fatty acid chain length. No response was elicited by hexadecanoic acid (C16:0), the major fatty acid component. Octadecanoic acid (C18:0) showed the highest assembling effect at a concentration ~ 1 equivalent ($p<0.001$). The very long chain hexacosanoic acid (C26:0), was significantly attractant at low doses (=1 equivalent), although a repellent effect was observed at higher doses.
Cuticular lipids were also evaluated for their role on the copulatory behavior of *T. infestans*, *Rhodnius prolixus* and *T. sordida* and *T. garciabesi* (sordida complex). Male copulation attempts were quantitated in the presence of freeze-killed females, either intact, deprived of their cuticular lipids, or re-painted with a female extract. Results in all species tested showed that contact cues are important in mate recognition in this group, and that mate recognition does not depend on female behaviour. The cuticular recognition cues are removed by hexane wash. Sexual dimorphism in their cuticular lipids is currently being investigated. We conclude that cuticular lipids trigger aggregation and mate recognition in triatomines, probably involving the participation of contact chemoreceptors.

# Production and predator-induced release of defensive chemicals by plant bug *Lygus hesperus*

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Both sexes of adult western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae), released three defensive allomones in relatively large amounts when attacked by ants (*Pogonomyrmex rugosus* and *Solenopsis xyloni*) or when grabbed by forceps as determined by solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). The relative amounts of the volatized compounds, hexyl butyrate, (*E*)-4-oxo-2-hexenal, and (*E*)-2-hexenyl butyrate, absorbed by SPME as a percentage of the largest were 100%, 44%, and 4%, respectively, from females, and 83%, 37%, and 3% from males. Both ant species were repelled by the defensive discharges (confirmed by SPME) when the ants attacked *L. hesperus* adults. Sexually mature *L. hesperus* were individually extracted in pentane to quantify the mean amounts of hexyl butyrate (14.9 µg/female; 10.3 µg/male), (*E*)-4-oxo-2-hexenal (2.7 µg/female; 3.1 µg/male), and (*E*)-2- hexenyl butyrate (1.2 µg/female; 0.6 µg/male). (*E*)-4-oxo-2-hexenal was unstable in solvent when in contact with a macerated adult, but relatively stabile when solution was decanted within minutes. Nymphs and young adults up to a few days post ecdysis appear chemically defenseless, suggesting a tradeoff between producing.allomones and faster growth/maturation. A cost of defensive secretion is suggested for mature females but not for mature males because heavier females produced more defensive compounds than lighter females.

# Semiochemicals involved in the sexual communication of the parasitoid *Nasonia vitripennis*

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Compared to other insect taxa little is known about the semiochemistry involved in the sexual communication of parasitic Hymenoptera. Only a few female-derived sex pheromones have been identified so far mediating long-range orientation or courtship behaviour in males. We studied the gregarious parasitoid *Nasonia vitripennis* Walker (Pteromalidae) parasitizing puparia of several necrophorous Diptera. We found chemical cues from three different sources to be involved in the sexual communication of *N. vitripennis*:
(a) We report the first identification of a male-derived sex pheromone in a parasitic wasp. The pheromone strongly attracts and arrests virgin females. Furthermore, we studied the site of pheromone biosynthesis, the age dependency of pheromone titers, and the behavioural context of pheromone release.
(b) A female-derived courtship pheromone composed of cuticular hydrocarbons elicits a characteristic courtship sequence in males. The male response to the courtship pheromone is synergized by additional physical cues since extracts applied to three-dimensional models (extracted males) elicit stronger responses than those applied to two-dimensional discs of filter paper or an insect cuticle [1].
(c) Volatiles from host puparia attract both males and females. The fact that male parasitoids do neither oviposit nor perform host feeding, suggests that the male response to host-associated volatiles is part of the mate finding strategy.

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# Tracking down aphid alarm pheromone synthase: a botanical approach

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When aphids are under threat from predation they secrete a cornicle droplet containing *E*-ß-farnesene, *E*ßF (Pickett & Griffiths 1980). This hydrocarbon acts as an alarm pheromone which, when detected by neighbouring aphids, causes them to disperse (Pickett *et al* 1992). Although the aphid which has secreted the pheromone is still likely to be eaten, its companions will escape and some of these will be of the same clone produced by parthenogenetic reproduction – hence the genotype will be protected (Hamilton 1964).
We are seeking to identify key genes and enzymes involved in the synthesis *E*ßF, but so far homology-cloning has not been successful in obtaining the genes involved (Adams 2002). Therefore we are developing an assay based on enzyme activity to screen aphid gene expression libraries for cDNAs encoding an enzyme that converts farnesyl pyrophosphate (FPP) into sesquiterpenes, the class of hydrocarbon to which *E*ßF belongs. In this assay, FPP is $^{3}$H-radiolabelled so that products from it can be detected by scintillation counting. Initially the assay has been developed with previously characterised plant sesquiterpene synthases: germacrene-D from goldenrod (*Solidago canadensis*) and several from patchouli (*Pogostemon cablin*) (Prosser *et al* 2002 and Michel *et al* 2005, respectively).
Homology-cloning has been successful in obtaining cDNAs encoding enzymes which act in steps further upstream in the biosynthesis of *E*ßF. FPP is the precursor of most sesquiterpenes and is produced by the prenylation of isopentenyl pyrophosphate (IPP), which in turn can be supplied via two pathways: mevalonic acid and methylerythritol phosphate pathways. Partial cDNA sequences encoding enzymes thought to synthesise FPP from IPP have been cloned from several aphid species, which share homology with other insect FPP synthases as well as a very high level of similarity to each other.
Aphids are one of the most agriculturally important insect pest species in the temperate world, largely because of their role as virus vectors, their high fecundity, telescopic generations and very importantly the emergence of resistance to many pesticides. Characterisation of genes involved in aphid alarm pheromone synthesis will provide valuable insight into the terpene biosynthetic pathway in this insect, about which very little is known to date and in turn this could contribute to a diversity of control strategies.

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# Biosynthesis of mandibular gland pheromones in social hymenopterans

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In the course of our studies in chemical ecology of social bees in Asia, 2-alkanones (C7 and C9) and 3-hydroxyalkanoic acids (C8 and C10) were identified in worker mandibular glands of three Japanese *Bombus* species. 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. These results prompted us to test a hypothesis that 3-hydroxyalkanoic acids are precursors of 2-alkanones. Deuterated 3-hydroxy fatty acids, palmitic and stearic acid were synthesized 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 and 2-alkanones. 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.

# Endocrine regulation of pheromone production in the pinyon Ips (Coleoptera: Scolytidae)

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Bark beetles are among the most economically important forest pests in the northern hemisphere, and rely on monoterpenoid aggregation pheromones to coordinate host colonization and mating. In this study, we investigate the interplay between feeding on host phloem and the induction of de novo pheromone biosynthesis in *Ips confusus*, the pinyon Ips. *I. confusus* has become a major pest in the southwestern United States, destroying hundreds of thousands of acres of pinyon pines. Juvenile hormone (JH) III regulates pheromone production in a number of bark beetles. Interestingly, it appears that JH III alone does not stimulate pheromone biosynthesis in male *I. confusus*. We have found that feeding on host phloem, but not JH III treatment, strongly induces pheromone production in male *I. confusus*. In males, feeding also stimulates the activity of a number of mevalonate pathway enzymes including 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-R), which is thought to be the most highly regulated enzyme in the pathway. While feeding and JH III both significantly up-regulate mRNA levels of *HMG-R* and other mevalonate pathway genes, JH III treatment alone does not up-regulate enzyme activities of some enzymes. Thus, some other regulatory factor in addition to JH III is required for pheromone production in *I. confusus.*

# Transcriptional analysis of the pheromone gland of the turnip moth, *Agrotis segetum* (Noctuidae)

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Throughout the order of Lepidoptera, the female sex pheromone commonly consists of a blend of olefinic fatty-acid derivatives (alcohols, aldehydes and acetates). Small differences in the pheromone blends between species accounts for the species specificity. The pheromone components can differ in chain length, desaturation positions and functional groups. The ratio between the pheromone components of a particular blend further adds to a private communication channel.
In order to elucidate how a particular pheromone blend can be achieved on a molecular level we investigated the transcriptome of the turnip moth, *Agrotis segetum*. This noctuid uses a "typical" moth pheromone, which for *Agrotis segetum* is a blend of (*Z*)-5-decenyl acetate, (*Z*)-5 dodecenyl acetate, (*Z*)-7 dodecenyl acetate and (*Z*)-9 tetradecenyl acetate (Löfstedt et al., 1986).
We have launched a program for global transcriptional analysis of the pheromone gland. For this purpose two cDNA libraries for *Agrotis segetum* have been costructed, one representing the female pheromone gland and one representing the rest of the insect body. By EST (Expressed Sequence Tags) analysis in both libraries altogether 2645 clones were analyzed and among those a uniset of 786 putative gene representatives was identified. By comparison of EST counts for these gene representatives, which may be regarded as rough estimators of expression levels, we found the transcriptomes of the two tissues to be clearly different (Figure 1). One third of the gene representatives were exclusively found in the pheromone gland library and could potentially be involved in the process of pheromone production. Half of the gene representatives were found only in the body library whereas one fifth were found in both libraries.
Genes putatively involved in fatty-acid biosynthesis as well as genes with putative functions in cell communication and signal transduction were more abundantly found in the pheromone gland library as compared to the body library. Among genes that were highly and exclusively expressed in the pheromone gland were genes putatively encoding a ? 11-desaturase, a Juvenile Hormone Binding Protein, a FAD-linked oxidase and a Troponin c homolog (involved in signal transduction). The uniset of 786 gene representatives is now employed for the construction of a first-generation *Agrotis segetum* microarray that will be used for more detailed analysis of transcriptional programs in the pheromone gland.

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# Selective use of two desaturases in the sex pheromone production in the genus *Ostrinia* –inference from crossing experiments between *O. furnacalis* and *O. scapulalis*

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In the genus *Ostrinia*, the Asian corn borer *O. furnacalis* females use (*E*)-12- and (*Z*)-12-tetradecenyl acetates (E12 and Z12) as the sex pheromone components, while the adzuki bean borer *O. scapulalis* females use (*E*)-11- and (*Z*)-11-tetradecenyl acetates (E11 and Z11). The difference in the double bond positions in these components is attributable to the type of desaturases involved in the pheromone biosynthetsis; ? 14-desaturase is involved in the production of pheromone components in *O. furnacalis* while ? 11-desaturase is involved in *O. scapulalis*. Interestingly, in *O. furnacalis*, not only the ? 14-desaturase gene (*OfuZ/E14*) but also the ? 11-desaturase gene (*OfuZ/E11*), which is not involved in the sex pheromone biosynthesis in this species, was found to be expressed in the pheromone glands (Roelofs et al., 2002). We have found that both ? 11-desaturase and ? 14-desaturase genes are also expressed in the pheromone gland of *O. scapulalis* (*OscZ/E11*, Fukuzawa et al., 2006; *OscZ/E14*, Fukuzawa, unpublished). In the present study, we conducted crossing experiments between *O. furnacalis* and *O. scapulalis* to examine the inheritance of the sex pheromone type. We were able to obtain hybrid offspring (F1) from the cross between *O. furnacalis* female and *O. scapulalis* male. Surprisingly, the major component in the pheromone gland of the F1 was tetradecanyl acetate (87%), although the pheromone components of their parents, E12, Z12, E11 and Z11, constituted only 2 to 5%. Back cross and F2 experiments showed that the type of the pheromone gland components, i.e., *furnacalis* type, *scapualalis* type, or hybrid type, is mainly regulated by an autosomal dominant gene. This gene appeared to be involved in the specific inhibition of one of the two desaturases. These results suggest that the shift inthe sex pheromone production system between *O. furnacalis* and *O. scapulalis* may have occurred by the mutation of a few genes.

# Identification of the pheromone biosynthesis activating neuropeptide (PBAN) produced by the Japanese giant looper, *Ascotis selenaria cretacea*

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Biosynthesis of lepidopteran sex pheromones is regulated by pheromone biosynthesis activating neuropeptide (PBAN) produced in a suboesophageal ganglion (SG). For about ten species which secrete Type I pheromones (unsaturated fatty alcohols and their derivatives synthesized *de novo* in a pheromone gland), structures of their PBANs have been determined by the analysis of isolated peptides or PBAN-encoding genes. The genes commonly contains five peptides including FXPR(K)L at the C-termini; *i.e.*, diapause hormone (DH) homologue, α-neuropeptide (NP), β-NP, γ-NP, and PBAN. However, PBAN has not been characterized for the species in the family of Geometridae, which secrete Type II pheromones (polyunsaturated hydrocarbons and their epoxides). Biosynthesis of the epoxy pheromones is quite different from that of the Type I pheromones. Namely, 3,6,9-triene, which is a precursor of epoxy pheromone of the Japanese giant looper (*Ascotis selenaria cretacea*) and is produced outside of the pheromone gland (probably in oenocytes or a fat body), transport to the gland *via* hemolymph after association with lipophorin. Only the epoxidation proceeds in the pheromone gland. Our previous study indicated that PBAN did not regulate the biosynthetic step but activated incorporation of the precursor into the gland. We were interested in this mode of action of PBAN, and clarified the neuropeptide of *A. s. cretacea*-PBAN (*Asse*-PBAN) as a first step.

A cDNA isolated from brain-SG complexes of *A. s. cretacea* encodes 181 amino acids including PBAN, DH and three NPs. The *Asse*-PBAN sequence consists of 28 amino acids. It is shorter than those of known PBANs, and its homology is less than 50 %. The length of β-NP (8 amino acids) was interestingly very short and the homology is low. While the cDNAs identified for other species show GRR sequence as a cleavage site between β-NP and PBAN, the corresponding sequence in of *A. s. cretacea* is GR. Furthermore, N-terminal amino acid of *Asse*-PBAN is Q. Therefore, two questions now arise: (1) whether β-NP and *Asse*-PBAN are cleaved or not, (2) in the case of their cleavage, N-terminus of *Asse*-PBAN is cyclized or not. To identify the structure of the pheromonotoropic neuropeptide acting in the females, we are going to analyze an HPLC-active fraction of the extract of brain-SG complexes by MALDI-TOF-MS.

# Sex-pheromone biosynthesis pathways of the pine caterpillar moth, *Dendrolimus punctatus* (Walker) (Lepidoptera: Lasiocampidae)

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Most *Dendrolimus* species occur in China where five out of the 28 species present are important pests in forestry. Identification of pheromone components in this genus started in the 1980's aiming at developing pheromone applications. Pheromone components reported in Lasiocampidae species are usually (*Z,E*)-5,7-dodecadiene carbon chains with different functional groups (alcohol, acetate, propionate and aldehyde). The major components of *D. punctatus* sex-pheromone were identified as the mono-unsaturated (*Z*)-5-dodecadienol and the di-unsaturated (*Z,E*)-5,7-dodecadienol. This species thus most probably utilizes two desaturases with various substrate specificities that contribute to this unusual Lasiocampidae-specific pheromone signature. Based on incorporation of labelled fatty acids, Zhao et al. (2004) proposed several biosynthetic routes potentially leading to the *D. punctatus* major sex-pheromone components. Production of both compounds probably involve chain elongation of 16:Acid to 18:Acid, followed by its subsequent desaturation by a ? 11 desaturase. The (*Z*)-5-dodecenol compound is produced by 3 cycles of chain-shortening reactions on Z11-18:Acid followed by a reduction step. In contrast, the production of (*Z,E*)-5,7-dodecadeniol requires the action of a second desaturase gene, desaturase which could use distinct unsaturated fatty acids as substrate (see figure). Despite consistent efforts on analyzing gland extracts labelled with various fatty acids, no consistent conclusion could be drawn regarding the biosynthesis of (*Z,E*)-5,7-dodecadienol. We took another approach to elucidate the sex-pheromone biosynthetic routes including the molecular characterization of desaturase encoding-genes from *D. punctatus* female pheromone glands and their functional expression in a recombinant yeast system.
By taking advantage of the known degree of homology shared by all members of the desaturase gene-family, an approach that has proved to be successful in many cases (e.g. Knipple et al., 1998; Roelofs et al., 2002), we could amplify five cDNAs sequences using degenerated oligonucleotide primers in a PCR- based approach. Subsequently, respective ORFs were amplified, ligated in the pYES2.1 vector and transformed in a mutant yeast system (Invsc1, Invitrogen). PCR products were expressed and cells were supplemented with various fatty acids. GC-MS analyses of methyl esters showed that a ? 11 gene is acting on various saturated substrates (16:Acid, 18:Acid) thus confirming that a ? 11 desaturase is indeed introducing a double bond into 18:Acid. Ongoing experiments will elucidate which gene among the four supplementary identified is involved in the biosynthetic route leading to this family-unique di-unsaturated (Z,E)-5,7-dodecadiene compound.

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# The labial gland of *Bombus lucorum* males, containing desaturase, produces *in vitro* only saturated esters

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Sex pheromone of the bumblebee males is produced by the cephalic part of the male's labial gland. To better understand the pheromone biosynthesis, labial glands of *B. lucorum* males were incubated *in vitro* with $^{2}$H- and $^{13}$C-labelled saturated fatty acids as well as with a series of alcohols of different structures. Formation of esters was observed in most cases. Ethyl esters and to a small extent also methyl esters were formed from deuterium-labelled fatty acids. From a series of alcohols, only methanol, ethanol, and benzyl alcohol gave the corresponding esters. The other alcohols tested (1-propanol, 2-propanol, allyl alcohol, 1-butanol, 1-hexanol, and 1-decanol) did not react under the same conditions. Thus, the esterase present in the labial gland seems to be highly substrate-selective.
Although the main pheromonal component in *B. lucorum* is ethyl tetradec-9-enoate, no unsaturated esters were found in incubation experiments. To know whether desaturases are present in the labial gland, we isolated a total RNA. The cDNA obtained by a reverse transcription and degenerative primers designed against the conservative domains of desaturases were used for PCR amplification of desaturase genes. We succeeded to isolate a conservative DNA fragment of the gene encoding ?$^{9}$ desaturase. The 3´and 5´ends of the gene were prepared by RACE method (rapid amplification cDNA end). The whole gene encoding ?$^{9}$ desaturase was cloned into YEpOLEX plasmid, which is currently tested for a functional expression of insect enzyme in a desaturase deficient *Saccharomyces cerevisiae* strain.

# Biosynthesis of neral, an alarm pheromone of an astigmatid mite, *Carpoglyphus lactis* (Acari: Carpoglyphidae), and its key enzyme, $NAD^+$-dependent geraniol dehydrogenase

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Neral, [(*Z*)-3,7-dimethyl-2,6-octadienal] has been identified as an alarm pheromone in an astigmatid mite, *Carpoglyphus lactis*. It has been shown that geraniol was first oxidized to geranial by $NAD^+$-dependent geraniol dehydrogenase (GeDH), and then geranial was isomerized to neral in *C. lactis*. In this study, *C. lactis* GeDH was purified through seven chromatography steps, and its enzymatic characters were elucidated. Purified *C. lactis* GeDH subjected to SDS-PAGE gave a single band with a molecular weight of 42800. The molecular weight of the native enzyme was estimated to be 56200 by gel filtration chromatography, suggesting that the enzyme functions as a monomer. The optimum pH and temperature for oxidation of geraniol by the enzyme were determined to be pH 9.0 and 25 °C, respectively. *C. lactis* GeDH was shown to selectively oxidize geraniol in the presence of $NAD^+$. On the other hand, the enzyme could not utilize not only nerol, geometric isomer of geraniol, but also other allylic alcohols such as citronellol, *E*,*E*-farnesol, and 3-methyl-2-buten-1-ol as substrates. These results suggested that *C. lactis* GeDH could selectively recognize the geometric isomers and the carbon length of substrates. The N-terminal sequence of the first 40 amino acids of *C. lactis* GeDH was determined and then the full-length cDNA encoding *C. lactis* GeDH was obtained by PCR and RACE. The deduced amino acid sequence of *C. lactis* GeDH showed homology with zinc-dependent alcohol dehydrogenases found in mammalians. This is the first example of GeDH from an animal origin, and the primary structure determination of GeDH.

# Morphogenetic factors and signalling compounds of Zygomycetes

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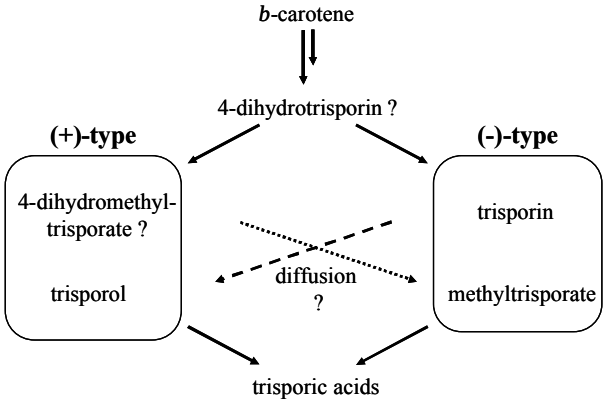
Zygomycetes are fungi living in soil, decaying plant or animal material. If environmental conditions deteriorate and mycelia of opposite mating types are present, the fungus reproduces sexually by formation of zygosporangia. For this sexual reproduction, zygomycete fungi interact via a series of carotene derived compounds, namely trisporic acids and their biosynthetic precursors. So far the postulated sequence of molecular interactions is based on the logical arrangement of isolated compounds and includes an essential diffusion process between the mating partners. The production and metabolism as well as the individual function of the chemical signals is largely unclear.




We developed a novel and highly flexible synthetic building-block strategy which gives a systematic access to structurally modified trisporoids along with putative early biosynthetic precursors [1]. To identify the full range of precursors and unambiguously follow their transformation, also labelled trisporoids were synthesised and an analytical approach to monitor the (labelled) metabolites by GC-MS was developed.
First administration experiments with *Blakeslea trispora* in liquid culture containing single and mated strains and labelled precursors, suggest a different signalling scenario than previously published. Future experiments are needed to understand the interactions and to investigate whether or not the type of chemical communications found in *B. trispora* is also representative for other members of zygomycota.

[1] Schachtschabel *et al.* (2005) *Phytochemistry* **66**: 1358-1365

# Deuterium isotopic effects in the sex pheromone biosynthetic pathway of the processionary moth

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*Thaumetopoea pytiocampa* sex pheromone glands contain desaturases that, after several sequential reactions, catalyze the formation of an unique enyne fatty acid, which is an immediate sex pheromone precursor. Thus, this compound is formed by two concomitant $?^{11}$ desaturations of palmitic acid to form 11-hexadecynoic acid followed by a $?^{13}$ desaturation to give rise to the pheromone intermediate (*Z*)-13-hexadecen-11-ynoic acid. Alternatively, in the same pheromone biosynthetic pathway, palmitic acid can be $?^{11}$ and $?^{13}$ desaturated to afford (*Z*,*Z*)-11,13-hexadecadienoic acid. We have observed that incubation of properly deuterated probes of palmitic acid with deuterium atoms in the $?^{11}$ and $?^{13}$ desaturation positions of palmitic acid originated ratio changes among the acetylene, enyne and diene acid intermediates in the biosynthetic pheromone pathway. This could be a caveat to be considered when deuterium labelled tracers are used in biochemical studies.

# Bark beetle cytochromes p450: characterizing a novel *Ips pini* myrcene hydroxylase

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Insect cytochromes P450 are physiologically important in the synthesis and degradation of endogenous compounds, as well as the metabolic detoxification of exogenous substances, including host plant chemicals and insecticides. We are investigating bark beetle (Coleoptera: Scolytidae) P450s in the pine engraver beetle (*Ips pini*) and the mountain pine beetle (*Dendroctonus ponderosae*). Pine trees are the obligate hosts of these two beetles. Understanding bark beetle-host tree interactions and the expected evolutionarily related pathways for monoterpene detoxification and monoterpenoid pheromone biosynthesis are important for uncovering new modes of controlling bark beetle outbreaks.

Fourteen unique *I. pini* and *D. ponderosae* P450 cDNAs have been cloned and sequenced utilizing expressed sequence tag (EST) libraries of these two species. A multi-disciplinary approach has been applied to the *I. pini CYP9T2* gene. Results are consistent with *CYP9T2* encoding a myrcene hydroxylase that functions near the end of the male *I. pini* pheromone biosynthetic pathway: (I) microarray analyses and quantitative real-time PCR experiments show that basal levels are higher in males than females and the transcript is upregulated in fed and juvenile hormone III treated males, (II) preliminary molecular modeling shows that a monoterpene fits well in the active site, and (III) a functional assay using microsomes containing recombinant baculoviral-mediated P450 and housefly (*Musca domestica*) reductase demonstrates it is a myrcene hydroxylase that converts deuterated myrcene to ipsdienol. Expression profiling, molecular modeling and functional assays of additional bark beetle P450s are in progress.

# Isolation and functional characterization of ?11-desaturase of *Manduca sexta*

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Desaturases are ubiquitous enzymes that introduce double bonds into a fatty acyl (FA) chain and play a key role in the maintenance of the proper structure and fluidity of biological membranes. Desaturases from female abdominal glands of moths (Lepidoptera) play a principal part in pheromone biosynthetic pathways. Desaturases of Lepidoptera are iron-containing integral membrane enzymes of the endoplasmic reticulum that require molecular oxygen, cytochrome *b5* and NADH-cytochrome *b5* reductase to produce specific pheromones. This work is focused on identification and characterization of desaturases involved in pheromone biosynthetic pathways of *Manduca sexta*, a tobacco hornworm moth with a very complex pheromone blend (mono-, di-, and trienals).
The cDNA prepared by a reverse transcription of RNA isolated from the female's abdominal glands and a set of degenerate primers against the conservative domains of acyl-CoA desaturases of different organisms were used for PCR amplification of desaturase gene. The 3' and 5' ends of this gene were isolated by RACE method using the gene specific primers. The desaturase sequence shows 70% identity and 85% similarity to *Helicoverpa assulta* Δ11-acyl-CoA desaturase on the protein level. The analysis of the DNA sequence showed that we have succeeded to construct a gene encoding APTQ desaturase of *M. sexta.* The full-length desaturase gene was cloned into YEpOLEX plasmid, which was used for expression of the desaturase in *S. cerevisiae* cells. The insect desaturase formed (11*Z*)-hexadecenoate in *S. saccharomyces* cells, presumably from the yeast-derived palmitate pool. In cells transformed by constructs producing high amounts of (11*Z*)-hexadecenoate, hexadeca-10,12-dienoates were detected and (10*E*,12*Z*)-isomer was a major compound produced. These results show that the APTQ desaturase can act as a 1,4-conjugase and can produce FA precursors for 10*E*,12*Z*-hexadecadienal, a principal component of the *M. sexta* pheromone blend.

# Alkaloid biosynthetic origin and nuptial gift in *Epilachna paenulata* (Coleoptera: Coccinellidae)

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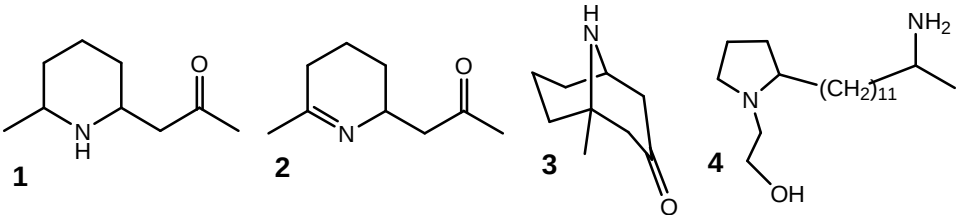
Coccinellid beetles contain a variety of defensive alkaloids that render them unpalatable to several predators. *Epilachna paenulata* (Coleoptera: Coccinellidae) is a South American ladybird beetle that feeds on plants of the family Cucurbitaceae. The defensive chemistry of *E. paenulata* was characterized as a mixture of piperidine, homotropane and pyrrolidine alkaloids. Whole body extracts of adult beetles contain four major alkaloids: 1-(6-Methyl-2,3,4,5-tetrahydro-pyridin-2-yl)-propan-2-one (**1**); 2-(2'-oxopropyl)-6-methylpiperidine (**2**); 9-aza-1-methyl-bicyclo[3.3.1]nonan-3-one (**3**) and 1-(2´´-hydroxyethyl)-2-(12´-aminotridecyl)-pyrrolidine (**4**). Comparative studies of the defensive chemistry of eggs, larvae, pupae and adults showed differences in alkaloid composition and concentration among all life stages. Eggs contain the highest concentration of **1** and adults contain mainly the homotropane **3**. We fed newly-emerged, virgin adult beetles with ($^{13}$C)-labelled acetate, and performed crosses between $^{13}$C-fed and control males and females. GC-MS analysis of alkaloids from $^{13}$C-fed males and females showed high incorporation of $^{13}$C into the alkaloids, evidenced from a 20-30% increase of isotopic peaks in diagnostic fragment ions. Analysis of eggs from different crosses showed that labelled alkaloid from both parents are incorporated into the eggs, indicating that males *E. paenulata* transfer a nuptial gift that contains alkaloid to the females.



# Pheromone production in female *Nereis succinea*

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Female specimens of the ragworm, *Nereis succinea*, employ a tetra-peptide, cysteinyl-glutathione (CSSG) as a mate recognition pheromone and gamete release inducing pheromone during reproduction. We used injection of radio-labelled reduced glutathione (GSH) to confirm the biosynthetic pathway of pheromone production to be the oxidation of reduced glutathione with cysteine to form the mixed disulfide, CSSG. We also investigated the hypothesis that females produce almost all their sex pheromone during the reproductive event itself, rather than e.g. during the maturation or metamorphosis to the sexually active heteronereis form. The rate of excretion was determined by HPLC and biological assays. During spawning the female worms excreted on average 55 µg/h CSSG. The pheromone release rate was directly correlated to female size with the largest females releasing over 100 µg/h CSSG. Collecting swarming specimens in the field (Isefjord, Denmark) revealed substantial variation in weight/size of the spawning (reproducing) specimens. Interestingly, a significantly greater proportion of small males appeared in the early stages of the spawning events. We discuss the potential role of size dependent pheromone release rate and male responses in mate choice, pair formation and the possible presence of sex pheromone production based alternative mating tactics in *Nereis succinea*.

# Two-step regulation of pheromone biosynthesis by the pheromone biosynthesis-activating neuropeptide in the moth *Heliothis virescens*

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The control of pheromone biosynthesis by the neuropeptide PBAN was investigated in the moth *Heliothis virescens*, by following the fate of both native and labeled compounds over time. When decapitated females were injected with [2-$^{14}$C] acetate, females co-injected with PBAN produced significantly greater quantities of radiolabeled fatty acids in their pheromone gland than females injected with saline. This indicates that PBAN controls an enzyme involved in the synthesis of fatty acids, probably acetyl CoA carboxylase. Decapitated females injected with PBAN showed a rapid increase in native pheromone, and a slower increase in native pheromone precursor, (*Z*)-11-hexadecenoate. Total native palmitate and stearate (both pheromone intermediates) showed a significant decrease after PBAN injection, before their titers were later restored to initial levels. In contrast, the acyl-CoA thioesters of these two saturated fatty acids increased during the period when total titers of these acids decreased. When a mixture of labeled palmitic acid and heptadecanoic acid (an acid that cannot be converted to pheromone) was applied to the gland, PBAN-injected females produced greater quantities of labeled pheromone and pheromone precursor than did saline-injected ones. The two acids showed similar time-course patterns, with no difference in total titers of each of the respective acids between saline- and PBAN-injected females. When labeled heptadecanoic acid was applied to the gland alone, there was no difference in titers of either total heptadecanoate or of heptadecanoyl-CoA between PBAN- and saline-injected females, indicating that PBAN does not directly control the storage or liberation of fatty acids in the gland. Overall, these data indicate that PBAN also controls a later step involved in pheromone biosynthesis, probably the reduction of fatty acyl moieties. The control by PBAN of two enzymes, near the beginning and end of the pheromone biosynthetic process, would seem to allow for more efficient utilization of fatty acids and pheromone than control of only one enzyme.

# Absolute configuration of quercivorol, the aggregation pheromone of Platypus quercivorus (Coleoptera: Platypodidae)

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The mass attack of the ambrosia beetle, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae), have resulted in the widely spread and continuous heavy mortality of oak in Japan since late 1980's. The gallery elongation of *P. quercivorus* and the infection of newly isolated phytopathogenic fungus, *Raffaelea quercivora*, disordered the water flow in attacked oak trees, especially *Quercus crispula* Blume. Field experiments indicated that the chemical communication using aggregation pheromone accomplished the mass attack by the beetles to hosts.

To clear the chemical structure of the pheromone, the volatiles from boring dust of the beetle were analyzed by gas chromatography (GC)-electroantennogram detection (EAD) and GC- mass spectrometry, and synthetic racemic mixture of the EAD-active compound was used for field trap experiments. The results of all these experiments indicated that *trans*-*p*-menth-2-en-1-ol is the aggregation pheromone of the beetle, but the stereochemistry of it has not yet been cleared.

To elucidate the absolute configuration of the aggregation pheromone of the beetle, (1*S*,4*R*)- and (1*R*,4*S*)-*p*-menth-2-en-1-ol were synthesized from (S)- and (R)-limonene.

The retention time of the natural pheromone on chiral GC column was coincided with that of synthetic (1*S*,4*R*)-isomer, but not with that of synthetic (1*R*,4*S*)-isomer. Preliminary field trap experiment revealed (1*S*,4*R*)-isomer is attractive for *P. quercivorus*. We concluded that quercivorol, the aggregation pheromone of *P. quercivorus*, is (1*S*,4*R*)-*p*-menth-2-en-1-ol.

# Anthrasteroid hydrocarbons of the sheep tick *Ixodes ricinus*

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Tick mating is a complex procedure regulated by sex pheromones. In the hard ticks (Ixodidae), at least three types of sex pheromones are involved in mating: an attractant sex pheromone, a mounting sex pheromone, and a genital sex pheromone. The attractant sex pheromone is typically released from feeding females and stimulates searching behavior in males. The mounting sex pheromone is present on the surface of the engorged female tick's body and serves as a recognition cue for males searching for females. The genital sex pheromone provides a conspecific identification and induces spermatophore production in males. The sheep tick *Ixodes ricinus* (Linnaeus, 1758; Ixodidae, Prostriata) is an important vector for many serious human diseases.
In a search for chemical compounds involved in reproduction behavior of the sheep tick, *Ixodes ricinus,* the composition of the tick cuticle surface was investigated. Compounds specific for engorged females were found. The structure of the main female-specific component was determined using MS, NMR, and derivatization (hydrogenation) as an anthrasteroid hydrocarbon, 14α(H)-1(10-6)-abeo-cholesta-3,5,7,9(10)-tetraene. The identity of other female specific compounds is currently investigated. The biochemical pathway and possible roles of novel compounds in the sheep tick biology will be discussed.

# Sex pheromone and cuticular hydrocarbons of the painted bug, *Bagrada hilaris* (Heteroptera: Pentatomidae)

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*Bagrada hilaris* Burmeister (Heteroptera Pentatomidae), the painted bug, is a phytophagous pest which occurs across Southeast Asia and Africa. In Europe it has been observed only in Malta and in the island of Pantelleria (Sicily - Italy) where it feeds mainly on caper plants (*Capparis spinosa* L.). The aims of this study were: **1.** to investigate the volatile chemical compounds produced by adult *B. Hilaris,* focusing mainly on sex-specific compounds which may act as a sex pheromone, and **2.** to analyse cuticular hydrocarbons of adults, adopting both solvent and solvent-free extraction methods. Behavioural observations were carried out with a vertical *Y*-olfactometer, while chemical analyses were performed with a GC (HP 5890) equipped with FID and a GC (HP 6850) interfaced with a MS detector (HP 5973N). Both GCs were quipped with a J&W DB5-MS column (30 m length x 0.25 mm ID, 0.25 μm film thickness) and all instrumental parameters were optimized using hydrocarbon standards (C9-C36). In laboratory bioassays, female *B. hilaris* were more attracted to odours from conspecific males than clean air control, while male *B. hilaris* showed no preference for either the odours of conspecific females or males. Volatile chemicals of live male and female *B. hilaris* were collected on activated charcoal traps from adults held in glass aeration chambers, eluted with hexane and analyzed by gas chromatography. The main compound in extracts from both females and males was (*E*)-2 octenyl acetate, which was confirmed by comparison with an authentic standard obtained from J.G. Millar. Samples of cuticular hydrocarbons of adult *B. hilaris* were obtained from hexane extracts of dissected body parts of males and females and use of SPME (Solid Phase Micro Extraction) techniques. Analysis of the cuticular hydrocarbons collected showed that male and female *B. hilaris* produced sex-specific blends of compounds but that the profiles were unaffected by the method of collection. Laboratory and field tests to clarify the role of these compounds in the semiochemistry of *B. hilaris* are in progress.

# Chemical ratios and release rhythms in the sex pheromone of an aphid

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In aphids, with one exception all studied species use the same monoterpenoid chemicals in their sex pheromone communication and these are released either as a single chemical or as a blend of two depending on the species. We have collected sex pheromone from different batches of rosy apple aphid, *Dysaphis plantaginea*, and have identified by GC, GC-MS and NMR, the presence of (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone in a 3.70:1 ratio (78.7%). Although chemical ratios appear to be a factor for species recognition in aphids, there are many examples showing that the signal integrity cannot rely on ratios alone. It has therefore been suggested that timing of sex pheromone release is an important factor for species recognition and thus reproductive isolation. However, little data is available regarding periodicity of sex pheromone release in aphids. We therefore constructed a simple sequential sampling device that allowed us to capture at hourly intervals headspace samples from an aeration chamber containing 95 same-aged sexual females. Uninterrupted patterns of sex pheromone release over 20 consecutive days are presented and data show that the aphids release high levels throughout the photophase and low levels throughout the scotophase. This reveals a periodic up and down-regulation of pheromone metabolism that cannot be completely switched off. Analysis has found no evidence for a narrow 'calling-window' because pheromone release increased significantly during the first three hours of photophase and thereafter approximately plateaued until the onset of scotophase. Pheromone output peaked on the eighth day of the adult stadium with a mean of up to 8.4 ng of pheromone being released per aphid per hour. The ratio of the two components was broadly stable, however, there was a significant decrease in the relative amount of the alcohol beyond the fourteenth day of the adult stadium. This is the first report on the chemical ratios for this species and is also the most detailed temporal study on aphid sex pheromone release. Our finding that there is no 'calling-window' would support the existence of other hypothesised recognition mechanisms, for example, synergism between host-plant volatiles and aphid sex-pheromone, and/or, the presence of additional unidentified minor pheromone constituents.

# Sex pheromone of *Elater ferrugineus* LINNÉ (Coleoptera: Elateridae)

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*Elater ferrugineus* L. is one of the largest and most notable click beetle species of Central Europe. Its development takes place in hollows of old deciduous trees and lasts up to six years. The larvae are partly carnivorous and preferably prey on the first stages of several scarab beetle species.
Due to massive habitat loss, *E. ferrugineus* is regarded as an endangered and red list species in most parts of its distribution area. Adult beetles of *E. ferrugineus* live very hidden and are hardly ever found in the field, while the larvae are recorded only occasionally when inhabited hollow trees are felled or otherwise opened. This usually goes along with destroying the habitat.
Therefore, we examined the sex pheromone of *E. ferrugineus* in order to develop pheromone traps for monitoring purposes in nature conservation. Beetles reared from larvae collected at different field sites were dissected and pheromone gland extracts of single females were examined using GC-MS. All samples showed a similar complex mixture of 7-methyl octyl esters with both saturated and unsaturated acid moieties from 6 to 11 carbon atoms.
A synthetic blend of the four main compounds was tested in the field and proved to be extremely attractive on *E. ferrugineus* males. This provides a non-invasive and highly effective monitoring method for this frequently overlooked species.

# Detection of stored product pests by SPME-CGC

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*Tribolium castaneum* (Coleoptera: Tenebrionidae) is a major stored product pest, usually feeding on flour and damaged grains, causing a significant economic loss. The red flour beetle produce defensive pheromone secretions upon disturbance, contaminating the material they feed on. The major components of the volatile blend are methyl- 1,4 benzoquinone (MBQ), ethyl- 1,4 benzoquinone (EBQ) and 1-pentadecene, produced both in abdominal and prothoracic glands. A number of analytical techniques had been applied to quantitate the unstable quinones as an indirect estimation of food product damage. The tenebrionid *Ulomoides dermestoides* has been recently introduced in Argentina, showing potential ability to contaminate stored grains; no reports are available on their volatile organic compound (VOC) secretions. Solid phase microextraction (SPME) technique was used for the collection of the VOC released by both beetles. SPME samples were analyzed by capillary gas chromatography (CGC) and CGC coupled to mass spectrometry (MS) employing alternatively three different fiber coatings: carboxen/polydimethylsiloxane (CAR/PDMS), PDMS/divinylbenzene (PDMS/DVB) or PDMS. Insect VOC were sampled from unstressed insects, the same insects were then stressed by agitation, finally a third extraction was performed during a heating treatment. The pheromone blend released by one *T. castaneum* specimen is easily detectable by any of the fibers. The relative amount of each component varied as a function of the extraction procedure and the fiber employed. After agitation disturbance, mixed coating fibers showed large signal intensities for the polar benzoquinones (BQ). The highest BQ relative amounts (81.5 ± 7.8%) were adsorbed with the CAR/PDMS fiber. The highest total BQ recovery was about 1 µg/beetle with any of the three fibers. *U. dermestoides* showed a similar VOC pattern, with prevalence of both quinones together with 1-pentadecene. Total BQ amounts detected per beetle were significantly lower (~ 0.3 µg) than in the red flour beetle. The SPME-CGC technique is a simple and appropriate tool to detect stored product pest contamination.

# Epicuticular hydrocarbons of *Leptinotarsa decemlineata* (Say): A sexual dimorphism

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Insect epicuticular hydrocarbons play a key role as odorants in several species. During last years they have been found to be involved in chemoreception processes such as nest recognition of *Apis mellifera*, sexual pheromones in several Diptera, and the aggregation phenomenon of the desert locust *Schistocerca gregaria*.
In the present study we characterise the epicuticular composition of the Colorado Potato Beetle, *Leptinotarsa decemlineata*. Despite this insect is considered as one of the most dangerous pest species in agriculture, so far no sexual pheromone has been reported. In a previous work we identified a new volatile amino-aromatic compound from the elytra of males and females. Here we focus our attention on the pattern of hydrocarbon extracted from the cuticle of male and female elytra in order to characterize a possible sexual communication. In a previous research a sex-dependent composition of cuticular hydrocarbons of *L. decemlineata* has been already described but the methodologies we adopted and the results differ substantially from the reported ones. We extracted cuticular hydrocarbons from the elytra of single animals in different life stage, e.g. freshly emerged, mating and ovipositing females. We analyzed the heptane extracts by the mean of GC-MS using a specific temperature program reaching 310 °C. The chromatograms were interpreted with NIST Standard Reference Database, and in comparison with standards chemicals. The results confirmed that there is no *n*-alkanes in the epicuticular hydrocarbon mixture of *L. decemlineata*, but -contrary of what previously reported- females did not show a higher quantity of hydrocarbons with more than 34 carbons atoms. In our experiments males showed a simpler mixture of hydrocarbons, where hydrocarbons with higher molecular weight are actually predominant in comparison with females. Moreover, three hydrocarbons are exclusively or highly more abundant in females. These three compounds appeared in the first half of the chromatogram, therefore with a relatively low molecular weight. The same data were also analyzed by using principal component analysis (PCA) Pirouette® software (Infometrix). A PCA scores plot showed a clear separation between male and female hydrocarbons.

# Queen signal of the stingless bee *Melipona beecheii* (Apidae, Meliponini)

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The social organization of advanced eusocial insects largely depends on chemical signals that act as a communication channel between the single individuals of a colony (e.g., Wilson 1971). For example, the queen pheromone of the honeybee is well investigated and does have an important function in the regulation of reproduction (Free 1987). In order to identify the queen signal of the stingless bee *Melipona beecheii* we studied the volatile odor bouquets of workers, virgin queens, and physogastric queens of this species in Costa Rica. Scent was collected by placing bees individually in tightly closed, clean glass vials for 30min. After removing the bees, each vial was put in a freezer at –8°C for 1h to condense chemical compounds at its wall. Subsequently, the inside of the bottles was rinsed with 1.5ml pentane for 90s to extract the compounds. By means of gas chromatographic analyses coupled to electroantennographic detection (GC-EAD) we found that 16 compounds from the physogastric- and virgin queens' odor bouquets elicit a response in the chemoreceptors on the workers' antennae. The relative proportions of these compounds in the odor bouquets of physogastric and virgin queens differs. Furthermore, most of these substances are absent in the workers. Our results indicate that the active, queen specific substances may act as a queen signal. They are likely to be involved in two important contexts: (i) as information that the colony is queen right and (ii) for discrimination of workers from virgin queens, which normally are killed by the workers unless the physogastric queen dies or the nest is divided in order to initiate a daughter colony. Future bioassays with synthetic compounds of identified odor components will test these hypotheses.

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# A terpene and terpene-derivatives survey from bumblebees (*Bombus terrestris*): Implications for insect-insect communication

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Terpenes and terpene-derivatives molecules have been already described as forming part of the food alert pheromone of bumblebees (Mena *et al*., 2005).
It has been carried out a survey of terpenes and terpenes-derivatives molecules from bumblebee workers, adults and larvae, which can allow us to start to understand the communication between bumblebees about nutritional food levels.
Bumblebee colonies submitted to food deprivation have been sampled in comparison with other similar colonies having food *ad libitum*. By using SPME techniques linked to GC-MS/MS analysis, we have obtained the following results:

| Compounds | Adult workers terguites before eating | Adult workers terguites after eating | Bumble bee larvae food deprivation (nanograms) | Bumble bee larvae having food (nanograms) |
|---|---|---|---|---|
| *alpha*-pinene | 748,6±191,8 | 648,8±220,9 | 587,56±125,21 | 101,83±55,98 |
| *R*-limonene | 696,8±126,1 | 735,8±165,5 | 227,38±97,25 | 128.7±48,54 |
| 1,8-cineole | LOQ | 622,4±134,4 | 145,54±64,24 | 134.23±45,25 |

Average±SE of 10 analyses per sample.

The number of terpenes and terpene-derivatives which can be perceived by bumblebee's olfactory organs is still a matter of study and discussion, but, in any case, the variation in amount of some of the compounds can be used by bumblebees as signals for food alert or as a deprivation of food signal.

[1] Mena et al. (2005): Chemical compounds of the foraging recruitment pheromone in bumblebees. *Naturwissenschaften* **92**: 371–374

# Chemical compounds of the food-alert and recruitment pheromone in the bumblebee *Bombus terrestris* and its physiological role

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The bumble bee *Bombus terrestris* is an annual bumblebee that do not communicate spatial co-ordinates of food sources, but successful bumblebee foragers do inform nestmates about the general availability and the scent of rewarding food sources (Dornhaus and Chittka 1999, Nature 401: 38 1999; 2001 Behav. Ecol. Sociobiol. 50:570–576).
We have investigated the chemical compounds of the emission from tergal glands of bumblebees. A total of ten *B. terrestris* colonies were used containing, each one, 30-50 workers. Each nest was contained in a wooden box, which was connected to a foraging arena by a Plexiglas tube of 30 cm length. Nest box and foraging arena had transparent Plexiglas covers, so that the behaviour of the bees could be observed. The arena contained a feeder at certain times of the experiment.
Three different trials were carried out (Mena et al., 2005, Natürwis. 92: 371-374). The first was a control, bumblebees remained in the boxes without eating and the air was analysed. For the second trial, a sugar solution was placed at the interior of the feeding arena and the air was analysed. The third trial consisted in identifying the origin of such alarm pheromone, by dissecting 5 foragers that had just returned to the nest from the feeder, and compared the compounds found in extracts of their tergites with those from 5 foragers that had not been offered food. Significant differences were founded in eucalyptol, Z-ocimene, and E,E-farnesol (Wilcoxon-test, n=10 pairs, T=0, Z=2.803, $p<0.005$ in all three cases), which we have identified as the compounds of the food alert pheromone of bumblebees. These compounds have been assessed by behavioural tests, demonstrating an increase in the foraging activity of bumblebees, being eucalyptol the compound that provokes the most increase.
In addition to the above discoveries, we have found (Roldán et al., 2005, (J.M.Guerra, A. Roldán & A. Mena, eds.), I Short Course on Pollination of Horticulture Plants, pp: 121-132) that the emission of the compounds of the alert food pheromone is independent from the behaviour of running and fanning into the nest and that each individual bumblebee worker releases the pheromone just after eating, either syrup or pollen.

# Sharing of trails, nests, and resources between *Camponotus* and *Crematogaster* ant species – mechanisms and evolution of behavioural tolerance

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The literature on interactions between ants is dominated by reports on antagonistic behaviour such as competition, predation and social parasitism between species and conspecific colonies. For interspecific aggression and nestmate recognition, the composition of cuticular hydrocarbons has been shown to be crucial. One approach to understand aggressive behaviour is to study cases where it is missing. In many tropical communities different ant species can be found on the same trail without displaying any mutual aggression, or even share the same nest (parabiosis). The most prominent examples worldwide are several ant species from the highly common and species-rich genera *Camponotus* and *Crematogaster* for which inter*generic* trail sharing and parabioses are widespread both in the neotropics and paleotropics. Only few studies have investigated trail sharing or parabiotic relationships between ants. It is unknown whether parabioses are based on common underlying principles, and how they relate to trail- and resource sharing. Virtually nothing is known about mechanisms and causes of such associations and whether they can be classified as mutualism, commensalism or parasitism.
Our present results suggest that cuticular hydrocarbon profiles in parabiotic ants in Borneo (*Crematogaster modiglianii* and its associated several *Camponotus* species) differ substantially from any other ant studied to date. Long-chain hydrocarbons such as alkanes are poorly developed or even absent in hexane extracts of these parabiotic ants, while various still unknown chemicals are common. This novel discovery opens up a variety of hypotheses about the role of cuticular profiles found in parabiotic species for nestmate and partner recognition or chemical camouflage, and whether such divergence from 'normal' cuticular signals is unique or widespread in other parabiotic associations elsewhere.

# Acquisition and developmental mechanisms in social behaviours of honey bees

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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. In response to the stimuli, individuals recognize the environmental conditions surrounding the colony. Semiochemicals play crucial roles in recognition and pheromones do in communication. Individual recognition and communications among colony members would lead to wide variety of social behaviors. Therefore, understanding the basic mechanisms of social behaviors attracts attentions of researcheres in the field of micro machines and brain sciences. How the flexibility of the honey bee colony arises, is one of the most attractive issues in our research. In this paper, aquisition and development in behavioral repertory between these two honey bees were investigated in connection with differences in recognition and responses to semiochemicals. In Japan, there are two *Apis* species, domestic honey bee (*Apis cerana japonica* Rad.; Acj) and imported honey bee, *Apis mellifera* L.(Am). There are behavioural differences between the two species. For example, Am collect propolis but Acj does not. Acj performs hygienic allo-grooming behavior against *Varroa* mites but Am seldom does it. When Acj workers with different ages (0, 7, 14, 17 days after eclosion) were exposed to *Varroa* mite extract, day 0 workers did not respond (0%), however 7 days and older workers responded (100%). It was observed that the older workers responded more quickly and persistantly upon exposure to the extract. Therefore, allo-grooming behaviour in Acj develops in accordance with the age after eclosion. Development of allo-grooming behaviour in Acj may be under hormonal control as is age-polyethism.

# Reversed innervation of pheromone specific antennal lobe neurons in the European corn borer (*Ostrinia nubilalis*)

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The European Corn Borer is a well known example of pheromone polymorphism and possible speciation through reproductive isolation in the pheromone communication channel. Two strains, the E and the Z strain, do not interbreed freely in sympatry, as they produce and prefer a reversed ratio of the pheromone components E11-14:OAc and Z11-14:OAc. Here we studied how the difference in male preference correlate with differences in wiring of olfactory input and output neurons in the antennal lobe.
Intracellular recording and staining techniques were used to establish the structure and function of male antennal lobe (AL) projection neurons (PN). Physiologically characterized neurons were filled with neurobiotin and their dendritic arborization patterns were reconstructed using confocal microscopy in alfa-synapsin overview stained ALs. In addition, innervation patterns of single, physiologically undefined olfactory receptor neurons (ORN) were established by neurobiotin staining.
The AL of males in both strains have approximately 60 glomeruli, the macroglomerular complex (MGC) situated at the entrance of the antennal nerve, is morphologically similar in the two strains, but differs substantially from earlier reports. It consists of two major compartments, a large, medial compartment folded around a smaller, lateral one. ORNs display a uniglomerular innervation pattern in the MGC. PNs innervating each of the two major interdigitated MGC glomeruli are specifically tuned to the two major pheromone components, such that E- or Z11-14:OAc-specific PNs have dendritic branches in either glomerulus. In the Z-strain Z11-14:OAc-specific PNs arborize in the larger, medial MGC glomerulus, whereas E11-14:OAc-specific neurons innervate the medial, smaller one. In the E strain this topology is reversed.
We postulate that the E and Z responding ORNs have exchanged the expression of their olfactory receptors, whereas the ORN as well as the PN innervation patterns in the AL has been conserved between the two strains.

# Picking a fly's nose: Differential olfactory learning and discrimination ability in *Drosophila* siblings

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The wiring of *Drosophila melanogaster*'s olfactory circuitry is increasingly understood, from olfactory input to relaying to second and third order neurons. We asked the question of how a fly uses input from across sensory cells in discrimination between odorants. However, generally flies do not respond in a robust repeatable manner to single odorants. We therefore used an operant learning paradigm to enhance the repeatability of the responses. We exposed flies to pairs of odorants which were combined with either a reward or punishment. In subsequent assays we asked whether they were able to discriminate between the two odorants. Flies showed robust learning, persisting over many days. The positive association was stronger than the negative. Whereas esters were readily learned, flies showed poor associative memories to several other odorants. We compared the odorants learning and discrimination ability of *D. melanogoaster* and its closely related sibling species *D. sechellia*. As this specialist sibling species expresses fewer types of neurons sensitive to esters, we wondered how this affected the odorant discrimination ability. Surprisingly, we found that in our assays this specialist species did not effectively learn odorants. However, its innate responses to single odorants, representative of its only host, *Morinda citrifolia*, were very robust. Possible causes are discussed.

# Biosynthesis of (3Z,6Z,9Z)-octadecatriene: A compound of the pheromone blend of *Erannis bajaria* (Lepidoptera:Geometridae)

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The pheromone blend of *Erannis bajaria* (Lepidoptera) consists of (3Z,6Z,9Z) octadecatriene (3Z,6Z,9Z-18:H) and (3Z,6Z,9Z)-nonadecatriene (3Z,6Z,9Z-19:H)[1]. Odd numbered hydrocarbon pheromones occur often and their biosynthesis is well studied. The biosynthesis of 3Z,6Z,9Z-19:H requires *a*-linolenoic acid (9Z,12Z,15Z-18:acid) which is elongated by malonyl-CoA to the corresponding 11Z,14Z,17Z-20:acid. Final reductive decarboxylation provides 3Z,6Z,9Z-19:H[2]. In contrast, knowledge about the biosynthesis of even numbered hydrocarbon pheromones is scanty. In the case of 3Z,6Z,9Z-18:H it was proposed that a direct reduction of the acid furnishes the hydrocarbon[2].
The results of our studies concerning the biosynthesis of 3Z,6Z,9Z-18:H in *Erannis bajaria* are in contradiction to this assumption. Feeding experiments with deuterium labeled 9Z,12Z,15Z-18:acid, 9Z,12Z,15Z-18:alcohol, 10Z,13Z,16Z-19:acid and 11Z,14Z,17Z-20:acid were performed followed by GC-MS analysis of extracts of the pheromone gland. The investigations showed incorporation of deuterium labeled 10Z,13Z,16Z-19:acid and 11Z,14Z,17Z-20:acid in the even numbered alkatriene. But we did not observe any incorporation of the other two possible precursors in several trials. The lack of incorporation of deuterium labeled 9Z,12Z,15Z-18:alcohol is understandable because another biosynthetic pathway than reduction of the alcohol seems to be favored. The fact that experiments with deuterium labeled 9Z,12Z,15Z-18:acid were not successful is explainable with always low incorporation rates.
Based on the results of our study we conclude that 9Z,12Z,15Z-18:acid is elongated to the respective 11Z,14Z,17Z-20:acid, followed by a-oxidation to the 10Z,13Z,16Z-19:acid. Decarboxylation finally furnishes the even numbered pheromone 3Z,6Z,9Z-18:H.

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[2] J. G. Millar **(**2000) *Annu. Rev. Ent.* **45**: 574-604.

# Investigation on potential chemical signals related to the sexual behaviour of *Rhodnius prolixus* Stal (Heteroptera: Reduviidae)

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*Rhodnius prolixus* is the main vector of Chagas disease in northern South America and in part of Central America (Schofield, 1994). Triatomine adults posses a pair of metasternal glands (MG) that open in the ventral metatorax (Schofield & Upton, 1977). The function of the MGs of *R. prolixus*, as well as the identity of their secretions is currently unknown. Therefore, the objective of this study is to identify the compounds produced by the MGs of *R. prolixus*, as well their emission in behavioural contexts probably related to the sexual communication of this species. In order to identify the compounds produced by the MGs, chemical analysis of the MGs content was performed using SPME and GC-MS. Moreover, the effect of age, nutritional and reproductive status in the synthesis of these compounds was also analyzed. Furthermore, we investigated whether some of these volatile compounds from MGs are emitted spontaneously by females at the start of their activity phase. Finally, as pairs emit odours that aggregate males (Baldwin et al., 1971) the same investigation was carried out with mating pairs. Sixteen compounds were identified in the MGs of *R. prolixus* and they were isoprenoids, ketones and alcohols. The same compounds were found in the glands of both sexes, except for a ketone and an alcohol which were only detected in samples of males and females, respectively. In 70% of the copulations, at least one MGs' compound was found, being the main MG peak detected in 45% of samples. We observed that up to seven compounds were spontaneously emitted by females. We suggest that the compounds produced by the MGs of *R. prolixus* are involved in the sexual communication of this species. We propose that long distance orientation and subsequent recognition between sexes can be potentially mediated by chemical signals originated from the MGs. The identification of compounds produced by the MGs, as well as their emission in sex related contexts, represents a first step in the study of the apparent use of sexual pheromones by *R. prolixus*. A potential use for manipulating the behaviour of this species makes them candidates for developing chemical baits for improving vector control strategies.

# SYMPOSIUM 4

# CAUSES AND CONSEQUENCES OF VARIATION IN LEPIDOPTERA SEX PHEROMONES

# Patterns of variation in lepidopteran sex pheromones

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The female-produced sex pheromone of nearly all moth species is comprised of two or more components, with the ratio of components ranging between tight to loose regulation. It is generally accepted that "off-ratio" females should suffer fitness costs in the form of a reduced probability of being mated. This would impose a strong selective force to limit variation in the blend. The described cases for moth sex pheromones for which it was possible to calculate a co-efficient of variation, were used to analyze the following questions: (1) How variable are pheromones and how do they compare to estimates of variation for other traits (i.e., life-historical, behavioral, and morphological); (2) Is the amount of variation in the pheromonal signal independent of the number of species in the clade; (3) Is the amount of variation in the pheromonal signal independent of pheromone chemistry; (4) Are there any ecological patterns to pheromone variation; and (5) Are there any phylogenetic patterns to pheromone variation? The results of this analysis are interpreted and compared to the assumptions and predictions of our current paradigm of moth pheromone evolution. Data on pheromone variation in the almond moth, *Cadra cautella* will be presented to illustrate the type of information useful to improving our appreciation of moth pheromone variation.

# Genetic mechanisms of variation in lepidopteran sex pheromones

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Species-specific sex pheromone blends are critical components of moth mating systems. They mediate mate location and contribute to reproductive isolation. More recently diverged species typically have more similar pheromone signals than less closely related species. The "gradual" model suggests that the type of genetic variation present at the micro-evolutionary level (within clades) can explain variation at the macro-evolutionary level (among clades, i.e. phylogenetic patterns). Essentially, it proposes that major differences in trait values develop via the accumulation of many genetic changes of small effect. An alternative "saltational" model suggests that differences among clades are due to a qualitatively different type of variation, that is, genetic changes with large effects on trait value. We review the literature on the genetic basis of variation in pheromones and, using the above general models, discuss mechanisms for variation and the significance of additive genetic variance and major gene effects. Finally, we will attempt to place what is known about pheromone genetics in the context of what is known in general about the genetics of morphological, behavioral, and life history traits.

# Evolutionary causes and consequences of variation in lepidopteran sex pheromones

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Signals are unique among adaptations in that they are at least in part the product of a co-evolutionary process with the response profiles of the responder. Thus understanding variation in female-produced lepidopteran sex pheromones must include a consideration of male response profiles. Theory predicts that for optimum recognition and attraction to occur, strong congruence must exist between the female signal and male response profile. In general, a male's orientation behavior to the pheromone signal is thought to result in strong stabilizing selection on the female's pheromone. Conversely, theory also predicts that, because lepidopteran mating systems involve female-biased investment in the zygote, selection is predicted to favor male response profiles broad enough to include most female signals in a population. We analyze the known cases of male response profiles (in no-choice and choice bioassays, and in field trapping trials) and attempt to resolve this dichotomy. We address the following issues: (1) Does variation in the signal have any consequences in terms of male orientation behavior; (2) What does the literature on male response profiles suggest about the type and strength of selection acting on sex pheromones; and (3) What types of associations are known about male response profiles and female variation? Some new information on the orientation preferences of the male almond moth, *Cadra cautella*, to variations in its pheromone blend suggest the kinds of experiments that can be useful that to understanding these issues.

# Synthesis and characterization of 3,13- and 2,13-octadecadienyl compounds for identification of the sex pheromone secreted by clearwing moths

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In addition to the nocturnal species, whereas diurnal insects can possibly find their mating partners by means of visual cues, females of diurnal species in the family of Sesiidae (Lepidoptera), which have clear wings and mimic bees, also secret a species-specific sex pheromone to attract males. In Japan, about 40 sesiid species have been recorded, and attraction of thirteen species to the lures baited with known sesiid pheromones has been documented. In addition to these attractants, an effective method has been developed to disrupt the mating communication of two harmful pests in Sesiidae, *Synanthedon hector* and *S. tenuis* as further agricultural application of their lures. Identification of pheromone components in females, however, has never been published for any of the Japanese sesiid species because the larvae burrowing in stems of plants are not found readily.

As a first step in studies on sexual communication systems of the diurnal species, we synthesized all geometrical isomers of 3,13- and 2,13-octadecadienyl compounds and accumulated analytical data on the sesiid pheromones to support chemical studies on the Japanese species. Although acetoxy derivatives of the 3,13- and 2,13-dienes showed almost the same mass spectra, alcohols were distinguished by comparing the relative intensities of $[M-18]^+$ at *m/z* 248, indicating direct differentiation of the two positional isomers without derivatization. Furthermore, it was confirmed that each geometrical isomer eluted from a high-polar GC column with a different retention time.

Base on these data, a pheromone gland extract of *Nokona pernix* was analyzed by GC-EAD and GC-MS, and two EAG-active components were identified, *viz.*, the E3,Z13-18:OH and Z3,Z13-18:OH in a ratio of 9:1. As a further study, we revealed sex pheromones of *Macroscelesia longipes* and *M. japona* including two common components, E2,Z13-18:Ald and E2,Z13-18:OH, which were mixed in a quite different ratio. This identification is the second dicoverery of these 2,13-dienes from the sesiid species, indicating that these compounds are also key compontents of the sesiid pheromones.

# A genetic factor unrelated to female sex pheromone influences the assortative mating of the pheromone races of the European corn borer (*Ostrinia nubilalis*)

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The European corn borer (ECB) is considered as a textbook example illustrating sex pheromone polymorphism and its evolution in Lepidoptera. The genetic bases of sex pheromone production by females and of perception and behavioural response to this pheromone by males are well described and distinguish the 'Z' and 'E' pheromone races. Females of the Z race produce a 97%Z11-14:OAc/3%E11-14:OAc blend to which males of this race respond preferentially while females and males of the E race communicate *via* a blend in opposite proportions. These differences were considered as a sufficient explanation for the fact that members of both races mate assortatively when they occur in sympatry. However, the extent of assortative mating in the field varies from almost complete isolation in France to low-level hybridisation in the US and even to random mating in some parts of Italy. Such variability suggests that the female sex pheromone is not the only factor influencing assortative mating between E and Z individuals. We hypothesised that a genetic factor unrelated to this pheromone system contributes to assortative mating trough close-range interactions. We conducted crosses and back-crosses between moths of the E and Z races to obtain females with either identical pherotype but otherwise different genetic backgrounds, or different pherotype but otherwise close genetic backgrounds. The mating performances of F1 and backcrossed F2 females were tested to isolate the effect of the putative additional genetic factor. Significant differences in the mating success of females with identical pherotypes but different genetic backgrounds was found. Conversely, no difference in mating success between females with different pherotypes but close genetic backgrounds was found. Our results support the predictions of a model assuming that assortative mating between members of ECB pheromone races is influenced by at least one autosomal locus expressed in females and segregating independently from the locus determining the E:Z proportion in the pheromone blend they emit.

# Analysis and manipulation of odor-plume structure from a piezo-electric pheromone release system and its effects on upwind flight of male moths to pheromone

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Piezo-electric sprayers for pheromone release enable the precise release of chemical stimuli as an aerosol, from a solution. These systems consists of a drawn glass capillary tip, with an opening of 10-50 μm internal diameter, which is vibrated at ultrasonic frequency (~120 kHz) by a piezo-ceramic disc. This disperses the solution in micro-droplets, which results in an aerosol which in turn evaporates completely within 5 cm of the tip. Plume structure has been demonstrated to be an important factor in the orientation of moths to an odor source. This sprayer system is routinely set-up with the downwind end shielded from insects by a protective mesh, but what effect this has on the plume structure produced by the system or in fact what plume structure the system produces while uncovered has not previously been documented. We investigated the odor plume structure of a typical piezo-electric sprayer system, set up to release ethanol, in a wind tunnel using a fast response mini-photoionizaton detector. We recorded the plume structure of four different sprayer configurations: the sprayer alone, a 5 cm circular upwind baffle, a 1.6mm steel mesh shield, and a 3.2mm steel mesh shield. We measured a 12 mm x 12 mm core at the center of the plume, and both a horizontal and vertical cross-section of the plume, all at 100 mm, 200 mm and 400 mm downwind of the release point. Marked differences in plume structure were found between all configurations in terms of conditional relative mean concentration, intermittency, ratio of peak concentration to conditional mean concentration, and size of the plume. We then measured the flight responses of the almond moth, *Cadra cautella*, to the different sprayer configurations, to identify whether these differences in plume structure had any effects on moth flights. Differences in plume structure between the sprayer configurations resulted in varying flight responses. This study provides further evidence that pheromone plume structure has effects of moth orientation behavior and demonstrates that care is needed when setting up experiments using a piezo-electric release system to ensure the optimal conditions for behavioral observations.

# Male odour drives female choice in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae)

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Sexual selection theory predicts emergence of female mate choice mechanisms due to high-energy investment in reproduction compared to males. Thus, males display coloured ornaments, songs etc. to be chosen as mates. Moths are no exception and odours released by males in courtship have been suggested to play a key role in female acceptance. Here we report on our investigations on the role of male pheromone in female choice in the so-called Z strain of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae). To assess whether females are able to distinguish between males we performed behavioural experiments in which one female was exposed to one or three males differing by their age (from zero to four days old). In both pair and quatuor experiments four-day old males obtained significantly more mates than others. GC-MS analysis of extracts from the abdominal hairpencils, putative pheromone release organs, allowed us to identify a blend of four acetates: (Z)-9-hexadecenyl acetate, (Z)-11-hexadecenyl acetate, (Z)-14-hexadecenyl acetate and hexadecanyl acetate. Removal of the hairpencils resulted in a drop of male mating success. Female acceptance was re-established by exposing female to natural or synthetic male odour loaded on a filter paper.
The amount of (Z)-11-hexadecenyl acetate in the male *O. nubilalis* hairpencil extracts increases with male age. This observation is likely to be the key of female choice in this species. The male pheromone components in *O. nubilalis* have a chemical structure similar to the conspecific female pheromone (a blend of (Z)- and (E)-11-tetradecenyl acetate) suggesting that similar enzymatic machinery is involved in production of the female and male pheromones. Interestingly, (Z)-14-hexadecenyl acetate is for the first time demonstrated as a pheromone component. This compound is most likely biosynthesized through the action of a ? 14 desaturase. Such a desaturase is involved in the female sex pheromone biosynthesis of *Ostrinia furnacalis*, a congener of *O. nubilalis* [1]. Traces of ? 14 desaturase mRNA have also been found in the *O. nubilalis* female pheromone gland [2] although this desaturase is not involved in the biosynthetic route leading to the female pheromone. Our findings suggest that the ? 14-desaturase gene in *O. nubilalis* is under sex-specific transcription/translation repression in *O. nubilalis* rather than being nonfunctionalized as proposed earlier [3].

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# SYMPOSIUM 5

# PLANT-HERBIVORE INTERACTIONS

# Cyanogenic glucosides in plants and insects and their role in plant-insect interactions

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Cyanogenic glucosides are amino acid derived natural products widely distributed in the plant kingdom. Among the more than 3.000 plant species known to contain cyanogenic glucosides are important crop plants like sorghum, barley, cassava, clover, flax, lotus and almonds. The biosynthetic pathway involves two membrane bound cytochrome P450s and a soluble UDPG-glucosyltransferase that are assembled in a metabolon to secure "conveyor belt" synthesis of the cyanogenic glucoside without diffusion of toxic intermediates into the cytosol. Plants producing cyanogenic glucosides, also contain β-glycosidases that upon cellular disruption of the plant tissue catalyze their conversion into keto compounds and hydrogen cyanide. This binary system - two sets of components which separately are chemically inert – provides the plants with an immediate chemical defence response to herbivores causing tissue damage. However, the trait of cyanogenesis is about 430 million years old enabling co-evolution of cyanogenic plants and their herbivores and pests. The great potential offered by transgenic plants to elucidate such interactions was illustrated by transfer of the entire pathway for synthesis of the cyanogenic glucoside dhurrin from sorghum to *Arabidopsis* using genetic engineering. The presence of dhurrin in transgenic *Arabidopsis* deterred feeding by *Phyllotreta nemorum*, thus unambiguously demonstrating that cyanogenic glucosides may confer resistance against selected herbivores. In contrast, the feeding behavior of *Plutella xylostella* did not change. Other insects like *Zygaena filipendulae* are able to sequester cyanogenic glucosides present in their host plant and to use the plant derived cyanogenic glucoside in their own defence. *Z. filipendulae* strives to achieve certain threshold contents and ratios of cyanogenic glucosides and is able to compensate for varying contents and different ratios of cyanogenic glucosides in the food plants by preferential *de novo* biosynthesis of the compound in demand. The defence system of the plant has thus been taken over by the insect. Accordingly, removal of a natural product from a plant does not necessarily increase its sensitivity towards important herbivores and detailed analyses are required to predict what happens with respect to plant-herbivore resistance when the pathway for cyanogenic glucoside synthesis is introduced into a new crop species or when the pathway is blocked in a cyanogenic crop plant like cassava.

# Terpenoid synthases and cytochrome p450 enzymes in the interacting genomes of conifers, bark beetles and fungal pathogens

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Conifer trees display a large array of defenses against insect pests and insect associated pathogens. Insect induced defenses in species of spruce (Picea spp.) include a myriad of combinations of constitutive and induced, chemical and physical, direct and indirect, as well as local and systemic defenses. Among some of the most prominent insect-induced defenses in conifers are terpenoid (traumatic resinosis; terpenid volatile emissions) and phenolic secondary metabolites. Traumatic resinosis involves methyl jasmonate or ethylene-inducible de novo differentiation of specialized anatomical structures (traumatic resin ducts, TRD) for induced terpenoid accumulation in the developing xylem. Phenolic defenses involve the induction of polyphenolic parenchyma (PP) cells of largely unknown chemical contents. Insect induced volatile emissions are based on passive release of resin terpenoids and on the active de novo formation and emission of non-resin monoterpenes and sesquiterpenes. In targeted biochemical characterization of insect-induced secondary metabolite defenses, we have functionally characterized a large family of conifer terpenoid synthase genes as well as cytochrome P450 genes for conifer diterpene resin acid formation and measured their expression in response to real and simulated insect attack. Large-scale genomics resources for species of spruce (>200,000 ESTs; ca. 6,400 FL-cDNA; and a 22k-cDNA microarray) and optimized proteomics tools have been developed in our conifer genomics program (www.treenomix.ca) and applied for a comprehensive analysis of conifer defense response to insect attack. In parallel, we have established an EST resource for a bark beetle associated fungal pathogen representing a large number of CYP450 potentially involved in detoxification of conifer chemical defenses.

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# Sequestration of Plant-Derived Glycosides by Leaf Beetles: a Model System for Evolution and Adaptation of Chemical Defenses

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Plant-derived secondary metabolites play an important role in the coevolution of host plants and herbivorous insects. Several insects have overcome the chemical barrier of noxious plants and, thereby, have successfully colonized a niche that is not readily available to non-adapted competitors. Further advanced strategies additionally exploit the plant-derived toxins for the insects' own antipredator defense. The insects have to develop (*i*) a selective import system that selects for the appropriate compound(s) from the plethora of chemicals present in the food and (*ii*) a mode of hemolymph transport and storage capabilities for the toxic compounds that exclude negative interferences with the insects own physiological processes. Chrysomelid larvae provide a well suited system for study of the mechanistic basis of sequestration at different levels of evolutionary adaptation. Leaf beetles (Coleoptera: Chrysomelidae) constitute a very large group of species, each of which feeds on a restricted number of plant taxa. Their defensive secretions may contain a single or a few compounds, or they may constitute a rather complex blend, covering different types of compound classes from different biosynthetic pathways. By using species producing monoterpenes *de novo* (*Hydrothassa marginella, Phratora laticollis*), two salicin sequestering species (*Chrysomela populi, Phratora vitellinae*) living on populus or salix, and members of *C. lapponica* sequestering a broad range of plant-derived glucosides, we studied the molecular basis of sequestration by feeding the larvae structurally different thioglucosides resembling natural *O*-glucosides. Their accumulation in the defensive systems demonstrated that the larvae possess transport systems, which are evolutionarily adapted to the glycosides of their host plants. Minor structural modifications in the aglycon result in drastically reduced transport rates of the test compounds [1]. Moreover, ancestral leaf beetles producing iridoids *de novo* already possess a fully functional import system for an early precursor of the iridoid defenses. This will be exemplified by larvae of *Plagiodera versicolora* and *Phratora vitellinae* which import early precursors from leaves of their host plants (salix or populus) and use this in addition to their own *de novo* biosynthesis.
Evidence will be given, that a complex network of glucoside transport systems is present in the insect that import and export (Malpighian tubules) the plant-derived glucosides to maintain a low hemolymph of potentially toxic compounds.
Our data confirm an evolutionary scenario in which, after a host-plant change, the transport system of the leaf beetles may play a pivotal role in the adaptation on new hosts by selecting plant-derived glucosides that can be channelled to the defensive system

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# Special weapons - exceptional countermeasures: glucosinolate hydrolysis in plants and their insect herbivores

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The chemical diversity of the glucosinolate-myrosinase defense system arises not only from the variety of structures of parent glucosinolates, sulfur-rich thioglycosides found in plants of the Brassicaceae and some related families, but also from the multiple modes of glucosinolate hydrolysis employed by plants. One parent glucosinolate can be hydrolyzed to several different hydrolysis products, such as isothiocyanates, nitriles, or thiocyanates. While isothiocyanates have frequently been shown to be toxic to a variety of organisms, including insects [1], the ecological role of the alternative hydrolysis products is largely unknown. We have generated transgenic lines of *Arabidopsis thaliana* (Brassicaceae) that differ from wild-type plants only in the type of products formed upon hydrolysis of the endogenous glucosinolates. First feeding experiments with the model generalist insect herbivore *Spodoptera littoralis* (Lepidoptera: Noctuidae) indicate that nitriles may not be functioning in direct defense. Interestingly, nitrile formation at the expense of isothiocyanates is the major pathway of glucosinolate detoxification in a specialist insect herbivore, larvae of the cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae), which feed exclusively on glucosinolate-containing plants. Nitrile formation is due to a protein, designated nitrile-specifier protein (NSP), in the larval gut [2]. A survey of representative species showed that NSP acitivity is only found in Pierid species feeding on glucosinolate-containing plants while species outside the Pieridae do not possess NSP activity. While the nitriles derived from aliphatic glucosinolates in *P. rapae* are excreted with the larval feces, the nitrile derived from benzylglucosinolate undergoes a series of further metabolic reactions. Thus the mechanism employed by *P. rapae* to overcome the chemical defenses of its host plants is apparently much more complex than that of another specialist insect herbivore, *Plutella xylostella* (Lepidoptera: Plutellidae), that produces a single enzyme which efficiently convert all glucosinolates to the non-toxic desulfoglucosinolates [3].

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# Plant defenses utilized by insects: functional and evolutionary aspects of the pyrrolizidine alkaloids

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Pyrrolizidine alkaloids (PAs) are produced constitutively by plants against herbivores. The occurrence of PAs is restricted to certain unrelated families within the angiosperms. To herbivores like mammals and insects these ester-alkaloids are toxic due to bioactivation within the liver. However, specialized insects evolved the ability not only to feed on PA-containing plants but also to accumulate plant-derived PAs in their own body against predators. We study the evolution of the PA-pathway in plants and of the mechanisms specialized insects evolved during their adaptation to PA-containing plants.
Homospermidine synthase (HSS), the first specific enzyme in the biosynthesis of the necine base moiety of PAs, was originally recruited from deoxyhypusine synthase (DHS), an enzyme involved in the posttranslational activation of the eukaryotic initiation factor 5A in primary metabolism. Recently, this gene recruitment has been shown to have occurred several times independently within the angiosperms and even twice within the Asteraceae. Immunolocalization experiments have shown that HSS is expressed differently, despite catalyzing the same step in PA biosynthesis.
Senecionine *N*-oxygenase (SNO) is a specific enzyme within PA-adapted arctiid insects that transforms PAs into their metabolically save *N*-oxide form. SNO belongs to the family of flavin-dependent monooxygenases (FMOs). We have analyzed FMO cDNA sequences from various arctiids that form two separate clusters for which we assume that one is encoding PA-specific SNO.

# EAG and behavioural responses of *Capnodis tenebrionis* L. to host-plant volatiles

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Electroantennograms (EAGs) and wind-tunnel bioassay responses were recorded from the flatheaded woodborer, *Capnodis tenebrionis* L. (Coleoptera: Buprestidae) to a broad range of volatile host-plant compounds. Volatiles emitted by aereal parts of five *Prunus spp.* (*P. armeniaca*, *P. persica*, *P. amigdaly*, *P. avia* and *P. domestica*) were collected and analyzed by capillary GC and GC/MS. We identified 151 chemical constituents in the *Prunus spp.* essential oils and headspace volatiles. Identified compounds mainly included green leaf volatiles (GLVs), short-chain aldehydes, phenol derivatives and terpenoid compounds. Gas chromatography coupled with electroantennographic detection (GC-EAD) using antennae of adult female and males *C. tenebrionis* was employed to screen olfactory stimulants present in headspace collections but the response profile is restricted to a small number of volatile components that evoke substantial EAGs. A comparison beetwen responses from antennae of males and females to *Prunus spp*. headspace volatiles revealed that males responded to the same suite of components as females. The EAD-active compounds that elicited the greatest EAGs were common to all five host plant species: Benzaldehyde, (*Z*)-3-hexenol, (*Z*)-3-hexenal, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenyl butyrate, ethyl benzoate, methyl salicylate, and terpinen-4-ol. A blend with the active compounds was tested in wind tunnel at two different concentrations (100µg /µgl; 10µg /µl) and it was compared with real odour from vegetal material of five species of host plants. The most active compounds were also tested individually at the same concentrations. On the basis of ubiquity of the EAG-active volatiles identified to date in host plant headspace collections and the results obtained in wind tunnel, we suggest that *C. tenebrionis* could use several of these volatile compounds to locate and identify appropriate host plants.

# Role of the host plant, *Vicia faba*, and induced volatiles in host location and sexual communication of *Lygus rugulipennis*

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*Lygus rugulipennis* Poppius (Heteroptera: Miridae), is the most common phytophagous species of the genus *Lygus* in Europe. As for many herbivores, the different steps of the *L. rugulipennis* sexual behaviour (meet, court and mate) occur on host plants. Herbivore females might use plant volatiles to find a suitable site to feed and oviposit, whilst males might use them to locate a suitable feeding site and to optimize mating. In this work, using *Vicia faba* L. as host plant, the attraction of *L. rugulipennis* females and males to odours from healthy plants, from plants damaged by conspecifics (feeding damage by males or virgin females; feeding plus oviposition damage by gravid females), from the plant-conspecifics complex or from females or males in the absence of host plants, were investigated in wind tunnel and/or in a vertical open Y-olfactometer. The volatile compounds emitted were collected and analysed using GC and GC-MS, and *L. rugulipennis* responses to the plant - conspecific females complex was investigated through coupled GC-EAG.
Females were attracted in olfactometer to volatiles from healthy plants and, both in wind tunnel and olfactometer, to volatiles from the plant-conspecifics complex, suggesting the existence of an aggregation mechanism. They were less responsive to volatiles released by damaged plants without conspecifics. Instead, males were attracted in wind tunnel towards damaged plants, and the presence of conspecifics on plants did not enhance attraction. Both females and males were less attracted towards the conspecifics in the absence of plants.
The volatile profile of the plant - females complex showed, compared to healthy plants, an increased emission of hexyl acetate, (Z)-*ß*-ocimene, (E)-*ß*-ocimene, methyl salicylate and (E)-*ß*-caryophyllene, indicating an induction of plant compounds which mostly depended on the combination of feeding and oviposition. The same complex also showed an increase in the pheromonal blend released by mated females (hexyl butyrate, (E)–2–hexenyl butyrate and (E)–4–oxo–2–hexenal) compared to females without host plants.
It is concluded that the increased emission of plant volatiles and female pheromone during *L. rugulipennis* feeding and oviposition on the host would help the conspecifics to find suitable food source and mates.

# Host-plant selection by the broad mite, *Polyphagotarsonemus latus* and how it is affected by plant defenses

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Broad mites (BM) exhibit a specific phoretic relationship with whiteflies. which enables broad mites to make long distance shifts from one host plant patch to another. Although both species are considered polyphagous their host plants are not necessarily identical. For example, in tropical and subtropical regions, BM favor greenhouse crops such as peppers and cucumbers, but not tomatoes, while *Bemisia tabaci* is a well-known tomato pest.
To study the host selection of the phoretic mites we placed whiteflies loaded with mites on leaves of different host plants and monitored the rate of mite detachment from their phoretic vector. We have compared mite response to a number of plants: young and old cucumber (*Cucumis sativus* L.) leaves, and three tomato (*Lycopersicon enculentum)* varieties: cv M82, cv Moneymaker, and cv Castlemart. The suitability of the plant was determined by monitoring the adult fecundity and offspring development. Phoretic mites eventually abandoned its vector regardless of the host genotype, but the rate of detachment was host-dependent and significantly correlated with BM performance on the plant. The lowest rate of detachment usually occurred on tomato. These results indicate that BM are able to choose their host actively. Subsequently, we compared mite detachment on wild type (wt) tomato, *L. esculentum* Mill cv. Castlemart and on the jasmonic acid (JA) pathway mutant *def-1* that is impaired in JA accumulation upon wounding or herbivory. BM detachment from the phoretic vector occurred faster on *def-1*, compared to wt leaves, reaching 50% in less then two hours. Treatment of *def-1* with MeJA reduced the detachment BMs' rate to the level comparable to that observed on wt. After one week, the number of BM progeny/female was lower on wt than on the *def-1* indicating the efficacy of a direct defense mechanism in tomato. It therefore appears that BM is able to discriminate between resistant and susceptible plants, preferring the JA-defective ones.
Taken together, we conclude that the JA signaling pathway provides BM with essential information for the recognition of a susceptible host. It is suggested that plant volatiles are playing an important role in guiding BM detachment from the phoretic vector once a suitable plant is reached. However, putative attractant or repellents remain to be identified.

# The microhabitat hypothesis to explain differing chemically based defence strategies in herbivorous Hymenoptera

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Among Tenthredinidae larvae, two taxa show differing defence strategies. On the one hand, the Nematinae possess eversible ventral glands which emit volatiles. These are especially effective in repelling arthropods such as ants. On the other hand, the Phymatocerini can display easy bleeding during which a hemolymph droplet is emitted. This liquid acts as a feeding deterrent on arthropods such as ants and wasps. The chemically based defence strategy of both Nematinae and Phymatocerini is not clearly effective against predatory birds. However, the Nematinae contain a series of species (from several genera) showing a brightly coloured body and living gregariously. These two traits are not encountered in the Phymatocerini. This leads to the paradox that only the Nematinae show traits obviously directed towards visually hunting predators such as birds, whereas the chemical defence itself from both Nematinae and Phymatocerini probably evolved primarily due to the attack from arthropods such as ants. Most Nematinae species live on trees and shrubs, and Phymatocerini live on low plants. We suggest that Nematinae larvae are subject to a relatively strong predation pressure from birds, which forage commonly in open habitats. In contrast, arthropods such as ants are often epigeous and actively forage on low plants on which Phymatocerini are living. Thus, the habitat structure and the linked habit of foraging predators may explain why bright colours and gregariousness evolved in Nematinae larvae only. Some paleozoological data and the geographic distribution of both Tenthredinidae taxa support this "microhabitat hypothesis".

# Early herbivore alert: insect eggs induce plant defence

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When herbivorous insects lay their eggs onto a plant, this is a first step to initiate a new herbivore generation that will damage the plant. However, plants are able to "notice" the eggs and to face the attack already at this early stage. Direct and indirect plant defence strategies against egg depositions are known. Direct plant responses have noxious effects on the eggs or egg-laying females, while indirect plant defence acts by oviposition-induced plant cues that attract or arrest egg parasitoids. Histological studies of egg-laden leaves show that in the systems studied so far very different layers of a leaf come into contact with the eggs and thus, different cells may be able to perceive the presence of eggs. These cells perceive an elicitor present in a secretion enveloping or attaching the eggs. In one system, there is strong indication that this elicitor is a small protein. The plant response to eggs affects both the primary and secondary plant metabolism. Photosynthesis activity was found to be reduced, and the pattern of released volatiles, especially terpenoids, changes. In pine induced by egg deposition of a pine sawfly, the transcription of a specific terpene synthase seems to be enhanced, resulting in minute changes of the quantitative terpenoid volatile pattern. The egg parasitoid is only attracted by this oviposition-induced pine volatile pattern, but not by volatiles from non-attacked pine. How do the parasitoids recognize such minute changes in a highly variable odorous environment ? Future studies need to elucidate whether they contrast the pattern of non-induced plant odour with the one of the oviposition-induced plant rather than relying on key components *per se*.

# Volatile-mediated priming of an indirect defence in nature

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Leaf damage induces in many plant species the secretion of extrafloral nectar (EFN) and/or the release of specific odours (volatile organic compounds, VOCs). Field studies in México have demonstrated that induction of both traits benefits Lima bean (*Phaseolus lunatus* L.) at its natural growing site. EFN and VOCs attract carnivorous arthropods, thereby increase the predation pressure on herbivores present on the plants, and consequently function as indirect plant defences. Plants parts regularly treated with jasmonic acid (JA, a plant hormone inducing the secretion of EFN and the release of VOCs) grew faster, had less dead shoot tips and less herbivore damage, and they produced more fruits than untreated parts of the same lant individuals.
While the defensive role of VOCs is widely accepted, the question as to whether VOCs can elicit defensive responses in yet undamaged, neighbouring plants has been debated controversially since the first description of this phenomenon. Recent experiments in the field revealed that VOCs released from induced Lima bean leaves at natural concentrations are sufficient to induce secretion of EFN in neighbouring leaves of the same species, and they prime these leaves to respond with augmented EFN secretion once they are damaged themselves. The effect of VOCs on neighbouring leaves of the same shoot was stronger than that of a putative plant-internal signal, highlighting the role of VOCs as externally transported signal for within-plant communication.
VOCs released from induced leaves are sufficient as a resistance-inducing signal at natural concentrations as occurring under field conditions. Plants – or parts of plants - therefore can use volatile signals in their environment to assess the actual risk of herbivory, and they can use this information to adjust their own defensive strategy accordingly.

# Intraspecific variation in a herbivorous mite accounts for differential defense of tomato plants

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It has been well established that plants defend themselves against herbivorous arthropods through jasmonate (JA)-dependent production of compounds detrimental to the herbivore. Therefore we hypothesized that successful herbivores must be either resistant to these compounds, or not inducing them or both. To test this hypothesis we made use of JA-deficient *def-1*, wild type (wt) and *35S::prosystemin* tomatoes (*Lycopersicon esculentum*) to select lines of the herbivorous spider mite (*Tetranychus urticae*) with different phenotypes. These spider mite lines are discriminated by traits that render them resistant or susceptible to tomato JA-defenses but also by traits that cause them to be inducers or non-inducers of these defenses. We have characterized different lines with similar reproductive success on *def-1*. Only one of these lines classified as susceptible to wt defenses as it has only a high reproductive success in the absence of JA-defenses. Moreover, we discovered two wt resistant phenotypes that both cope with JA-defenses of wt tomatoes in discrete ways. The first type is truly resistant against JA-defenses given the fact that its fecundity is always high and independent of established JA-defenses. The second type is susceptible to constitutive JA-defenses on *35S::prosystemin* plants, but does not induce JA-related direct or indirect defenses in wt plants, while it causes as much damage and produces as much offspring on these as it does on *def-1*. Our results show that lines of two-spotted spider mites vary in the way they cope JA-mediated plant defenses.

# Discriminating bites from wounds: A molecular approach

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Plants as immobile organisms are challenged by a multitude of pathogens and insect herbivores, and, thus, need to recognize the attacker to produce an appropriate defense response. To separate physical and chemical signals originating from insects during herbivory, we have developed a mechanical device, MecWorm, programmable to replicate very closely the pattern, time course, and quantity of damage caused by different insects. We have discovered that the impact of continuous mechanical damage on the plant defense response has been largely underestimated, necessitating a very cautious rating of the different individual stimuli emanating from insects during herbivory. These findings will change our view of how plants can perceive feeding by herbivorous insects and how in turn insects are able to manipulate plant defenses. To assess the relative contribution of insect-mimicking wounding on induced transcript changes, we compared transcript profiles from *Arabidopsis thaliana* leaves damaged by *Plutella xylostella* larvae, a worldwide pest on cruciferous plants, and by MecWorm *versus* undamaged control plants on the whole genome level. We observed a massive impact of both MecWorm treatment and *P. xylostella* herbivory on the plant transcriptome, with changed transcript levels of several thousand genes compared to control plants. Most important, continuous MecWorm damage reproduced transcriptional changes for many previously herbivory-assigned genes in a quantitative, time-dependent, and fully repeatable manner. Transcript levels of other genes, however, differed strikingly between MecWorm and *P. xylostella* damage, demonstrating that insect-derived chemical cues do play a role in eliciting changes in the plant's transcriptome during insect herbivory. Various examples will be presented and discussed.

# Enzymes involved in the homeostasis of *N*-acyl amino acids in the gut of herbivorous insects

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Feeding insects elicit in the attacked plants the emission of volatile organic compounds which attract carnivorous and parasitoid insects. *N*-acyl glutamine (*N*-Acyl-Gln) conjugates of fatty acids and glutamine which are present in the spit of certain herbivorous insects are discussed to play a role in the elicitation of plant defense mechanisms. In search for the enzymes involved in the homeostasis of *N*-Acyl-Gln in the insect gut, we identified two groups of enzymes – firstly an insect protein isolated from *Spodoptera littoralis* and secondly an enzyme from the insect gut bacterium *Microbacterium arborescens*. Both proteins catalyze the hydrolysis as well as the synthesis of a broad range of *N*-acyl amino acid conjugates and show a high degree of adaptation to the conditions present in the gut.
The insect protein (66.7 kD) has been isolated from the cytosolic fraction of the gut homogenate. The amino acid sequence displayed homology to α-glycosidases from *Helicoverpa armigera.* Some of the homologous proteins are GPI-anchored.
Various microbial species from the gut of *Spodoptera exigua* larvae were also found to be able to hydrolyze or synthesize *N*-Acyl-Gln. One enzyme was isolated and characterized from the gut bacterium *Microbacterium arborescens* SE14. This enzyme turned out to be a homo-dodecamer with 10 iron atoms per subunit. The corresponding gene shows homology to Dps proteins (DNA-binding protein from starved cells). Although the enzyme has no DNA binding capacity it is able to protect DNA against oxidation by $H_2O_2$ by a yet non identified mechanism.
The involvement of gut bacteria in the homeostasis of a plant defense elicitor introduces an additional trophic level into plant-herbivore interactions.

# Investigation of plant-aphid interactions using a proteomic approach

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Plant – insect relations are mainly regulated by the evolution of the defence mechanism's developed by plants and the ways herbivore insects adapt themselves to these defensive systems. Plant defence can be direct or indirect, localised or systemic. A common property of these mechanism's is the broad range of phytophagous agents, including insect pests, which are efficiently controlled by the defensive produced molecules. To cope with the induction of several direct defence molecule productions, herbivores developed several detoxification enzymatic systems such as the gluthathione S-transferases and monooxygenases. Here we studied the chemical ecology of aphid (such as *M. persicae*) – plant relations using a proteomic approach. The aphid switch from a host plant to others from Solanaceae and Brassicaceae family was first investigated to assess the metabolic changes and potential adaptations in aphids according to particular host plant species. Specific association's between aphids and their host plant were previously shown to be related to the presence of particular bacterial symbionts. The respective role of the aphid and their related symbionts in the adaptation to the host plant was also investigated considering the proteome variations of aphids in presence or absence of endosymbionts. Finally, the particular role of aphids in plant defensive responses due to its sucking feeding behaviour was investigated focusing on the protein composition of aphid saliva. The complex protein mixtures related to the different aphid materials was separated by two dimension electrophoresis methods and the related spots of proteins significantly varying were selected and identified by mass spectrometry (ESI-MS-MS and Maldi-Tof-MS-MS) coupled with data bank investigations. The impact of the down regulated or overexpressed aphid proteins involved in different metabolic pathways was discussed. This broad proteomic approach is a very reliable tool to study the biologically involved proteins from aphids in response to several environmental changes, and particularly the insect - host plant interactions.

# Tritrophic interactions in soybean: effects of ambient UV radiation

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Plants attacked by herbivorous insects elicit the emission of a blend of volatile organic compounds (VOCs) that acts as a signal to parasitoids and predators of the herbivores. This can be regarded as a facultative mutualistic symbiosis between plants and the herbivores' natural enemies. Abiotic stress factors may modulate the emitted VOC-blend and could therefore alter tritrophic interactions between plants, herbivores and natural enemies.
In plants, solar UV-B radiation can damage biomolecules and thus induces the synthesis of protective compounds (e.g. flavonoids). This and other responses may change the suitability of the plant as a host to herbivores. UV-B was also shown to act synergistically with wounding on jasmonic acid-inducible plant defenses (e.g. proteinase inhibitors). Therefore, we hypothesized that UV-B may indirectly (altered feeding behavior) and/or directly (impact on JA-signaling and consequently VOC emission) change higher trophic interactions.
Specifically, we asked: Is there an effect of UV-B i) on soybean secondary compounds (protective compounds, VOCs) ii) on the performance of the herbivore *Spodoptera frugiperda* and iii) on the host searching behavior of the parasitoid *Cotesia marginiventris*? To address these questions we placed climate chamber raised soybeans (*Glycine max* var. London) under filters that permitted or excluded ambient solar UV-B radiation. Pulse Amplitude Modulated Fluorometry (PAM) revealed that soybeans showed maximal difference in the induction of light absorbing compounds after five days of exposure. Subsequently, five-day exposed plants were used in all further experiments.
The composition of light-protective compounds was analyzed by high performance liquid chromatography (HPLC) and an increase in some of the analyzed flavonoids could be found.
VOC collection and parasitoid observation was done simultaneously in a six-arm-olfactometer. No significant effects of UV-B were found on herbivore-induced VOCs. Also, the difference in UV-B exposition did not alter herbivore performance on these plants. As predicted from VOC analyses, the host-searching behavior of female parasitoids was not modified by UV-B.
In summary, we showed that ambient UV radiation affected the plant's physiology but not higher trophic level interactions.

# Fluoride ions as elicitors of plant defence reactions

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Many plants react to herbivore feeding, attack by pathogens or abiotic stress factors with specific and differentiated defence reactions. An intensely studied defence strategy is the emission of volatile compounds mainly terpenoids serving to attract predators or warn neighbouring plants.
A rare but exquisite defence compound is found in plants such as *Dichapetalum cymosum* producing fluoroacetate, a strong toxin for mammals inhibiting the aconitase of the citrate cycle.
While investigating the ecological role of fluoroacetate I observed that fluoride ions strongly elicit the production of volatiles in Lima bean (*Phaseolus lunatus*) whereas the mammalian toxin fluoroacetate does not. Sodium fluoride (100 µg/ml) induces the emission of a volatile pattern in Lima bean plantlets similar to that observed after treatment with the peptaibol alamethicin. High amounts of the terpenoids 4,8-dimethylnona-1,3,7-trien (DMNT), 4,8,12-trimethyltrideca-1,3,7,11-tetraen (TMTT) are found together with methyl salicylate although the latter was less pronounced than after treatment of Lima bean plants with the peptaibol alamethicin.
In order to elucidate details of the signalling pathways involved in the plant's reaction to fluoride inhibitors were used. After preincubation of Lima bean plantlets with the phospholipase $A_2$ inhibitor aristolochic acid, fluoride application led to a drastically reduced volatile emission. In presence of phenidon an inhibitor of lipoxygenases the plants were not able to induce the volatile production any more. Additionally, $Ca^{2+}$-ion fluxes and the oxylipin profile were monitored.
In summary, fluoride treatment of Lima bean results in the activation of plant defence mechanisms with a lipid derived signal involved in the induction of the volatile emission.
It will be interesting to investigate how fluoroacetate producing plants adapted to fluoride uptake. Furthermore, fluoride can be used as a new tool to study details of plant signalling networks.

# The ecology and evolution of chemical defense in poison frogs and their dietary arthropods

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Certain aposematically colored frog genera of five families from five continents contain defensive alkaloids in their skin, and evidence for two of these families indicates that these lipophilic alkaloids are perhaps completely derived from their diet, as captive-born poison frogs have no alkaloids. Our studies focus on poison frogs endemic to Madagascar (*Mantella* spp.); we recently reported that like their Neotropical dendrobatid counterparts, *Mantella* depend heavily upon dietary ants as the source of their defensive skin secretions, as evidenced by GC-MS spectra of *Mantella* spp. and sympatric arthropods, and frog stomach content data [1]. Our report included one millipede and three ant species as some sources of such alkaloids in *Mantella* frogs; as these same alkaloids occur in unrelated ants and frogs of the Neotropics, this evidence supports the convergent evolution of such New and Old World tropical ecosystems [1]. *Mantella* stomach contents consisted of about 70% ants, and thus local ant diversity is expected to influence chemical diversity detected in frog skin exudates [1]. Occurrence of nicotine in one *Mantella* individual suggested a possible plant-herbivorous insect-frog predator toxin food chain [1]. Our current study shows that individual frogs vary in alkaloid content, and that such variation is likely a reflection of arthropod prey available in the local environment [2]. As such, the toxic molecules detected in poison frogs may provide a measure of local ecosystem health. Individual frogs within a locale are more similar to one another than are individuals of localities that differ in elevation and habitat disturbance [2]. *Mantella* sampled at a pristine locality had significantly greater skin alkaloid diversity than frogs sampled at disturbed and fragmented localities [2]. Further, in a comparison of frogs sampled at two locales 10-15 years apart, the frog alkaloid profiles were relatively consistent at the pristine locality and highly variable at the disturbed roadside locality, suggesting increased leaf-litter arthropod species turnover [2]. Frogs from our study were sampled with both lethal full-skin extraction and with a non-lethal Trancutaneous Amphibian Stimulator; the latter is now proven effective for sampling alkaloid diversity in frogs and its use is recommended for future studies [1][2].

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# Coping with cardenolides in host plants: How many tricks are there to evolve tolerance or resistance?

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Cardenolides (cardiac glycosides) are potent plant toxins that act by a dose-dependent inhibition of the $Na^+/K^+$-ATPase, a transmembrane carrier that is ubiquitously present in animals and responsible for the maintenance of membrane potentials. The cardenolide binding pocket of the $Na^+/K^+$-ATPase is apparently highly conserved across the whole animal kingdom. Plant cardenolides are therefore a potential threat to all insects using cardenolide containing host plants. This paper focusses on *Chrysochus* leaf beetles that have adopted sequestration of plant cardenolides following a host switch to North American Apocynaceae. By analytical chemistry, tracer feeding experiments and molecular biological analyses of the cardenolide binding pocket of the $Na^+/K^+$-ATPase as well as of the carriers involved in cardenolide transport we disentangle the different steps in the evolution of tolerance to the toxins. Our data indicate that in *Chrysochus* an amino acid substitution in the cardenolide binding pocket of the $Na^+/K^+$-ATPase known to confer resistance must have occurred first. However, there is some indication that the ancestral species may have been preadapted to tolerate cardenolides. A second decisive modification changed the balance between cardenolide uptake and cardenolide excretion via the Malphighi tubules. While in the ancestral species cardenolides do not accumulate in the body and cannot be transferred into the glands releasing defensive secretions, they are taken up and transported into the secretions of the cardenolide adapted species. The decisive adaptation on the way to cardenolide sequestration is therefore a change in gene expression in the responsible carriers. These carriers have been identified as members of the OATP family (organic anion transporting proteins). The situation in *Chrysochus* is compared to the know physiology of other specialized insects on cardenolide plants.

# Throwing light on sequestration processes in leaf beetle larvae

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Leaf beetle larvae live in the foliage of their host plants where they are highly exposed to predation. Chrysomelina larvae release a deterrent secretion from nine pairs of dorsal glands to avert predatory attacks. To derive repelling substances, larvae rely on *de novo* synthesis or sequestration. Plant-derived glucosides serve as precursors for the production of the deterrents in the glandular reservoirs of the sequestering species. Recently, it has been shown that even *de novo* synthesizing species are able to sequester glucosides. Systematic feeding studies with thioglucoside analogues of the plant-derived glucoside precursors revealed that the import of the compounds is very specific and requires specialized transport proteins [1]. To analyse the characteristics of the different transport systems located in the insect gut and in the membrane of the defensive system, we used the two leaf beetle species *Phaedon cochleariae* (*de novo* production of iridoids) and *Chrysomela populi* as an example of a sequestering species. The selectivity of the first carrier, the gut tissue, was assayed *in vitro*. We could demonstrate that the thio-analogues of the natural precursors are taken up into the tissue with high selectivity. To classify the type of transport proteins, *in vitro* assays with mid gut tissue and inhibitors of certain transport proteins were performed. Next, the uptake process of the glucosides from the hemolymph into the defensive gland was studied by injection of the test compounds into the larval hemolymph. As a matter of fact, the pattern of the uptake of the test compounds from the hemolymph followed the same selection criteria as previously found in the feeding studies [1]. Moreover, significant amounts of the injected thioglucosides were present in the feces demonstrating that also an export system with a similar substrate preference must exist in the larvae.

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# A grasshopper and its beneficial drug source: the African story of *Zonocerus variegatus* and the neophyte *Chromolaena odorata*

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Since the 1970s, the pestilence of dry season populations of the polyphagous grasshopper *Zonocerus variegatus* has impacted negatively on agriculture and forestry in sub-Saharan Africa. Current chemo ecological knowledge indicates that this is attributed to the sequestration of pyrrolizidine alkaloids (PAs) from the flowers of the introduced perennial pioneering shrub, *Chromolaena odorata* (Asteraceae) native to the tropical Americas. The quickly spreading shrub provides *Z. variegates* with an efficient chemical protection against its natural enemies. This phenomenon was investigated in *Z. variegatus* by studying the effects of diet with and without PAs from *C. odorata* flowers on its morphology and reproductive biology. The results showed that diet of *Z. variegatus* from a sole PA source did not support its development. However, diet with PAs as a component significantly improved growth, morphology and fecundity. Mortalities were significantly lower in insects raised on diet with PAs as a component. Also, *Z. variegatus* that had access to PAs in their diet produced significantly more but lighter eggs. In addition, the PAs from *C. odorata* and eggs of *Z. variegatus* were analysed by capillary gas chromatography-mass spectrometry. The PA profile and quantity in flowers of *C. odorata* is given and compared with the profile found in *Z. variegates* eggs. Some possible evolutionary implications regarding the interaction of *Z. variegatus* and *C. odorata* in sub Saharan Africa are discussed.

# Phytoecdysteroids taste bitter

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About 5% of plant species belonging to most groups produce analogs of insects moulting hormones. These hormones agonists or antagonists share a common chemical structure and are called phytoecydsteroids (PEs). While a number of studies focused on their toxicity, very few data exist as concern how they are detected and avoided. PEs are avoided by larvae of both species of moths, the European corn borer, *Ostrinia nubilalis* Hbn., and the grapevine moth, *Lobesia botrana*. Several of their taste sensilla house one deterrent cell sensitive to PEs. This neuron responds vigorously to 20-hydroxyecdysone (20E) and several other PEs, with a threshold of $10^{-6}$ M. This sensitivity is already expressed in first instar larvae and 20E remains deterrent through all larval stages of these two species. We then tested if adults detect 20E and if females avoid ovipositing on a substrate with 20E. We have found on the tarsi of both species, taste sensilla housing one cell detecting 20E in the same concentration range as in the larvae. When given a choice between treated and non-treated glass spheres, used as a surrogate to berries, *L. botrana* females avoid laying eggs on spheres treated with 20E. In contrast, *O. nubilalis* females do not seem to make the difference between treated and non-treated areas, when 20E is sprayed on an artificial substrate. However, *O. nubilalis* females consistently avoid laying eggs on maize sprayed with 20E, in choice conditions. These studies show that 20E are detected by these insects both in during their larval stages and during their adult life. It suggests that for these insects, 20E represents a token stimulus to avoid plants producing PEs. This observation is striking given the relatively modest toxicity of 20E, when included in the diet of these insects. Given the number of plants producing PEs in different genera and scattered observations in the literature concerning the detection of PEs, it is possible that the detection of PEs has been conserved in many phytophagous insect species, not only in larvae but also in adult insects.

# Variation in composition and attractiveness of floral scent in a highly polymorphic Andean cactus, *Echinopsis ancistrophora*

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The adaptive radiation of select Angiosperm lineages provides opportunities to study the, mechanisms by which plant-animal interactions evolve, especially when these involve shifts between pollination syndromes. Floral volatiles have long been thought to contribute to the specificity of such interactions, through selective attraction or repellence of specific visitors, but rarely have been studied in such a context. The Andean cactus species *E. ancistrophora,* usually divided into 4 subspecies, is noteworthy for its remarkable geographic variation in floral depth, color, nectar production, and anthesis time. While floral traits in most populations suggest adaptation to bees or hawkmoths, some have unusual, intermediate combinations of floral traits that do not fit into our common understanding of floral syndromes. For example, flowers of *E. ancistrophora ssp. cardenasiana* are long-tubed and pink, lack nectar, open after midnight and remain open the entire day. Such decoupling of floral traits provides unusual opportunities for testing the function and importance of floral characters that typically are correlated.

We analyzed floral scent composition and emission rates in all four subspecies, including 13 populations of one subspecies, in which both bee and moth-adapted flowers and morphological intermediates thereof were represented. Patterns of floral volatile emission differed between subspecies, but within-group variation also was high in some populations. In *E. ancistrophora ssp. ancistrophora*, differences between bee and moth-pollinated populations were not significant on the level of compound classes, but Mantel tests indicated significant correlation between syndrome and floral scent, independent of geographic distances between populations.

In order to test the functional importance of scent variation, we performed wind tunnel tests with hawkmoths (*Manduca sexta*), comparing the attractiveness of scent from single flowers of bee versus moth-adapted *E. ancistrophora ssp. ancistrophora*, and the closely related, bee pollinated pink flowers of *ssp. cardenasiana*. The odors of all white flowers of *ssp. ancistrophora* were equally attractive to moths, regardless of pollination syndrome, whereas the scent of *ssp. cardenasiana* was not attractive. Thus, the scent from individual flowers of *E. ancistrophora ssp. ancistrophora* is sufficient to attract hawkmoths from a distance, even if cactus populations are small or few flowers are in bloom. However, other floral traits, such as anthesis time, nectar quality and tube length, will determine whether moths encounter open flowers, are rewarded by them, and effectively pollinate them, respectively.

# Pollen odors as cues to pollen-foraging bumble bees

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In the flowers of some plant species that are used as pollen sources by bumble bees, the pollen odor is chemically distinct from the scents of the petals, suggesting that bees could use pollen odor in selecting which flowers have more pollen - and thus which flowers to visit. To determine if bumble bees discriminate between flowers with different amounts of pollen (and pollen odor), wild *Bombus terrestris* were offered a choice of pollen-rich and pollen-poor flowers of *Rosa rugosa* in the field. The bees significantly preferred flowers with higher amounts of pollen, with some caveats related to weather and time of day. The reliance of pollen-foraging bumble bees on olfactory cues from pollen, and even from anthers, when selecting flowers may in part underlie the difficulties in using bumble bees to cross pollinate male-sterile and male-fertile lines of tomato to obtain hybrid seed, as suggested by chemical analyses of tomato floral scents.

# The impact of insect-plant coevolution on plant community structure

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Coevolutionary theory proposes that the diversity of chemical structures found in plants is, in large part, the result of selection by insect herbivores. Because herbivores tend to feed on plants with similar chemistry, they should impose pressures on plants to become chemically different or bias community assembly toward chemical divergence. Using a coevolved interaction between a group of chrysomelid beetles and their host plants I tested whether coexisting plant species in communities of the Mexican dry forest tend to be chemically more dissimilar than random. Results indicate that in areas where herbivores have tight, one-on-one interaction with plants, communities have a strong pattern of chemical disparity.

# Impact of UV-radiation on a beetle's preference for white mustard

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The qualitative and quantitative composition of plant metabolites is highly influenced by exogenous factors which thereby can affect the feeding behaviour of herbivorous insects. The impact of ultraviolet radiation (UV-A and B) on white mustard (*Sinapis alba*, Brassicaceae), and – mediated by the plant - on a herbivorous specialist, the mustard leaf beetle (*Phaedon cochleariae*, Coleoptera: Chrysomelidae), was investigated. Green house-grown plants were exposed for two days at natural conditions in foil tents, which excluded or allowed UV-A and UV-B radiation. While the concentration of flavonoids increased drastically with UV exposure, glucosinolate concentrations and activity of glucosinolate-hydrolysing enzymes, myrosinases, were only little affected. Larvae reared on young leaves of differently treated plants (with and without UV) did not differ in their performance, e.g., showed similar developmental times and body masses. However, naïve larvae preferred strongly the diet low in flavonoids. Preference behaviour of larvae and adult beetles was further investigated with bioassay-guided fractionation to determine which compounds influence feeding.

# Glucosinolate polymorphism in *Barbarea vulgaris*

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In natural populations of *Barbarea vulgaris* we found two distinctly different glucosinolate profiles. The most common glucosinolate profile is dominated (94%) by the hydroxylated form, (*S*)-2-hydroxy-2-phenylethyl-glucosinolate (glucobarbarin, BAR-type), whereas in the other type 2-phenylethyl-glucosinolate (gluconasturtiin, NAS-type) was most prominent (82%). NAS-type plants have a 108 fold increase of gluconasturtiin concentration in rosette leaves compared to BAR-type plants. The glucosinolate composition of both chemotypes is consistent throughout all plant organs and after induction with jasmonic acid. In 12 natural populations that we sampled in Germany, Belgium, France and Switzerland solely BAR-type plants were found. However, 8 out of the 15 Dutch populations that were sampled contained 2-22% NAS-type plants. Controlled crosses showed that the chemotype was heritable and determined by a single gene with two alleles. The allele coding for the BAR-type was dominant and the allele for the NAS-type was recessive. The different glucosinolate profiles will yield different hydrolysis products upon damage, and therefore we expect them to differentially affect the multitrophic interactions associated with *B. vulgaris* in their natural environment. To clarify these interactions for BAR-type and NAS-type plants several greenhouse experiments with above and belowground herbivores are performed. Additionally a long term extensive field survey shows us the effect of this polymorphism on insects and nematodes in a more natural environment.

# Mechanistic approach to understanding the chemical diversity of conifer terpenoids

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Conifer trees produce a great diversity of terpenoid compounds, many of which are important in plant-herbivore interactions. This chemical diversity is a product of both a large family of terpene synthases as well as the multiple products produced by many of these enzymes. Although terpene synthases use geranyl diphosphate, farnesyl diphosphate, or geranylgeranyl diphosphate as substrates, predicting product profile from sequence alone is almost impossible. These enzymes generally have high sequence similarity and the specific amino acids present in the active site have an exquisite effect on product profile. Diterpene resin acids are major constituents of conifer oleoresin and are implicated in the constitutive and induced resistance of conifers to herbivores and associated pathogens. They are formed from the conversion of geranylgeranyl diphosphate to diterpenes and subsequent oxidation to carboxylic acids. Two diterpene synthases in Norway spruce (*Picea abies*), isopimaradiene synthase (Iso) and levopimaradiene/abietadiene synthase (LAS), have very similar amino acid sequences (92% identical) yet have completely different product profiles. Iso produces only isopimaradiene while LAS produces a mixture of sandaracopimaradiene, palustradiene, levopimaradiene, dehydroabietadiene, abietadiene, and neoabietadiene. Using reciprocal domain-swapping and site-directed mutagenesis, heterologous expression in *E. coli*, and characterization of recombinant enzymes, we have identified amino acid residues that are important for determining the different product profiles of these two enzymes. Although single amino changes can result in changes to product profiles, multiple changes are necessary for complete reversal of product profiles. This study identifies how easily the diversity of products and thus the potential differences in terpenoid-mediated plant-herbivore interactions can result from relatively small mutations in enzyme sequence.

# Analysis of the chemical composition of *Pinus* spp needles using comprehensive two-dimensional techniques: GCxGC and GCxMS

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The pine processionary moth *Thaumetopoea pityocampa* is considered as the most destructive pine defoliator in the Mediterranean area. Host species appear to influence the distribution and population dynamics of the defoliator, but the mechanisms responsible for its performance are not yet understood. The characterization of the chemical composition of pine species is a key tool to decode the process of host selection and performance by *T. pityocampa*. However the characterization of the chemical composition in pines is a very difficult task, due to the high complexity of the matrices. These studies are usually performed by means of one-dimensional gas chromatography (1D-GC) and mass spectrometry (GC-MS) and often result in complex chromatograms with tens or hundreds of peaks that, in ideal conditions, must be separated. The analytical capabilities of traditional 1-D-GC is partially limited by the separation power of the columns and consequently the active compounds may be hidden due to co-elution with sample components and baseline noise and result in wrong identifications, or poor matches in the MS library searching due to "impure" mass spectra.
A new multidimensional technique, with the potential to overcome these problems, denominated comprehensive two-dimensional gas chromatography (GCxGC) is recently gaining importance for the optimized analysis of complex samples. This new technique uses two capillary columns with independent separation mechanisms that are serially connected by a modulator, allowing to the total resolving power of both columns to be available to drastically improve the peak capacity of the chromatographic system and consequently the separation efficiency and detectability of analytes. The concept of multidimensional / comprehensive chromatography can also be extended to some detectors that also have separation capability, such as the mass spectrometer. For Mass Spectrometry (MS) the term "separation" requires a mechanism that is able to produce a "characteristic single label" for the individual components in the mixture. However the mass spectra produced by electron ionization (EI at 70 eV) cannot meet this requirement, because EI produces multiple fragments and similar classes of compounds will have similar fragmentation patterns. However, when MS is applied using a soft ionization technique, such as field ionization (FI), only one type of ion (the parent ion) is produced in the mass spectrum of each component allowing this combination of GC and MS (GCxMS) to be used as a separation/ identification tool.
In this work, the application of GCxGC and GCxMS to the study of the chemical composition of *Pinus* spp needles, upon the performance of *T. pityocampa* will be described. The results show that comprehensive techniques significantly improve resolution as compared to one-dimensional separations.

# Defensive gene expression in willow tree, *Salix eriocarpa*, induced by herbivory of the willow tree beetle, *Plagiodera versicolora*

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The willow tree beetle, *Plagiodera versicolora*, is one of the most dominant herbivores on the Salicaceae. The plants are able to defend themselves against *P. versicolora* directly by producing secondary metabolites and proteins that negatively affect the beetle and/or indirectly by releasing volatiles that attract their carnivorous natural enemies. The majority of the defensive chemicals are constitutive, but a few are induced and thus their production would be under the control of specific pathways activated in response to herbivory.
To better understand the molecular basis of these mechanisms, we searched for defensive genes in *Salix eriocarpa*, one of the major willow tree species in Japan. Total RNA was extracted from *S. eriocarpa* leaves infested by *P. versicolora*, and then first-strand cDNA was synthesized by reverse transcription. To isolate defensive gene fragments, PCR primers were designed from published *Populus* sequences or other public databases. After PCR amplifications from the first-strand cDNA, DNA products were cloned and sequenced. We isolated putative DNA fragments of lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL), dihydroflavonol 4-reductase (DFR), polyphenol oxidase (PPO), trypsin inhibitor (TI), farnesyl pyrophosphate synthase (FPS), cellulase (CEL) and chitinase (CHIT). Temporal and spatial expression patterns of these putative defensive genes in plants that were intact (controls) and defoliated by either beetle adults or larvae, as well as artificially wounded were analyzed by RT-PCR. The expressions of *LOX*, *DFR* and *TI* were strongly induced locally 3h after artificial wounding and systemically by 24h later. *LOX*, *DFR* and *TI* expressions were also observed at higher levels in plants attacked by herbivores than in controls, but these levels were lower than in artificially damaged plants. These results suggest that both *P. versicolora* adults and larva suppressed plant defense reactions chemically through oral secretions and/or physically by a specific trenching pattern. The molecular basis of the *S. eriocarpa* - *P. versicolora* interaction will be discussed.

# Influence of aphid infestation on volatile emission and synthesis of feeding deterrents in cotton

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Several plant species release volatile compounds after being attacked by herbivorous insects. In cotton (*Gossypium hirsutum*), these herbivore inducible compounds include acyclic terpenoids, (*Z*)-3-hexenyl acetate, indole, and others. We found that cotton plants infested by the cotton aphid *Aphis gossypii* emit a different volatile blend compared to cotton plants infested by *Spodoptera littoralis* larvae and uninfested control plants. We detected significant changes in the emission of myrcene, (*E*)-ß-farnesene and d-cadinene after aphid infestation compared to control plants. d-Cadinene was emitted in significantly larger amounts in response to aphid infestation while the amounts of myrcene and (*E*)-ß-farnesene were reduced compared to uninfested control plants. Cotton plants infested by aphids and consecutively by lepidoptera larvae showed a significant effect on the emission of volatiles compared to volatiles emitted by plants only infested by Lepidoptera larvae. Cotton plants that were first infested by aphids for several days and consecutively by *Spodoptera* larvae showed an increase in emission of myrcene, n-octylacetylene, and d-cadinene compared to plants only exposed to *Spodoptera* feeding. A reduction of volatile emission from plants previously infested with aphids and subsequent *Spodoptera* feeding was observed for the compounds linalool-oxide, (*E*)-2-hexenyl butyrate, and (*Z*)-3-hexenyl-2-methyl-butyrate. The volatile emission in response to aphid or Lepidoptera infestation indicated a differentiated response of the plant to different types of herbivores that may also affect the response to subsequent infestations. Cotton plants do not only respond to herbivory with the emission of volatiles, but also synthesize a number of feeding deterrents like the terpenoid aldehydes hemigossypolon, heliocides H1, H2, H3 and H4 that are synthesized via the sesquiterpene cadinene. While *Spodoptera* herbivory induced the synthesis of larger amounts of hemigossypolon, heliocides H1 and H2, we found that aphid infestation reduced the levels of hemigossypolon and the heliocides H1 and H4 compared to undamaged plants.

# Delayed induced responses of birch glandular trichomes to mechanical defoliation and simulated winter browsing

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Changes in morphology and chemistry of leaf surface in response to herbivore damage may increase plant resistance to subsequent herbivore attack; however, there is lack of studies on induced responses of glandular trichomes and their exudates in woody plants and on effects of these changes on herbivores. We studied delayed induced responses in leaf surface traits of five clones of silver birch (Betula pendula Roth) subjected to various types of mechanical defoliation and simulated winter browsing. Glandular trichome density and concentrations of the majority of surface lipophilic compounds increased in trees defoliated during the previous summer. This induced response was systemic, since control branches in branch defoliated trees responded to the treatments similarly to defoliated branches, but differently from control trees. In contrast to defoliation treatments, simulated winter browsing reduced glandular trichome density on the following summer and had fewer effects on individual surface lipophilic compounds. Moreover, constitutive density of glandular trichomes was negatively correlated with induced total amount of lipophilic compounds per trichome, indicating a tradeoff between constitutive and induced resistance in silver birch. Induced changes in leaf surface traits had no significant effect on leaf damage by chewers, miners and gall mites, but increased susceptibility of birch trees to aphids. However, leaf damage by chewers, miners and gall mites in defoliated (but not in control) trees was correlated with concentrations of some fatty acids and triterpenoids, although the direction of relationships varied among herbivore species. This indicates that induction of surface lipophilic compounds may influence birch resistance to herbivores. Our study thus demonstrated both specificity of elicitation of induced responses of birch leaf surface traits by different types of damage and specificity of the effects of these responses on different types of herbivores.

# Short-term effects of plant damage on aphid behaviour

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Host plant selection process, partly mediated by chemical coumpounds, is a major step in phytophagous insect life cycles as it will determine their fitness. Production of plant semiochemicals can be modified following plant damages. Previous works reported the effects of long periods of plant damage (e.g., effects of 4-days previous infestation) and long-term effects of plant damage (e.g., effects observed more than 24h after the damage). In our study, we investigated the effects of potato (*Solanum tuberosum* L.) plant damage on the host plant selection behaviour of the potato aphid *Macrosiphum euphorbiae* Thomas (Homoptera: Aphididae). In order to determine if plant defence mechanisms could occur shortly after a short-period damage and if they had visible repercussions on aphid behaviour, short duration damages were inflicted to the plant and aphid behaviour was monitored just after the plant damage. We also considered stresses of different natures: biotic (preinfestation by conspecifics or by Colorado potato beetles) and abiotic (scissor cuts). The aphid response to host plant volatiles was investigated using a darkened arena bioassay and the probing behaviour was assessed using the electrical penetration graph (EPG) technique.
Aphids were attracted towards their host plant (healthy or damaged) when presented vs. a blank. In a preference test (healthy plant vs. damaged plant), plants previously infested by conspecifics were preferred to healthy plants, but this preference was not observed for the other types of damage.
Aphid probing behaviour was not modified on plants previously infested by conspecifics whereas changes were observed with the two other types of damages.
Our data present evidence that aphid orientation and trophic acceptance can be immediately modified by short-period damages of abiotic or biotic nature inflicted to potato plants.

# Effect of plant induced volatiles on the behavior of the potato tuber moth *Phthorimaea operculella* (Gelechiidae)

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Plants respond to herbivory by releasing volatile compounds that attract predators or parasitoids, thus aiding in the control of the herbivore population. However, it is acknowledged that volatile chemicals also play important roles aiding in the discovery of plants by phytophagous insects. The objective of this work was to determine the role of these signals in plant-herbivore interactions of adults of the potato tuber moth *Phthorimaea operculella* (Zeller) and their host plants *Lycopersicum esculentum*, *L. hirsutum* and *Solanum tuberosum* (Solanaceae). By using a four-arm olfactometer coupled to a volatile extraction system, the individual responses of adults of the potato tuber moth were evaluated on induced whole plants at different induction times. The results indicated that healthy host plants tested in this study did not attract adults of the potato tuber moth; however, caterpillar-infested and mechanically induced *L. hirsutum* plants repelled both mated and unmated females. Nevertheless, plant repellence diminished after 24 hours of induction for this species. Caterpillar-induced plants also repelled unmated females only after 24 hours of induction. On the other hand, healthy potato tubers attracted significantly more mated females than damaged tubers. Our findings suggest that *P. operculella* mated females are capable to use volatiles emitted by tubers of *S. tuberosum* as a cue to locate appropriate sites for oviposition. There may be adaptative fitness advantages to mated females that avoid induced plants, since such plants are likely to activate direct defense mechanisms or attract host competitors to their offspring. It is possible that preference behavior of unmated and mated females differed in their sensitivity to volatiles released by induced host plants.

# The analysis of phytoalexins by high performance liquid chromatography-mass spectrometry (HPLC-MS) following induction in *Dodonaea viscosa*

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Some plants are known to be able to defend themselves by the accumulation of newly synthesized antifungal compounds called phytoalexins. As part of a larger project, screening Omani plants for phytoalexin induction, we have looked at phytoalexin induction in *Dodonaea viscosa*, which belongs to the family Sapindaceae. This plant is used for treatment of a number of diseases e.g., rheumatism, skin infections, diarrhea etc and its leaves are apparently effective in the treatment of toothache.
Different classes of constituent compounds have been isolated from *D. viscosa* including flavonoids, terpenes, coumarins etc. It is also said to contain saponins that are quite toxic.
Chemical induction was achieved using $CuCl_2$ solution, while biotic induction was carried out using spore suspensions of *Botrytis cinerea* and UV induction with wavelength 254 nm.
Phytoalexins were extracted and detected using (TLC)-*Cladosporium* bioassay followed by large scale preparative TLC. Eluted antifungal compounds were further purified by TLC and identified by HPLC-MS.
Two compounds, Amyrin and either Ursolic acid or Oleanolic acid were detected only in $CuCl_2$ treatment solutions; they were not detectable in $CuCl_2$ treated leaves, controls or the UV-treatment. These compounds were only produced upon induction with $CuCl_2$ and are therefore considered the phytoalexins from this species.
Pisatin and Genistein were present in the control, $CuCl_2$ and UV treatments in the leaf and the solution extracts. Sanguinarine was only detectable in the control and $CuCl_2$ treatments. Both Pisatin and Genistein are known to have antifungal activities in many plant species and are known phytoalexins of the leguminoseae. Sanguinarine is considered to have anti-bacterial and antifungal properties and has been reported as phytoalexin in *Papaver bracteatum*. Trihydroxydimethoxyflavone was detected in all samples including the control. Its levels were highest in the UV-treatment followed by the control and then the $CuCl_2$ treatments. These findings are similar to those from another species of the genus *Dodonaea*, namely *Dodonaea angustifolia*.
This shows that these biologically active compounds which were present in all samples regardless of the elicitor are constitutive antifungal compounds, while Amyrin and either Ursolic acid or Oleanolic acid appear to be the only phytoalexins.

# The chemical ecology of hiding insect eggs in a complex (plant odour) world

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How can herbivorous hosts hide their eggs from parasitoids? The oviposition site selection of phytophagous insects is based on a variety of biotic factors like the resource quantity and accessibility, as well as the offspring mortality risk due to parasitoids. We studied the egg-laying response of the generalist herbivore, *Galeruca tanaceti*, in relation to host plant availability and general plant diversity. Furthermore, the relevance of plant odours - blends of host plants and of non-host plants - for the egg-laying decision of the leaf beetle and for the orientation of its specialised egg parasitoid, *Oomyzus galerucivorus*, was experimentally investigated.
Egg clutch occurrence in the field was positively related to the presence and quantity of two host plant species. Moreover, the total number of herbaceous plant species in the close surrounding of egg clutches exceeded significantly the total number of plant species in control plots, indicating a beetles' choice for enhanced plant diversity.
In the laboratory plant odours significantly influenced the oviposition decision of *G. tanaceti*. In two-choice bioassays gravid leaf beetle females preferred substrates for oviposition that were surrounded by the host plant odour of *Achillea millefolium*, yarrow, compared to substrates surrounded by the odour emanating from a diverse plant species mixture (including yarrow). However, substrates located in an odour from a diverse mixture of plants received more ovipositions when it was offered against a pure grass odour.
The presence of *A. millefolium* enhances the probability of parasitization by the egg parasitoid *O. galerucivorus* in the field. A synthetic terpenoid mixture from yarrow odours attracts the parasitoid in the laboratory, however an enhanced odour complexity negatively affected orientation of the egg parasitoid. These results indicate the possible existence of a partial refuge for beetle eggs in complex odour environments. It is discussed how herbivorous females might decide between hiding their eggs from parasitoids in complex odour environments, on the one hand, and taking care of food plant supply for hatching larvae, on the other.

# Finding a moving host: Kairomones for *Cephalonomia tarsalis*, a parasitoid of the freeliving grain feeder *Oryzaephilus surinamensis*

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The parasitic wasp *Cephalonomia tarsalis* parasitizes larvae of the rusty grain beetle *Oryzaephilus surinamensis* feeding on wheat grains. These larvae are highly mobile walking between the grains in grain stores. Therefore *C. tarsalis* needs an effective mechanism for the location of its hosts. From the literature it is known that *C. tarsalis* females use non-volatile chemicals from host trails to locate hosts at close range. To investigate the origin of chemical cues used by *C. tarsalis* for long-range host finding, the reaction of wasps was tested to different odour samples from grain- and host derived samples in a 4-chamber-olfactometer.
Experiments revealed that odours of healthy and mechanically damaged wheat grains are equally attractive to the wasps. Host faeces are highly attractive and wasps are able to differentiate between host faeces and faeces of non-hosts, larvae of the granary weevil *Sitophilus granarius*. Fresh trails of host larvae on filter-paper are attractive for 30min after deposition. This indicates that host trails contain not only non-volatile, but also highly volatile chemicals.
From these results we hypothesize that over long distances *C. tarsalis* females locate the host habitat by moving towards grain derived odours. Within the grains, wasps search for faeces of their hosts. While moving in areas contaminated with host faeces wasps discover larval trails, which indicate the presence of hosts at most 30 min. ago. By following these larval trails wasps are finally able to locate hosts. Further studies will examine this hypothetical host finding scenario.

# Comparative Study of Electroantennogram Responses of *Microplitis croceipes* and *Cotesia marginiventris* to Host-Plant Volatile Compounds

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Parasitoids are known to rely on chemical cues (volatile signals) for foraging and host location. These volatile signals could be plant-based, originate from the herbivore host, or produced from an interaction between herbivores and their plant host. A current paradigm regarding the evolution of parasitoid foraging and host location strategies is that the degree of specificity of the signals needed by a parasitoid species for successful host location correlates with its level of host specialization. Specialist parasitoids utilizing a relatively few number of hosts are predicted to have a highly efficient host detection system than generalist parasitoids.

In order to test the hypothesis of differential response of parasitoids to different host-plant volatiles, we compared the electroantennogram (EAG) responses of both sexes of the specialist parasitoid, *Microplitis croceipes* (Cresson) and the generalist, *Cotesia marginiventris* (Cresson) to varying doses of select host-plant volatiles: two green leaf volatiles (*cis*-3-hexenol and hexanal), and three inducible compounds (*cis*-3-hexenyl acetate, linalool, and (*E,E*)-α-farnesene). The data showed a significant effect of sex on EAG responses of both parasitoid species with females and males showing differential EAG response to the test compounds; however mating had no significant effect on EAG response. Females of both species showed significantly greater EAG response than conspecific males to the two tested green leaf volatiles (*cis*-3-hexenol and hexanal), while the three tested inducible compounds (*cis*-3-hexenyl acetate, linalool, and (*E,E*)-α-farnesene) elicited significantly greater EAG in males than in conspecific females at high doses, supporting the hypothesis that female parasitoids are more responsive than conspecific males to green leaf volatiles released immediately after initiation of herbivore feeding damage, whereas males show greater response than conspecific females to inducible compounds released much later after initial damage. In general, *C. margiventris* females and males showed greater EAG response than counterpart *M. croceipes* to the tested compounds at various doses, possibly suggesting that the generalist parasitoid is more responsive than the specialist to the tested host-plant volatiles.

# Oxylipins in attacked plant leaves, their uptake and metabolism by feeding insects

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We present a rapid and reliable GC-MS based method for the identification and quantification of oxylipins including labile and highly reactive compounds. Key feature of the method is the *in situ* derivatization of oxylipins in the sample tissue with pentafluorobenzyl hydroxylamine. The procedure generates stable pentafluorobenzyl-oximes (PFB-oximes) that prevent degradation and rearrangement processes during sample preparation. Moreover, the mass spectra of the PFB-oximes greatly facilitate the identification of unknown oxylipins in complex matrices and allow monitoring of temporal and spatial changes of oxylipin patterns in response to stress factors [1].
In caterpillar attacked Lima bean (*Phaseolus lunatus*) leaves high levels of oxylipins were observed locally in the damaged leaf tissue. Upon feeding the insects ingest the plant-derived oxylipins and may have evolved ways of responding to them. Therefore, we investigated the oxylipin signatures in wounded plants as well as the insect's gut to study their role in plant-insect interactions.
The oxylipin profile found in the damaged plant leaves can also be found in the regurgitant and the feces of the generalist herbivore *Spodoptera littoralis*. Surprisingly, 12-oxophytodienoic acid (OPDA) present in the plant material is missing in the corresponding caterpillar's regurgitant and feces. Instead, another compound was found and identified as an OPDA-isomer (*iso*-OPDA) resulting from a shift of the ring-double bond between the side chains. We suggest that OPDA-isomerization takes place in the gut of *S. littoralis* larvae. Recent feeding experiments performed with OPDA and deuterium labeled OPDA evidence a rapid and highly efficient enzymatic isomerization process, since in the feces only *iso*-OPDA was found. This indicates a biological meaning of the isomerization process which needs further evaluation.

[1] B. Schulze, R. Lauchli, M. Mekem Sonwa, A. Schmidt, W. Boland. (2006) *Anal. Biochem.* **348:** 269-283.

# Genetic modification of green leaf volatile biosynthesis in plants: Its potential in integrated pest management

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Plants commonly emit green leaf volatiles (GLV) that consist of C6-aldehydes, C6-alcohols and their acetates. We have been studying the role of GLV in induced resistance to herbivores and pathogens. We previously reported that (1) *Cotesia glomerata*, a parasitoid of cabbage white butterfly (*Pieris rapae*) larvae is attracted to host damaged crucifer species using GLV, such as (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate[1)], and (2) gaseous GLV made *Arabidopsis* plants more resistant to *Botrysis cinerea*[2)]. In this paper, we report the effects of genetic modification of GLV biosynthesis in *Arabidopsis* on the attractiveness to the parasitoid *C. glomerata* and on the infection by the pathogen *B. cinerea*.

We hypothesized that genetic modifications enhancing or reducing GLVs production in *Arabidopsis* would alter the level of attractiveness of host damaged plants to *C. glomerata*, and consequently the incidence of *P. rapae* larval parasitism observed. We also hypothesized that such genetic modifications to GLV production would impact of the infection of *Arabidopsis* by *B. cinerea* .

We focused on hydroperoxide lyase (HPL), one of the key enzymes in the biosynthesis of GLV. In comparison with wild type controls, HPL sense *Arabidopsis* plants increased their resistance to *P. rapae* larvae, one of the herbivores of crucifer plants, by attracting parasitoids as well as their resistance to a necrotic pathogen *B. cinerea*. Conversely, compared with wild type controls, the plant resistances to *P. rapae* and *B. cinerea* significantly decreased following antisense manipulation. Thus, GLV biosynthesis serves as a multifunctional weapon for plants, acting against both their herbivores and their pathogens.

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# Investigating the ecological relevance of acylphloroglucinol production in *Hypericum* (St. John's Wort, Clusiaceae) species

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The economically valuable flowering plant genus *Hypericum* (Clusiaceae) is represented by approximately 400 species, the most well-known of which is *Hypericum perforatum* L. (Common St. John's Wort), which is sold as an herbal preparation to treat mild and moderate depression. One class of bioactive substances, acylphloroglucinol derivatives such as hyperforin, generally occur in highest concentrations in the flowers and fruit. HPLC profiling of leaf, flower and fruit samples from multiple populations of a related Canary Island species, *Hypericum canariense* L., was conducted to examine chemical variability. During this screening, the presence of an unknown compound produced in high quantity in flower and fruit samples was detected. Elevated amounts of this compound were observed in leaf samples that had been harvested from a subset of aphid-infested individuals. The antifeedant activity of this compound, an acylphloroglucinol derivative, against the generalist herbivore *Spodoptera littoralis* (Noctuidae) has been determined, and general perceptions regarding the occurrence of these types of compounds in *Hypericum* species are discussed.

# Ecology of the tree *Spondias purpurea:* Sexual expression, herbivory, nutritional quality and chemical defense

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The herbivory by insects is an antagonist relationship that potentially reduces the growth and host plant fitness. Specifically, the effects of folivory on dioecious plants have been poorly documented. The resource allocation theory predicts that the costs and resource allocation of chemical defense, nutritional quality, growth rate and plant reproduction are different between males and females. Therefore, it is possible to expect differences in the levels of herbivory among both sexes. However, some studies have demonstrated contradictory results. These variations in the levels of herbivory on different host plant species have been explain by the diversity of chemical plant defense, nutritional quality and age of leaves. In this study, we determine the relationship between the herbivory by folivorous insects, plant defense, and nutritional quality of leaves in three different canopy stratums of male and female individuals of a dioecious tree, *Spondias purpurea,* in a tropical dry forest, during two consecutives years (2004 and 2005). We found significant differences in the levels of folivory between sexes; the females had higher percentages of leaf area removed by insects in both years, and the superior canopy stratum in both sexes present lower levels of foliar herbivory than medium and inferior stratums. There is also an individual variation in the levels of folivory in both sexes. We found significant differences in the levels of herbivory between young and mature leaves in female and male trees. The total phenol, flavonoids, total tannins, hydrolizable tannins, gallotannins and proanthocyanidins concentration were similar between male and females trees. However, we found a negative correlation between the percentage of leaf area removed by insects and the total flavonoids concentration in both sexes, hydrolizable tannins and gallotannins in male trees and total tannins in female trees. The male trees had higher concentration of proanthocyanidins in the medium and superior canopy stratum. The mature leaves had higher concentration of hydrolizable tannins in female trees and the male individuals presented higher levels of total tannins in young leaves. The nutritional quality was measured as water and chlorophyl concentration. The higher water concentration was found in lower and medium stratum of females trees and young leaves of male trees. Finally, the female trees had higher levels of chlorophyl than the male trees. We also found a negative correlation between de damage level and the chlorophyll concentration in male individuals.

# Genetic variation of alkaloid production in *Conium maculatum* after re-association with the specialist moth *Agonopterix alstroemeriana*

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Studies in biological control and invasion biology rarely determine whether introduced plants may rapidly evolve in the area of introduction. Examining the evolution of plant chemical defenses after reassociation with a coevolved enemy is important not only to understand the dynamics of plant-herbivore interactions but also in predicting potential ultimate outcomes of classical weed control programs. A system in which chemical evolution may be examined involves the interaction between *Conium maculatum*, a Eurasian weed naturalized in North America that contains high concentrations of piperidine alkaloids (?-coniceine, coniine, conhydrinone) acting as chemical defenses, and its monophagous European associate *Agonopterix alstroemeriana.* In the United States, *C. maculatum* was largely free from herbivory until approximately 30 years ago when it was reassociated via accidental introduction with *A. alstroemeriana*. At present *C. maculatum* and *A. alstroemeriana* are found in a continuum of re-association times and intensities. To determine the degree to which variation in alkaloid content and resistance to *A. alstroemeriana* were genetically based we conducted a common garden experiment involving plants from three locations in the U.S. (Illinois, Washington and New York) that had experienced 12, 20 and 32 years of reassociation with *A. alstroemeriana* respectively. We analyzed alkaloid concentrations and recorded the natural colonization of *A. alstroemeriana* on *C. maculatum* by counting the number of leaf rolls at the end of the season. Additionally, a bioassay with larvae from our laboratory colony raised on IL, NY and WA foliage was conducted to determine the effects of secondary chemistry on insect fitness.

Total alkaloid production in *C. maculatum* was positively correlated with reassociation time between *C. maculatum* and *A. alstroemeriana,* with the longest historical association with *A. alstroemeriana* (NY) had highest alkaloid content; WA plants were intermediate, with the highest amount of variability among sites, and IL plants had lowest alkaloid concentrations. High variance among WA plants may be a result of transitioning from low alkaloid levels resulting from costs associated with investments in alkaloids in the absence of consistent herbivory, to a highly resistant state where variability has been stabilized due to selection by *A. alstroemeriana*. The number of leaf rolls present on *C. maculatum* was higher in IL plants compared to WA plants and larvae raised on foliage from IL experienced significantly lower mortality than larvae raised on foliage from WA.

Prolonged reassociation between *C. maculatum* and its specialist moth may increase toxicity of this noxious weed in its introduced range. In a previous experiment we documented differences in alkaloid concentrations among plants from IL, WA and NY collected *in situ* (Castells et al. 2005 J. Chem Ecol. 31). Here we show that at least part of these differences, higher alkaloids in those regions with longer reassociation times (NY > WA > IL), have a genetic basis. Plants with higher alkaloid concentrations experienced less damage by *A. alstroemeriana*. At the same time, a decrease in fitness was found when larvae were raised on high alkaloid plants. All of these results are suggestive of strong directional selection exerted by *A. alstroemeriana* on *C. maculatum* chemistry.

# 20-Hydroxyecdysone effect on the European corn borer oviposition?

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Phytoecdysteroids (PE) are plant secondary compounds that are found in about 5-6 % of the plant species in most plant genera. They are structural analogs of the molting hormone of insects and are usually considered as plant defense compounds against insects. One of the most common PE is 20-hydroxyecdysone (20E). We have found that larvae of the European corn borer, *Ostrinia nubilalis*, can avoid food and plants containing 20E thanks to their taste receptors. As in other insects, at high concentrations, 20E can produce abnormal molting and death. From an evolutionary point of view, we would thus expect *O. nubilalis* females to avoid laying eggs on plants producing 20E.
We have examined the oviposition responses of ECB to 20E on different substrates. On artificial substrates, females did not discriminate between 20E-treated and non-treated areas. However, when 20E is sprayed on 8-leaves maize plants, females given a choice, actively avoided to oviposit on the treated plant.
Presuming that legs play an important role in assessing the oviposition substrate, we evaluated the sensory responses of taste sensilla adult females to 20E with electrophysiological technique. We demonstrated that most ventral tarsal sensilla house one neuron that is sensitive to 20E, with a detection threshold of about $10^{-6}$ M.
In summary, adult ECB females can detect 20E with taste receptors apparently as sensitive as in the larvae, but this detection does not always modify their behavior. In one experimental situation, females are not deterred by 20E even at high doses, whereas they avoid very well plants treated with 20E. These observations leave open several hypotheses, for example that females ECB make sense to taste information only when smelling plant odors or that cuticular waxes may help to detect and avoid 20E. However these experiments on *O. nubilalis* as well as former observations on the European grapevine moth, *Lobesia botrana,* suggest that larval deterrents receptors are maintained in adults.

# Resveratrol and related stilbenoid phytoalexins associated with plant-insect chemical interactions

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Polyhydroxystilbenes belong to a small, but important, group of plant secondary metabolites. They possess an extensive range of biological activities. Such compounds are widely abundant in natural sources, but can also be prepared in various synthetic ways. In our case, special consideration has been given to derivatives of pinosylvin or *trans*- and *cis*-resveratrols, which occur as phytoalexins after a bacterial infection or other environmental stress of their source organisms, e.g., pines (*Pinus sylvestris*), grapevines (*Vitis vinifera*) or various herbs used as foodstuffs. Their bio-transformed complex dehydro-oligomers (dimeric, trimeric or even tetrameric analogues) are similarly interesting.

The precise mode of action of such compounds is largely unknown. There is evidence that one possible mechanism is interaction with the arthropod endocrine system, which is crucial for proper development in insects. We have compared the biological activities of selected stilbenoids with a series of natural and chemically modified ecdysteroids in the *Drosophila melanogaster* $B_{II}$ bioassay, in which potency reflects the affinity of binding of the test compound to the ligand-binding site of the ecdysteroid receptor (Dinan, 1999). In order to explore this phenomenon further, we have prepared and tested several new specific analogues and also steroidal and non-steroidal ecdysis-disturbing natural compounds (Dinan, 2001). From consideration of the structure-activity relationships, it was concluded that secondary metabolites other than ecdysteroids might have ecdysteroid antagonistic activity and be capable of affecting insect development.

The varied structures of stilbenoids, together with their large molecular size (in the case of oligomers), when compared to ecdysteroids (Lafont, 2002), but still retaining significant biological activity (Harmatha and Dinan, 2003), can provide new insights into ligand-receptor interactions, which may help to explain the unexpected effects of some other unusually large analogues at the receptor, e.g. dimeric ecdysteroids (Harmatha, 2002). Such compounds could be useful tools to influence gene regulation in insects and become potential lead compounds for the development of new types of insecticides.

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# ß-Glucosylation of DIMBOA in larvae of the rice armyworm, *Mythimna separata*

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Benzoxazinones are the major secondary metabolites found in poaceous plants, such as maize, wheat, and rye. The main benzoxazinone in maize and wheat is 2,4-dihydroxy- 7-methoxy-1,4-benzoxazin-3-one (DIMBOA). This compound is stored as a glucoside in the plants. When plant tissues are injured, the aglycone is released through the action of hydrolytic enzyme ß-glucosidase. DIMBOA shows many different biological activities, such as the reduced survival and reproductive rate of aphids, feeding deterrent effects against European corn borer. Although maize plants are chemically defended as described above, *Mythimna separata*, feed on the plants. In this study, we focus on detoxification of DIMBOA in *M. separata*.

When the fifth instar larvae of *M. separata* were fed on artificial diet containing DIMBOA, they grew up normally. When *Bombyx mori* larvae were fed on the diet, on the other hand, they died in two days. Interestingly, three kinds of glucosides [DIMBOA-2-*O*-ß-Glc, HMBOA (2-hydroxy-7-methoxy-1,4-benzoxazin-3-one)-2-*O*-ß-Glc and MBOA (6-methoxy-2-benzoxazolinone)-*N*-ß-Glc], were identified from the frass of *M. separata* by LCMS and NMR analyses. Those metabolites were not found in the frass of *B. mori*. Furthermore, when the midgut tissues of *M. separata* and UDP-glucose were incubated *in vitro*, the glucosides were also detected in the incubation. These results suggest that the capability to glucosylate DIMBOA analogues contributes to feeding of *M. separata* on maize plants, and UDP-glucose is used as a glucosyl donor.

# Effects of alkaloids from *Sophora alopecuroids* on the activities of digestive and protective enzymes in *Clostera anastomosis* (Lepidoptera: Notodontidae)

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The influences of total alkaloid and its single compounds isolated from *Sophora alopecuroids* on the activities of digestive and protective enzymes in *Clostera anastomosis* were studied. Results showed that the activity of SOD was significantly higher than those of controls and there was no effect on the activities of POD and CAT, when treated with the total alkaloid or matrine for 5 hours. As viewed from the variation trends of protective enzymes, the activities of POD and CAT after the treatment with sophoramine showed a gradual increase in prophase, a subsequent decease and an increment again in post-phase. However, the activity of SOD increased in prophase, subsequent deceased and then keep on at a lower level. The variation of CAT after treatment with sophocarpine was similar to those of treating with sophoramine. But activities of SOD and POD were not significantly changed within 21hours. Results also indicated that the total alkaloid significant affected the activities of digestive enzymes in the treated insects. The activities of proteinase and amylase were significantly higher than those of the controls in *C. anastomosis*, when treated with the total alkaloid for 24h. However, the tested six single alkaloids not significantly affected the activities of digestive enzymes in the treated insects. These results indicated that the dynamic balances among SOD, CAN and POD in the insect were disturbed by alkaloids of *S. alopecuroides.* It resulted in disfunction of the free radical clear system, and led to increasing of the level of free radical, and consequently injured the larvae.

# Specificity of plant responses: impacts of specialist vs. generalist feeding on the glucosinolate-myrosinase-system in a Brassicaceae

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The glucosinolate-myrosinase-system of Brassicaceae is known to hold a defensive function. Glucosinolates are preformed substrates which are hydrolysed upon tissue disruption by myrosinase enzymes. The resulting products are toxic for most herbivores. Nevertheless some insects evolved detoxification mechanisms which enable them to feed exclusively on Brassicaceae, e.g. the turnip sawfly *Athalia rosae*. The larvae of this hymenopteran species sequester glucosinolates in their haemolymph for their own protection against predators. Glucosinolates and myrosinases are constitutively present in all plant organs and can increase in levels by induction mediated by pathogens or herbivores. In a variable environment with a variety of antagonists, specificity of induction responses plays a key role for plants with limited resources. Induction of plant chemical defences which deter or poison generalists might be ineffective against adapted specialist herbivores. As response to the latter, regrowth of destroyed plant parts or investment in mechanical defences might be more favourable.
We investigated the specificity of induction patterns of chemical defences in *Sinapis alba* damaged by a specialist herbivore (*A. rosae*), a generalist herbivore (fall armyworm, *Spodoptera frugiperda*) or mechanical wounding (cork borer). After 24 hours of damage to young leaves, local as well as systemic changes in glucosinolate and myrosinase levels were monitored. The intensity of the responses was highest in damaged leaves. While feeding of *A. rosae* larvae evoked strong increases in levels of both parts of the system, caterpillars of *S. frugiperda* induced weak increases in glucosinolate levels only. In contrast, mechanical wounding moderately elevated glucosinolate levels, but myrosinase activities were strongly induced.
Induction responses in *S. alba* are dependent on the attacking herbivore and are distinct from a mere wound response. This alteration of responses is possibly mediated by the introduction of elicitors from the herbivore's saliva to the wounding site. Considering the arms race between plants and herbivores, induction responses in *S. alba* do not seem to benefit the plant, but rather its opponents: the sequestering specialist might profit from increased glucosinolate levels and thus increased protection due to sequestration. The generalist herbivore suppresses induction responses of hydrolysing enzymes in order to obtain less toxic food.

# Ontogeny of checkerspot chemical defense

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Larvae of some species of checkerspot butterflies (tribe Melitaeini) sequester certain iridoid glycosides from their hostplants, often in very large amounts. These compounds make both larvae and adults unpalatable to potential predators. The Baltimore Checkerspot, *Euphydryas phaeton* (Nymphalidae) commonly uses two hostplant species, *Chelone glabra* (Scrophulariaceae), the native host plant, and *Plantago lanceolata* (Plantaginaceae), an introduced host plant. Rearing Baltimore checkerspot larvae on these two plant species showed that iridoid glycosides are sequestered from both species, and that the amounts and kinds of iridoids sequestered differs depending on the hostplant species. Both larvae and adults contain iridoid glycosides and females also put iridoid glycosides into the eggs.

# Feeding- and life-stage-dependent content and ratios of cyanogenic glucosides in *Zygaena filipendulae* (Insecta: Lepidoptera)

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Zygaena use the cyanogenic glucosides linamarin and lotaustralin for their defense against predators. Zygaena larvae are able to *de novo* biosynthesize as well as sequestering linamarin and lotaustralin from their food plants (Fabaceae). This is a unique system of plant insect interactions. Zygaena food plants harbor a large variation in the content and ratios of cyanogenic glucosides and this study applies sensitive LC-MS analyses, free choice feeding experiments and developmental studies to examine the effect of this on *Z. filipendulae*. Populations of *Z. filipendulae* were feeding on Lotus plants with different content and ratios of linamarin and lotaustralin. Subsequently samples from different life stages of the populations were analyzed for cyanogenic glucoside content and ratios. The feeding preferences of *Zygaena filipendulae,* when presented with plants containing different content and ratios of cyanogenic glucosides, were also tested. The results imply that *Z. filipendulae* strive for threshold content and ratios of cyanogenic glucosides regardless of the content and ratios of cyanogenic glucosides in their food plant. *Z. filipendulae* larvae are able to compensate for different content and ratios of cyanogenic glucosides in their food plants by biosynthesizing the compounds the plants lack. Despite of this, the ratios of cyanogenic glucosides in *Z. filipendulae* is still affected by the food plant, caused by their need to sequester cyanogenic glucosides to conserve energy. This is supported by results showing that *Z. filipendulae* larvae feeding on an acyanogenic plant and therefore biosynthesizing all of their cyanogenic glucosides, spend an excess amount of energy causing decelerated development. More linamarin than lotaustralin was present in imagos, eggs and newly hatched larvae compared to later larval stages, implying that linamarin could either function as a better defensive compound than lotaustralin, or lotaustralin is more easily degraded and used for nitrogen storage. This study show that *Z. filipendulae* represent a good model Zygaena species, which readily switch food plant and whose *de novo* cyanogenic glucoside biosynthesis can easily be turned on and off depending on the food plant.

# The viceroy is not a cheater: Chemical confirmation of Müllerian mimicry between danaid and viceroy butterflies

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The viceroy-danaid butterfly association is a classic mimicry example presented in nearly all introductory biology textbooks. These relationships were originally classified as Batesian, or parasitic, but were later reclassified as Müllerian, or mutualistic, based on predator bioassays. The Müllerian reclassification implies both interacting species are unpalatable because of some type of chemical defense; however, the viceroy defensive chemistry remained uncharacterized. This absence of any biochemical evidence explaining the predator behavioral responses resulted in objections from the scientific community regarding the Müllerian reclassification. We show the viceroy butterfly (*Limenitis archippus*, Nymphalidae) not only sequesters non-volatile defensive compounds from its larval host-plant, the Carolina willow (*Salix caroliniana*, Salicaceae), but also, secretes volatile defensive compounds when disturbed. We developed LC/MS/MS methods to identify a set of phenolic glycosides shared between the adult viceroy butterfly and the Carolina willow and Solid Phase Micro Extraction (SPME) and GC/MS methods to identify volatile phenolic compounds released from stressed viceroy butterflies. In both approaches, all chemical structures were characterized based on their mass spectral fragmentation patterns and confirmed with authentic standards. The phenolic compounds we found are known to deter predator attack in other prey-predator systems including other willow-feeding insect species. Since these compounds have a generalized defensive function, our results support the Müllerian reclassification with the viceroy butterfly possessing different chemical defenses than its monarch (*Danaus plexippus*, Nymphalidae) and queen butterfly (*Danaus plexippus*, Nymphalidae) counterpart (phenolic glycosides vs. cardiac glycosides, respectively). The viceroy is not a Batesian mimic, rather it is a participant in the first clearly documented example of Müllerian mimicry where the co-mimics possess unrelated chemical defenses.

# Changes in essential oil composition and volatile emissions of *Minthostachys mollis*, induced by feeding punctures of *Liriomyza huidobrensis*

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Plants defensive mechanisms against herbivores include chemical changes following damage. This possibility was analysed for *Minthostachys mollis*, a Lamiaceae native to Central Argentina with medicinal and aromatic uses in the region. Effects of feeding punctures produced by *Liriomyza huidobrensis* adult females on the plant's dominant monoterpenes pulegone and menthone, were assessed by monitoring essential oil composition at 24, 48 and 120 h; emission of volatiles was also measured 24 and 48 h after wounding. Leaf puncturing resulted in reduced menthone throughout the experiment and increased pulegone concentration in *M. mollis* essential oil during the first 48 hours. The adjacent undamaged leaves showed similar changes, suggesting a systemic response. Composition of volatiles released from *M. mollis* damaged leaves was also altered, most noticeably by increasing pulegone and diminishing menthone emissions, resulting in a reversal of relative representation of both compounds.
It is likely that changes in both oil concentration and headspace serve important ecological roles during the leaf postwounding period. Moreover, from a commercial point of view, the effects of wounding on leaf oil composition could also have an important impact, since the quality of essential oils and volatile emissions can be altered.

# Plant-herbivore interactions of a generalist tropical herbivore, *Diaprepes abbreviatus* (Coleoptera: Curculionidae)

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The tropical weevil *Diaprepes abbreviatus* was introduced into Florida from the Caribbean in the 1960s. It has since become a major pest of citrus and ornamental plants in that state, and has expanded its range into California and Texas. This weevil threatens a number of important crops because of its high degree of polyphagy and lack of effective natural enemies. We have sought to advance the understanding of the chemical ecology of *D. abbreviatus* by studying its interactions with host and non-host plants. Genetic control of resistance was demonstrated in crosses between *Citrus* and *Poncirus trifoliata* (L.). However, production of resistant varieties is constrained by the relatively low level of resistance in sexually compatible citrus and the long generation time required for breeding citrus trees. While *D. abbreviatus* has become a pest of citrus, laboratory studies showed that other plants, native and introduced into its native range, are as good or better hosts than citrus. Attempts to show specific attraction to species of citrus and other plants or their odors have been unsuccessful. A tropical legume, *Tephrosia candida* DC was shown to be toxic to larvae and repellent to adults of *D. abbreviatus* in greenhouse and laboratory assays. Bioassay-driven chemical fractionation and a new bioassay method have contributed to the discovery of at least one compound(s) responsible for these effects. To date, no semiochemical has been identified for *D. abbreviatus*. Electroantennogram studies demonstrated antennal response to common plant volatiles such as linalool, geraniol, and citral. However, no behavioral attraction to these compounds was demonstrated. We have identified at least two compounds produced by *D. abbreviatus* that evoke a consistent antennal response in GC/EAG studies. These are being investigated as potential sex or aggregations pheromones.

# Behavioral and electrophysiological response of *Heteronyx dimidiata* and *H. crinitus* (Coleoptera: Scarabaeidae) to plant and beetle volatiles

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Various species of scarab beetles, belonging to the Melolonthinae, are serious pests of eucalypt plantations in Australia. Adult beetles emerge from the soil during spring, aggregate into swarms and attack tree seedlings. In Tasmania, the two main pest species are *Heteronyx dimidiata* and *H. crinitus*. Field observations suggest that plant kairomones and/or sex pheromones may be involved in swarm formation and host plant location. GC-MS analysis of SPME collections from damaged eucalypt leaves identified a number of volatiles, including: cis-3-hexenal, ethyl isovalerate, isoamyl acetate and isoamyl isovalerate, which could act as kairomones. GC-MS analysis of SPME collections from adult beetles also detected the presence of benzoquinone in both species and hydroxycineole in *H. crinitus*. We present the results of initial behavioral (funnel traps) and electrophysiological (EAG) studies to these and other putative attractants with the aim of developing a monitoring and/or mass trapping system for these pests.

# Characterization of the volatiles emitted from galleries under construction by females of *Tomicus destruens* (Woll.) (Coleoptera: Scolytidae) using SPME and GC-TOF-MS

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The pine shoot beetles *Tomicus* destruens (Woll.), is an important pine pest widely distributed throughout Europe. *T. destruens* has one generation per year. During their development, imagoes feed on young spring shoots and adults females produce a second type of damage, on host trees, by digging galleries between the bark and the sapwood, while laying their eggs.
Characterization of the volatiles emitted by host species has proven to be an important tool to decode the process of host tree selection by herbivore insects, some of which cause serious economic damage to pines. Variations in the relative composition of the *bouquet* of semiochemicals are responsible for the outcome of different biological processes, such as mate finding, egg-laying site recognition and host selection.
In this work the volatile compounds released from galleries under construction by *T. destruens* females on *P. pinaster* logs were studied with the aim of finding possible host-plant attractants, or aggregation pheromones.
The volatile compounds emitted from recently established galleries were extracted by means of headspace solid phase microextraction, using a 2 cm 50/30 mm divinylbenzene/carboxen/polydimethylsiloxane table flex fiber. The detection and identification of the volatile components were performed by gas chromatography-mass spectrometry with a time-of-flight mass analyzer (GC-TOF-MS), on a LECO Pegasus system, equipped with an Agilent 6890N GC and a time-of-flight mass spectrometer LECO Pegasus III. The data collected after the analyses were processed with automated mass spectral deconvolution using the ChromaTOF software (LECO, Co.).
About 200 compounds were detected and identified from the volatile fractions studied.

# Chemical composition of pine needles in relation to larval performance of the defoliator pine processionary moth

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The pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae) (Den. & Schifft) constitutes an economic pest and a health hazard in the Mediterranean region. Host species appear to influence the distribution and population dynamics of the defoliator, but the mechanisms responsible for its performance are not yet understood. In this work the effect of different chemical characteristics of the needles of four *Pinus* spp., *P. pinaster* Ait, *P. pinea* L., *P. halepensis* Mill and *P. brutia* Ten., upon the larval performance of *T. pityocampa*, between the 2nd and the 5th instars, were investigated.

The needles variables quantified were: force to fracture, considered as an indicator of toughness, water content, calories, total nitrogen and carbon, soluble and insoluble carbohydrates, amino acids and resin acids.

Negative correlations were calculated between food consumption by the larvae, survival and post-digestive efficiency, and the proportion of dehydroabietic acid contents of the needles. These larval parameters were not correlated with the total contents of resin acids of the needles. Using partial correlations, which allowed for the control of the effect of dehydroabietic acid, it has been concluded that food consumption was neither related to needle toughness, nor to any of the primary components analyzed. By contrast, the efficiency of assimilation by the larvae was negatively correlated with toughness and positively with the needles contents in nitrogen and in soluble sugars.

Larvae fed on *P. pinea* showed the highest survival and biomass gain. Globally, larval performance on *P. pinaster* was worst than on either *P. pine* or *P. brutia*, in spite of the highest food consumption observed on the former species. *P. halepensis* was the least suitable food, and concomitantly contained the highest proportion of dehydroabietic acid.

Results show that different traits of the needles of the pine species tested strongly influenced consumption, food utilization and conversion of assimilated food into biomass by *T. pityocampa* larvae. Additionally, they also point to the potential importance of dehydroabietic acid contents in larval performance.

# Similarities and differences in pheromonal and host-plant related chemical communication of flea beetles *Phyllotreta cruciferae* Goeze and *Ph. vittula* Redtenbacher (Coleoptera, Chrysomelidae)

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Both *Phyllotreta cruciferae* Goeze and *Ph vittula* Redtenbacher rank among the most important pest flea beetles in Europe. Remarkable similarities have been found in the pheromonal communication of the two species. Recently we reported that catches of both *Ph. cruciferae* and *Ph. vittula* increased in traps baited with allyl isothiocyanate when a mixture of male specific, pheromone candidate compounds (previously identified from *Ph. cruciferae*) were added. It appeared that for *Ph. cruciferae* only Compound **A** [(5R,5aS)-1,1,5,8-tetramethyl-1,2,3,4,5,6,5a-heptahydrobenzo[1,2-a][7]annulene] was the one for which pheromonal activity could be clearly shown out. The addition of only Compound **A** to allyl isothiocyanate was capable of increasing catches also in *Ph. vittula*, which suggests that Compound **A** may be the key pheromone component also in this species. In volatiles collected from male *Ph. vittula*, the presence of all male specific compounds found previously in collections from North American or European populations of *Ph. cruciferae* was verified.
On the other hand host-plant related chemical communication appeared to show significant differences between the two species. In preliminary screenings sizeable catches of *Ph. vittula* were recorded in traps baited with 3-butenyl isothiocyanate, or a mixture of 2-butenyl-, phenethyl-, 3-butenyl- and butyl isothiocyanates. Later studies revealed that *Ph. vittula* responded better to the above isothiocyanate mixture, than to allyl isothiocyanate, while *Ph. cruciferae* catches were always greater in allyl isothiocyanate baited traps. Of the four isothiocyanates in the mixture, 3-butenyl isothiocyanate may predominantly be responsible for attractivity of the mixture towards *Ph. vittula*. For practical applications, the use of a bait containing several isothiocyanates may be advantageous as it would efficiently attract both important pest *Phyllotreta* spp.

# Electroantennographic responses of *Cerambyx welensii* and *Prinobius germari* (Coleoptera: Cerambycidae) to synthetic plant volatiles

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*Cerambyx welensii* and *Prinobius germari* (Coleoptera: Cerambycidae) can be considered the most important xylophagous insects on *Quercus ilex* and *Quercus suber* in western Andalusia (Spain). The larvae feed in trunks and branches, making them collapse; as well, adults could act as vectors of both patogenous and wood-rotting fungi. The patterns of host-plant selection by these cerambycids are unknown, but it is known the relevance of olfactory stimulation in host selection by several xylophagous insects. This work is part of a larger project in progress aiming to determine the population behaviour and host selection cues of *C. welensii* and *P. germari*; we present a comparative study on the electroantennographic responses (EAG) elicited in both species by a group of synthetic plant volatiles (32 compounds). We employed complete insects, using glass microelectrodes-silver filaments. The group of compounds included the main monoterpenes emitted by *Quercus ilex* and *Q. suber*, several secondary volatiles, four GLV (green leaves volatiles), several foliar triterpenes of *Q. suber*, three acids from tannins, and other compounds (acetic acid, ethanol). Standard stimulus were (*E*)-2-hexenal for *C. welensii* and commercial turpentine for *P. germari* (in both cases, 50% v/v in hexane). Twenty compounds in *C. welensii*, and sixteen in *P. germari*, elicited significant responses. This group included foliar monoterpenes (except ρ-cymene, linalool and terpineol in *P. germari*), all the GLVs, 3-methyl-1-butanol and, for *C. welensii*, acetic acid. Significant differences between sexes were found in *C. welensii*, but only to cineole and ocimene. Cineole elicited the highest response in *C. welensii* (relative EAG >135% in females), β-pinene was the most stimulant in *P. germari* (relative EAG > 128%); both standards were among the most effective stimuli. Four of the five most important volatiles emitted by *Q. ilex* and *Q. suber* (α-pinene, β-pinene, limonene and myrcene) were perceived by both species. The pinene isomers elicited high relative responses (>80%) whereas myrcene and limonene had medium responses (<62%). These results suggest that olfactory cues can play a role in host location by these species. In this sense, the perception of the four main monoterpenes could contribute to a positive discrimination; the perception, especially by females, of characteristic volatiles of non-host that are absent or minoritary in *Q. ilex* and *Q. suber* (such as cineole, the turpentine blend or, also, (*E*)-2-hexenal) could originate a negative discrimination.

# Tracking the signal used by grapevine moth for host-finding

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Grapevine moth *L. botrana* females were attracted to green grapes and shoots of grapevine *Vitis vinifera* in a flight tunnel. Females were also attracted to headspace collections from grape, showing that they rely on olfactory cues during host search. Chemical analysis of grape headspace disclosed high variation in the odour profiles of different varieties and different phenological stages of the same variety. A synthetic mimic of grape volatiles, consisting of the compounds eliciting the strongest antennal responses, was as attractive as the host plant to mated *L. botrana* females. The essence of this attractant was boiled down to a blend of three ubiquitous terpenes showing that attraction to grape is encoded by a ratio-specific blend of at least three compounds. Grapevine moth is a generalist herbivore. Females were attracted also to its native host, the flax-leaved daphne *Daphne gnidium*. The compounds from *D. gnidium* which elicited antennal activity attracted more females than the corresponding blend of *V. vinifera* compounds. In addition, more females were attracted to the compounds co-occurring in *Daphne* and *Vitis*, than to the compounds which occurred in *Vitis* only. Attraction to widely occuring plant volatiles may thus in part account for a generalist feeding habit.

# Host location by *Telenomus busseolae* on the plant substrate

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In egg parasitoids, host foraging behaviour takes place on an immobile and inactive host stage. In the system studied the stemborer *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae), lays its egg masses under the leaf sheath of maize plants by sticking them without prior wounding. The egg parasitoid *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae), due to its morphofunctional adaptation, is able to reach these concealed eggs by crawling under the leaf sheath.
*T. busseolae* behaviour on the host plant was investigated and described using a state transition diagram, and behavioural bioassays were performed to study semiochemical and physical cues from the host and/or from the plant, eliciting host location after parasitoid landing on the plant substrate. Bioassays were carried out on maize plants, 12h or 24h after *S. nonagrioides* oviposition, either with or without moth scales, and on plants without egg masses but artificially contaminated with scales. Healthy plants were used as control. All behavioural observations were carried out in a glass cubic arena. Plants were put in the centre of the arena and a mated female of *T. busseolae* was released on the distal part of the third leaf. The main parasitoid behaviours were considered and both frequencies and duration were recorded. Data were collected and analyzed with The Observer Video-Pro Version 4.0 for Windows. The combined effects of cues from scales and from egg masses laid 24h earlier elicit in the parasitoid a significant increase, compared to the control, of permanence time on plant, searching time and host location probability. Instead, the presence of cues only from scales does not appear to be sufficient for the location of host eggs. In fact, in plants with scales but without egg masses the total parasitoid permanence time and the number of individuals reaching the stem were significantly lower compared to those on plants with host eggs. Therefore, under the combined presence of host eggs deposited 24h earlier and scales, the parasitoid appears to be more stimulated towards host searching. However, this response was not observed when only 12h had elapsed from oviposition, suggesting that from 12h to 24h a change of chemical composition and/or an induction of plant compounds, behaviourally active on the parasitoid, occur.
Further studies are presently in progress to understand whether volatile cues eliciting the final steps of host location in *T. busseolae* are host-derived or plant-derived, induced by host oviposition, and whether their activity depends on time elapsed after oviposition.

# Analysis of behaviour of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) towards host volatiles

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*Rhyzopertha dominica* is one of three bostrichid species (also *Prostephanus truncatus* and *Dinoderus bifoveolatus*) that are serious pests of stored cereal grains. The release of male pheromone by these species is known to be an important component of host selection; much less clear is the role of host odours. In *R. dominica* there are some indications that these odours are attractive, in the closely related *P. truncatus* there is evidence that they are insignificant and in *D. bifoveolatus* there is no evidence either way.

In order to contribute to a better understanding of host selection we have described clearly the behaviours of walking *R. dominica* under the influence of host odours. We videoed a test arena where beetles were released 20 cm downwind of either clean crushed wheat or infested crushed wheat to compare the strength of response in the presence of beetle products (especially male pheromone). The responses were analysed using EthoVision software. Males and females were tested separately at various wind speeds (0.1, 0.6 and 1.0 m/sec), after one to five days starvation and in the light and dark. We also tested beetles that had flown off cultures, i.e. had chosen to distribute from the food medium and so might be in a physiological state conducive to host selection.

The ability of beetles to locate the food source was not affected by lighting conditions but of the three wind speeds tested more beetles located the food at the highest wind speed of 1.0 m/sec. Typically, only about 40-50% of beetles could locate clean crushed wheat compared with 75-80% that could locate infested crushed wheat. Detailed investigations were made in the light at 1.0m/sec and no difference in the ability of the beetles to locate food was observed after one to three days starvation. After more than three days no females, and more than four days no males, reached the food. The velocity of males that responded to clean crushed wheat did not change significantly with up to four days starvation whereas non-responders were slower on the third and fourth day. Responding and non-responding females showed similar velocities after starvation. However, when the same beetles were retested on the same day they did not necessarily respond in the same manner as before, i.e. a responder to food may subsequently behave as a non-responder and vice versa, although the proportion of beetles responding was conserved. Beetles that had flown off cultures gave responses similar to other beetles.

It is clear that host volatiles are only one factor in host selection by *R. dominica.* The factors that determine whether an individual beetle will be a responder to host volatiles remain to be determined but at this stage it would appear that response may be linked to a transient physiological state.

# The relationship between the foretarsal morphology of *Papilio* butterflies and their main host plant leaf structure

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Foretarsal ventral surface observation of Japanese *Papilio* species revealed that the morphology of these areas is closely related to both phylogeny and behavior in these species. My results basically supported the classification that Japanese *Papilio* species divides into five subgenera -- *Papilio* (*P. machaon*), *Princeps* (*P. xuthus* and *P. demoleus*), *Achillides* (*P. maackii* and *P. bianor*), *Menelaides* (*P. helenus*, *P. polytes*, *P. protenor* and *P. macilentus*) and *Iliades* (*P. memnon*) that well correspond to the classification yielded from the interspecies hybridization studies by AE and other researchers in Japan. Moreover, female foretarsal morphology also corresponded to the physical features of their preferring host plant leaves. Some of these might show that *Papilio* species examined the plants by the chemical components not only on the leaves but also in the leaves.

# The role of $CO_2$ cues from plants in the moth-plant interaction and in the processing of odor information in the moth's CNS

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It has been proposed that carbon dioxide ($CO_2$) cues from hostplant flowers play a role in the foraging behavior of moths. Moreover, receptor cells in an olfactory organ, and neurons in the moth's first-order center for odor information processing (the antennal lobe, AL), are specialized for detecting and conveying information about the levels of this gas in the surrounding air. We asked if floral $CO_2$ levels have informational value about food (nectar) abundance for moths. For our studies we utilized a model system consisting of the hawkmoth *Manduca sexta* and one of its preferred hostplants, *Datura wrightii*. Immediately after measuring the $CO_2$ levels emitted by identified flowers from identified wild plants, we removed the nectar from those flowers and recorded volume and sugar concentration. Our data suggest that floral $CO_2$ levels have informational value about nectar resources in flowers from plants living in desert-like (hot and dry) conditions but not in those living in a cooler, more humid environment. Thus, under certain conditions, moths may make use of the $CO_2$ emitted by flowers to improve their foraging efficiency as it may provide information about nectar abundance in a flower before the moth spends time and effort probing in it. In addition, using extracellular multichannel recordings of AL activity, we investigated if $CO_2$ information is integrated with plant (e.g., flower) odor information in the moth's brain. Our results suggest that such integration happens already at the level of the AL. We found that $CO_2$ modulates neural responses to odors and vice versa. Moreover, a single AL neuron may integrate $CO_2$ information with information about only certain odors. Analysis of which odor information is integrated with $CO_2$ information, and the characteristics of the integrated responses, should provide clues about the relative roles of $CO_2$ and volatile organic compounds in the biology of moths and also should help us make predictions about roles of olfaction in the moths' plant-oriented behaviors. Thus, neurobiology should be expected to continue to contribute hypotheses worth testing by behavioralists and chemical ecologists.

# Early experience on apple increases the attractiveness of apple odours for the hawthorn psyllid *Cacopsylla melanoneura*

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The hawthorn psyllid *Cacopsylla melanoneura* spends most of the year on conifers, whereas reproduction and larval developement takes place on hawthorn (*Crataegus spp.*) or apple (*Malus domestica*) in early spring. In recent years it was found that *C. melanoneura* [1] and the apple psyllid *C. picta* [2,3], are vectors for the apple proliferation phytoplasma, causing major economic losses by inducing 'witches brooms' and tasteless dwarf fruits. In this study we investigated if chemical cues are responsible for location of their host for reproduction by overwintered female psyllids and in mate finding by both sexes.
All psyllids were collected from hawthorn by beating tray sampling in early spring. Both sexes of adult *C. melanoneura* were separated one day before testing and stored in plastic vials at 4 °C. Behavioural tests were done using a dynamic olfactometer consisting of a Y-shaped glass tube. Different odours were offered singularly or simultaneous (hawthorn twigs with or without psyllids, apple twigs 'Gala Royal', or empty) in the test arms of the olfactometer. The position of the test arms was turned every second trial, and after four trials the glass tube was exchanged. For each test ten individuals of the same sex were put simultaneously into the entrance arm of the olfactometer and were allowed to walk up and decide for one of the test arms for 15 min. Every individual that has passed a final marking on one of the test arms was counted. Results were analysed statistically by dependent *t*-test.
Behavioral responses of the psyllids were shown for both sexes. When females were tested for attraction to host plant odours, they favoured hawthorn odours when offered together with apple odour or an empty control, respectively. But they did not discriminate apple odour against an empty control. After having gained experience with apple odours by feeding and egg laying on apple for 2-4 days, apple odour became more attractive than hawthorn odour when offered simultaneously. If odours of hawthorn twigs infested by either females or males of *C. melanoneura* were provided, males were attracted by odours of hawthorn twigs infested both by males and females, compared to an uninfested control twig. In contrast, females were only attracted by odours of females sucking on hawthorn but not by hawthorn twigs infested by males. All presented results are statistically significant.
In this study the finding of Gross & Mekonen (2005) [4] was confirmed, which stated that plant odours influence the host finding behaviour of *C. melanoneura*. Further, females are able to discriminate host plants by their volatiles. The preference for host volatiles showed to be dependent on the last host experience. Additionally, our results indicate that host derived volatiles and/or pheromone compounds may be involved in mate finding of *C. melanoneura.*

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# Floral scent of willows guiding pollinators?

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The genus *Salix* (willows) is composed of woody plants with approximately 400 to 500 species. Normally willow species are dioecious with flowers arranged in catkins. *Salix* shows traits of insect as well as wind pollination. Since many willow species are known to be pollinated predominantly by insects, these pollinators are thought to play an important role in regulating intra- and interspecific gene flow. Quality and quantity of floral scent are assumed to be olfactory cues for attracting pollinators.
Floral odours were collected from male and female individuals of different willow species growing either in natural habitats or in the Ecological-Botanical Garden in Bayreuth, Germany. The volatiles were analysed using a highly sensitive dynamic-headspace MicroSPE method and gas chromatography coupled with mass spectrometry (GC-MS).
Visitors of catkins were caught during day (bees, such as *Andrena vaga*, *Colletes cunicularius*) and night (moths, such as *Orthosia* gothica, *O. munda*) and their response to the floral scent was tested by means of gas chromatography coupled to an electroantennographic detector (GC-EAD).
Floral scent of different willow species revealed more than 40 compounds, most of them being isoprenoids (e.g. *cis*- and *trans*-β-Ocimene, *α*-Pinene, D-Limonene) and benzoids (e.g., Salicylaldehyde). Generally, most species were similarly scented, however several species can be differentiated on the basis of their scent. The arising question is, if pollinators can differentiate the willow species on the basis of their scent, and scent could be a reproductive isolation mechanism by guiding insects to intraspecific pollination.
Indeed, when testing *Andrena vaga* bees on the scent of different willow species, the EAD responses were species specific. Different willows elicited different EAD responses.
The role of different volatiles in attracting specific pollinators is discussed.

# Scent variation, hybridization and speciation in sexually deceptive orchids of the genus *Ophrys*

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Orchids of the European genus *Ophrys* are pollinated by deception of male insects. An *Ophrys* species attracts only one pollinator species by producing an odour identical to the female sex-pheromone of its pollinator. Thereby reproductive isolation is achieved. Nevertheless, hybrids occur, and if the odour of a hybrid swarm by chance resembles the sex pheromone of another species, which responds as a new pollinator, hybrid speciation may occur. With the aim to study processes of speciation we investigated hybrid swarms between *O. fusca* and *O. iricolor* on Sardinia using behavioural experiments, GC-EAD analyses, morphometric and chemical analysis, and molecular markers (AFLP).
In the behavioural experiments most flowers of the parental species were attractive to its own pollinator species. However, certain flowers were also attractive to the pollinator of the second parental species and some attracted both pollinator species. Hybrid plants were either attractive to one or both pollinator species. Morphometric analyses of 17 flower characters showed only minor differences between the parental species. Hybrid flowers were more similar to flowers of *O. iricolor* than to *O. fusca* and did not form a distinct group. In GC-EAD analyses we found a mixture of ?? saturated and unsaturated hydrocarbons to be perceived by the male pollinators. A discriminant function analysis showed a significant difference in the flower odour between both parental species, while *O. iricolor* and hybrids showed a broad overlapping and could not be separated using floral scent. AFLP data of the same plant specimen showed a clear separation of *O. fusca* from *O. iricolor* and hybrids, which form a common cluster .
The clear odour difference between both parental species is probably maintained by a strong selection of the pollinator species while the broad overlap of morphological flower characters indicates a lower selective pressure by the pollinators. We assume that *O. iricolor* is on its way to adsorb *O. fusca* on Sardinia for the following reasons: In bioassays, more flowers of *O. iricolor* attracted the pollinator of *O. fusca* (*A. nigroaenea*) in addition to its own pollinator than vice versa. Furthermore hybrids are more attractive for *A. morio* than for *A. nigroaenea*. We therefore expect a higher rate of false pollination by *A. nigroaenea* as compared to *A. morio* and as a consequence a gene flow from *O. fusca* to *O. iricolor*.

# The temporal change in floral odors affects pollinator arrivals in *Homalomena propinqua* (Araceae)

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*Homalomena propinqua* (Araceae) is a common understory herb found in lowland dipterocarp forests in Borneo, and shows temporal heat generation at the top of spadices. Two species of beetles, *Parastasia bimaculata* Guerin (Scarabaidae) and *Dercetina sp*. (Chrisomelidae), are known to pollinate, and stay in chambers with mating and/or feeding on inflorescences. Mating site pollination systems are relatively specific in archaic angiosperms including Araceae, and are thought to have evolved through phytophagous insects that used deterrent chemicals as cues for mating sites and food. Therefore floral odors of *H. propinqua* also may affect on pollinator arrivals as some chemical cues, especially attractant substances. To examine the relationship between floral odors and pollinator behaviors, behavioral observations of pollinators, chemical analysis of floral odors and bioassay by five major compounds in floral odors were conducted.

As the results of behavioral observations, beetle pollinators visited inflorescences mainly at 7:00-9:30 am, just after sunrise, and this paralleled heat generation of spadices. *P. bimaculata* visited inflorescences on the first day of flowering (female-phase inflorescences) significantly more frequently than the other days, although *Dercetina sp*. visited each inflorescence throughout of flowering period. Chemical analysis showed floral odors of *H. propinqua* were composed of 18 compounds, and five major compounds were a-Pinene, 2-butanol, 2-methyl-3-buten-2-ol, 2-heptanol and Veratrole (1,2-dimethoxybenzene). As a result of bioassay, *P. bimaculata* was attracted by mixture of 2-butanol + Veratrole, and *Dercetina sp.* was attracted by Veratrole. Moreover, the quantity of compounds that attract *P. bimaculata* was most abundant on the first day inflorescences over the flowering period, and this was consistent with the behavioral observations of *P.bimaculata*. Meanwhile, the quantity of Veratrole that attract *Dercetina sp.* was the most scarce on the 3$^{rd}$ day, and this was not consistent with behavioral observation of *Dercetina sp*., which was attracted throughout the flowering periods. The attractant compounds of *P. bimaculata*, especially 2-butanol, are very specific, while the attractant compound of *Dercetina sp*., Veratrole, is a wide spread compound in floral odors.

These results indicate that *H. propinqua* may change in the emission of attractant compounds temporally, and attract pollinators on the female-phase inflorescences, as a result, promotion of efficient cross-pollination. Therefore, attractant strategies of *H. propinqua* may be adapted to attract *P. bimaculata*. However, *Dercetina sp*., which may be more of a generalish herbivore-pollinator, was also attracted in great numbers. Thus, *H.propinqua* may be adapted to attract both specific and generalish pollinators to ensure pollination and promote reproductive success.

# Chemical usurpation of volatile signals by parasites in a nursery pollination mutualism – the case of fig and fig wasps

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In nursery pollination mutualisms, floral scents play a major role in advertising host localisation and rewards for the specific pollinator. However, chemical signals responsible for the encounter of the mutualist partners in these interactions can be also used by specific parasites of the mutualism to locate their host. Each *Ficus* species (Moraceae) is involved in a specific and obligatory mutualism interaction with at least one pollinating wasp (Chalcidoidea, Agaonidae). Volatile olfactive compounds (VOC) emitted by receptive figs are responsible for pollinator attraction. Many highly specific non-pollinating chalcidoid species exploit the coevolved mutualists but nothing is known about the cues they use to locate their hosts. To investigate if they use the VOC emitted by the fig to attract pollinators, we performed bioassays on the role of VOC in host localisation both by pollinators and parasites. Using Y-tube olfactometers, we tested the olfactory attraction of the pollinator (*Ceratosolen marchali*) of the dioecious fig species *Ficus hispida*, as well as that of one parasite of the mutualism (*Philotrypesis pilosa*). We also tested for the specificity of the attraction using VOC emitted by two other fig species: *F. racemosa* (monoecious) and *F. tinctoria* (dioecious). The parasite uses the same signal as the pollinator to locate its resource. Moreover, both pollinator and parasite are only attracted by the specific VOC of their host fig species. A consequence of pollination by deceit characterising all dioecious fig species is that both the pollinator and the parasites can only reproduce in male figs. Strong selection should occur on these wasps to recognize and avoid the deceptive sex. However, we showed that only the parasite and not the pollinator can discriminate between VOC emitted by male or female figs. Compared to parasites, pollinators live only a short time (a few hours versus 7 days), so they should be under strong "selection to rush". Given limited discriminability due to inter-sexual mimicry of the figs, the best strategy should be to enter the first fig of the good species regardless of the sex. In the case of the parasite, however, selection to rush should be relaxed and they should be able to discriminate the deceptive sex. Headspace extraction and GC/MS analyses of the VOC emitted by receptive figs of the three species revealed that the main compounds, or their relative proportions, were often different among species, as well as between sexes in dioecious species.

# Informative values of chemical compounds of zucchini flowers

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Infochemicals have a semiotic meaning for insects, which varies depending on the species, sex, and even on the age. Moreover, the different plant organ origin of the scents has an important role to play on insect-plant relationships, adding a meaningful context to similar chemicals.
We have carried out a study of scent emitted by zucchini flowers (Mena et al., 2005). The effect of each individual compound on bumble bees, used as pollinators of zucchini crops, was tested performing behavioural tests with each individual compound. Such tests were tri-optional, considering the attraction, repellence or indifference of workers bumble bees, against controls. Compounds tested were *citral*, eucalyptol, R-limonene, α-pinene, ß-pinene, linalool, ocymene, hydroquinone,1,2,4-trimethoxybenzene, geranic acid, geranyl acetate, geranyol, 3-carene, E,E-farnesol, eugenol, *indole*, o-xylene, p-xylene, m-xylene, ethylbenzene and toluene, being written with bold typos the attractive ones, with italic the repellents and the rest indifferents.
When comparing these behaviour responses with those carried out by Peterson et al (1994) with *Diaphania nitidalis,* it can be seen that a given group of chemical compounds from trichome glands of leaves of zucchini can be attractants for bumble bees (for example citral, linalool, 3-carene, and eugenol), while only germacrene resulted attractive for the moth. In addition, the above cited study (Petersen et al., 1994) emphasizes that oviposition was significantly stimulated by a mixture of the VOCs presents in the whole leafs of plants (limonene caused a significant reduction). On the search for natural pest control in a same plant species like *Cucurbita pepo*, the match of these studies are good examples of the context meaning for each kind of insects, concluding that some specific VOCs induce different behaviours on *Bombus terrestris* than on *Diaphania nitidalis*, depending on the origin.

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# Chemical mediation of ecological interactions between the parasitic plant *Cuscuta pentagona* and its hosts

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Parasitic plants are important components of both natural and agricultural ecosystems, but little is known about the ecology of interactions between parasitic plants and their hosts. Flowering plants in the genus *Cuscuta* are obligate parasites with little photosynthetic capability and obtain nutrients by attaching to above-ground shoots of other plants. They are important worldwide pests of agriculture and can be difficult to control without also impacting host plants. We will discuss the chemical ecology of interactions between the parasitic plant *Cuscuta pentagona* and various host species including domestic tomato.

# Chemical interplay between fungi and collembola during interspecific basidiomycete interactions

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When mycelia of different basidiomycete species meet, recognition as non-self is accompanied by changes in morphology and production of extracellular enzymes, volatile and diffusible secondary metabolites. These are visually manifested as bright pigments in mycelium and culture substratum. We investigated the sites of collembola grazing and the production of volatile organic compounds (VOCs) in mycelial interactions between *Hypholoma fasciculare* and *Resinicium bicolor*. For VOC determination, mycelia were grown opposing each other across a porous polyester/polypropylene membrane overlaid with a Fluropore™ membrane (1µm pore size) supported over broth medium in a polypropylene bottle. The interspecific interaction produced 12 VOCs (collected by SPME - 100µm PDMS fibre - and quantified by GCMS) that were not found in single species samples, 4 were common to the interspecific interaction and *R. bicolor* samples, 3 to the interspecific interaction and *H. fasciculare*, 1 was unique for *R. bicolor* and 3 were identified in all treatments. Most VOCs were sesquiterpenes; a benzoic acid methyl ester, a benzyl alcohol, and a quinolinum type compound with a distinctive fragmentation pattern at m/z 203, 204, 206, 207 were also identified; 3 volatiles with m/z maxima of 163, 159 and 206-208 respectively, were unidentified. All VOCs detected increased in concentration over time and then declined. Only 4 of the metabolites were detected at 1 d. Initiation of all other VOCs occurred between 3 d and 17 d, though the majority of the interaction volatiles were detected from 9 d following mycelial contact.

Fungal metabolite production during interactions affects distribution of collembola. During the first 6 d following mycelial contact collembola (*Folsomia candida*) increased on *R. bicolor* mycelium while decreasing on that of *H. fasciculare.* The trend was reversed between 6 and 18 d. After onset of pigment production and post-8 d volatile production the collembola began to relocate from *R. bicolor* to *H. fasciculare*, implying that *R. bicolor* may be producing compounds that repel collembola and/or that *H. fasciculare* volatiles/diffusibles are attracting and/or arresting collembola. In conclusion, the complex chemical interplay between fungal mycelia during antagonistic interspecific interactions can also affect invertebrates.

# Chemical ecology of the Hessian fly and its grass hosts

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The Hessian fly has many interesting features, a number of which are relevant to the field of chemical ecology. First, the Hessian fly female produces a long-range sex pheromone that elicits male behaviour similar to sex pheromone-mediated behaviour of male moths. The major component of this sex pheromone was identified over 15 years ago but is not sufficiently attractive to serve as a monitoring tool in the field. GC-EAD studies indicate that there may be as many as six other components to the pheromone. Laboratory and small plot tests have indicated behavioural activity for four of the seven components. However, none of the tested blends was as stimulatory as female extract, indicating that additional components need to be identified and/or there are problems with ratios or stereochemistry. Other interesting features concern Hessian fly relationships with the grass hosts. Grass host species have resistance (*R*) genes, specific for defense against Hessian fly. *R* genes have strong phenotypic effects: 100% of first instar larvae die on plants carrying an effective *R* gene. Defense pathways are triggered after an interaction occurs between an *R* product (the 'receptor') and a foreign product produced by a parasite avirulence (*avr*) gene (the 'elicitor'). Relationships between these grass *R* genes and Hessian fly *avr* genes fit the gene-for-gene model. The well-known adaptation of Hessian fly to *R* gene-mediated resistance probably occurs when mutations in a matching *avr* gene disrupt the *R*-*avr* interaction. We describe current studies to identify 1) larval chemicals injected into plant cells, and 2) the effects of larval chemicals on attacked cells and plant growth. One important discovery is that the Hessian fly larva is a gall-maker, inducing a nutritive tissue at feeding sites. This result suggests a possible goal of *R* gene resistance: the *R* product triggers an early and localized defense that prevents the formation of the nutritive tissue, thereby also preventing larval-induced growth deficits. A final interesting feature of the Hessian fly is that the adult female is highly discriminating when selecting host species and physiological stage, but does not discriminate between grasses with and without *R* genes. We review our research on chemical ecology of Hessian fly host selection.

# Chemical ecology of moth pollinators of the prairie fringed orchid

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The western prairie fringed orchid (WPFO) is currently federally listed as a threatened species in the USA. It is believed that habitat loss and various land management practices, e.g. grazing, burning, weed control, have contributed to orchid decline. Based on flower morphology, it appears that that WPFO depends on insect pollinators for seed production. Pollen is packed into packets called pollinia, each of which has a sticky pad that attaches to the head of the visiting pollinator. Orchids typically have close relationships with a small number of pollinator species, with odors from orchids attracting pollinators to a significant nectar reward. When we began our studies in the Sheyenne National Grasslands in southeastern North Dakota, two species of hawkmoths (Lepidoptera: Sphingidae) had been identified carrying WPFO pollinia, the wild cherry sphinx, *Sphinx drupiferarum*, and achemon sphinx, *Eumorpha achemon*. Land managers believed that low pollination rates for the orchid resulted from the unreliable presence of these two pollinators. In our studies we used various experimental and trapping methods to address the following questions. Do only a limited number of hawkmoth species serve as pollinators? What role do orchid volatiles play in relationships with pollinators? What volatile chemicals are released by orchids? Are these volatile chemicals specific to WPFO? We have discovered four new moth pollinator species, including at least one species that is not a hawkmoth (Lepidoptera: Noctuidae, genus *Catocala*). Several of these species are migrants from southern regions. Two of the newly discovered WPFO pollinator species are pollinators of the closely related eastern prairie fringed orchid (EPFO) indicating that WPFO and EPFO do not exist as a "pollinator-isolated species pair". Overnight, in-field collection of orchid volatiles revealed three major components, methyl benzoate, 2-hydroxymethyl benzoate, and benzene methanol. Antennae of one of the newly discovered pollinators, the hermit sphinx *Sphinx eremitus*, responded to all three of these chemicals. We discuss whether orchid volatiles and moth sex pheromones could be used to monitor year-to year pollinator presence in the Sheyenne National Grasslands.

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