International Society of Chemical Ecology

19th Annual Meeting

University of Hamburg, Germany

August 3. – 7. 2002

Scientific Programme

and

Abstracts

Universität Hamburg

Meeting Host	**Wittko Francke**
Scientific Committee	**Ian T. Baldwin** **Wittko Francke** **Thomas Hartmann** **Monika Hilker**
ISCE-President	**Murray B. Isman**

Local Team:
Marianne Wenzel, Jan Bergmann, Karsten Fehler, Stephan Franke, Gaby Graack, Jenny Haftmann, Nicolas Heinzel, Carolin Kellersmann, Melanie Mosler, Stephanie Nagorny, Sven Possner, Alcindo dos Santos, Claudia Schulz, Susanne Sölter, Michael Specht, Jochen Titze, Till Tolasch, Gunnar Weibchen

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Programme

Saturday, August 3

Chemistry Department
Martin-Luther-King-Platz 6

13:00 – 18:30 **Registration at Fachbereich Chemie**

Zoology Department
Martin-Luther-King-Platz 3

18:30 Official Welcome
Wittko Francke, Meeting Host
Murray B. Isman, ISCE President

18:45 **Social Lecture**
Introduced by Gunnar Bergström

Jerrold Meinwald
From Fireworks to Pheromones

20:00 Get-together / Grill-party

Mounting of Posters

Sunday, August 4

Zoology Department

Symposium on Semiochemicals

Organizer and Chair: Wittko Francke

8:30 – 9:00 **K. Mori**:
Recent results in pheromone synthesis

9:00 – 9:20 **W. Kitching**:
Suites of novel hydrocarbons from sugar cane scarabs: structure, synthesis and stereochemistry

9:20 – 9.40 **B. Burger**:
Identification and synthesis of constituents of the semiochemical secretions of South African antelope species

9:40 – 10:00 **E. D. Morgan**:
Some painless chemistry from stingless bees

10:00 – 10:30 Coffee Break

10:30 – 10:50 **W. A. König**:
Stereochemical analysis of volatile natural compounds by enantioselective gas chromatography

10:50 –11:05 **W. Boland**:
Classification of terpenoid biosynthesis according to the MEP- or MVA-pathway by isotope ratio mass spectroscopy

11:05 – 11:20 **S. Schulz**:
Novel compounds from the cuticle of *Pieris* butterflies showing chemical dimorphism

11:20 – 12:15 **ISCE Silver Medal Lecture**

Chairperson: Wittko Francke

John A. Pickett
To attack or not to attack? Life as a chemical ecologist, or how pests avoid plants and animals that are unsuitable as hosts

12:15 – 13:45 Lunch Break

Sunday, August 4

Chemistry Department
Lecture Hall B

Contributed Lectures
Chairperson: Zoltán Imreï

13:45 – 14:00 **A. Svătos**:
Semi-synthetic preparation of sex pheromones from plants transformed with an insect desaturase

14:00 – 14:15 **A. Hooper**:
Lacewing chemical ecology – A push and a pull?

14:15 – 14:30 **G. Szöcs**:
Sex attractants for lepidopterous species: Pure enantiomers or racemates of epoxydienes

14:30 – 14:45 **S. Seybold**:
The chemical ecology of the eastern larch beetle, *Dendroctonus simplex* in Minnesota

14:45 – 15:00 **J. McNeil**:
Pheromone ecology of the potato aphid, *Macrosiphum euphorbiae* under laboratory and field conditions

15:00 – 15:15 **J. Staples**:
Semiochemistry of the conifer feeding sawfly, *Acantholyda erythrocephala* (L.) (Hymenoptera)

15:15 – 15:45 Coffee Break

Contributed Lectures
Chairperson: Sandrine Gouinguiné

15:45 – 16:00 **J. Steidle**:
Do generalist parasitoids use specific or general chemical cues for foraging?

16:00 – 16:15 **K. Shiojiri**:
Daily periodicity in production of plant volatiles affects daily rhythm of herbivorous and carnivorous insects

16:15 – 16:30 **E. Mateus**:
Needle monoterpene contents of eleven pine species and relative susceptibility to the attack of *Thaumetopoea pityocampa* (Den. & Schiff.)

16:30 – 16:45 **J. A. Byers**:
Host-tree finding in a bark beetle by avoidance of nonhosts

16:45 – 17:00 **J. B. Fernandes**:
Electrophysiological responses of female and male *Hypsipyla grandella* to *Swietenia macrophylla* essential oil

17:00 – 17:15 **J. Ruther**:
Chemical orientation in cockchafers

17:15 – 17:30 **C. Tosh**:
Reproductive cues of generalist and specialist aphids

Poster Session (Beer and Pretzel)

Sunday, August 4

Chemistry Department
Lecture Hall C

Contributed Lectures
Chairperson: Astrid Groot

13:45 – 14:00 **M. Isman**:
Larvae exposure to ovipositon deterrents alters subsequent oviposition behavior in generalist and specialist moths

14:00 – 14:15 **A. Renwick**:
Ovipositonal and electrophysiological responses of the diamondback moth (*Plutella xylostella*) to isothiocyanates

14:15 – 14:30 **C. Müller**:
Defense in herbivores of glucosinolate containing plants

14:30 – 14:45 **J.-L. Boevé**:
Defence strategy in sawfly larvae by integument and hemolymph adaptations and host plant chemistry

14:45 – 15:00 **J.-F. Picimbon**:
Chemosensory proteins

15:00 – 15:15 **B. Pophof**:
Pheromone binding proteins contribute to the excitation of olfactory receptor cells in moths

15:15 – 15:45 Coffee Break

Contributed Lectures
Chairperson: Lana Barkawi

15:45 – 16:00 **E. Plettner**:
Selectivity of pheromone-binding proteins in the gypsy moth, *Lymantria dispar*

16:00 – 16:15 **E. Jacquin-Joly**:
Elements of odor recognition in moths: Sensory neuron membrane protein (SNMP) and G protein

16:15 – 16:30 **P. Nagnan-LeMeillour**:
Molecular coding of behavioral antagonists by OBP in two noctuid species, *Mamestra brassicae* and *Helicoverpa zea*

16:30 – 16:45 **L. Field**:
Cloning of genes encoding odorant binding proteins and chemosensory proteins in insects

16:45 – 17:00 **G. Guiraudie**:
Role of the vomeronasal organ in the detection of pig appeasing compounds

17:00 – 17:15 **I. Said**:
Responses of single olfactory cells in the palm weevil, *Rhynchophorus palmarum* (Coleoptera, Curculionidae) to insect and host produced volatiles

Poster Session (Beer and Pretzel)

Monday, August 5

Zoology Department

Symposium on Insect-Plant Relationships

Organizer and Chair: Monika Hilker

8:30 – 9:00 **M. Dicke**:
Chemical ecology of induced plant volatiles: From mechanisms to functions

9:00 – 9:30 **P. Hatcher**:
Plant-Pathogene-herbivore-interactions: Ecological and chemical aspects

9:30 – 10:00 **S. Eigenbrode**:
Plant surface waxes and insect ecology

10:15 – 10:45 Coffee Break

10:45 – 11:00 **T. Turlings**:
Recruitment of parasitic wasps by caterpillar-injured plants

11:00 – 11:15 **M. Rostàs**:
Plant-mediated interactions between a phytopathogenic fungus and a leaf beetle on Chinese cabbage

11:15 – 11:30 **S. Colazza**:
The role of stink bug-induced host plant volatiles in the attraction of the egg parasitoid, *Trissolcus basalis*

11:30 – 12:15 **Silverstein-Simeone Lecture**

Chairperson: Wendell Roelofs

Thomas C. Baker:
Some emerging issues concerning ‘mixtures‘, ‘noise‘, and fine-grained odor plume resolution involving plant-odor and sex pheromone mixtures

12:15 – 13:45 Lunch Break

Monday, August 5

Chemistry Department
Lecture Hall B

Contributed Lectures
Chairperson: Glenn P. Svensson

13:45 – 14:00 **S. Gouinguiné**:
Electroantennogram responses of three parasitic wasps to induced odour of maize, cowpea, and cotton

14:00 – 14:15 **C. Tamò**:
A comparison of naïve and conditioned responses of three endoparasitoids of lepidopteran larvae to host-induced plant odours

14:15 – 14:30 **M. Riedel**:
Selective sampling and analysis of epicuticular wax crystals in *Nepenthes alata* pitchers

14:30 – 14:45 **T. Meiners**:
Chemically mediateds host-foraging strategies in egg parasitoids of specialist and generalist herbivores

14:45 – 15:00 **G. Pohnert**:
Phospholipase activity triggers the wound-activated chemical defense in the diatom, T*halassiosira rotula*

15:00 – 15:15 **E. Gross**:
Impact of herbivory by *Acentria* (Lepidoptera: Pyralidae) on polyphenols in the freshwater angiosperm, *Myriophyllum spicatum*

15:15 – 15:45 Coffee Break

Contributed Lectures
Chairperson: Junji Takabayashi

15:45 – 16:00 **I. Narberhaus**:
Physiological adaptations to host plant alkaloids in the leaf beetle genus *Longitarsus*

16:00 – 16:15 **G. Hol**:
Changes in alkaloids in *Senecio jacobaea* by artificial root herbivory; effects on the aboveground herbivore *Mamestra*

16:15 – 16:30 **M. Macel**:
Diversity in plant secondary metabolites: Selection of neutrality?

16:30 – 16:45 **M. Buennige**:
Chemical defensive strategies in galerucine larvae (Coleoptera, Chrysomelidae)

16:45 – 17:00 **A. Eben**:
Phagostimulation of diabroticina beetles (Chrysomelidae: Galerucinae) by pollen extracts and amino acids

17:00 – 17:15 **J. Dickens**:
Attracting volatiles for the Colorado potato beetle

Poster Session (Beer and Pretzel)

Monday, August 5

Chemistry Department
Lecture Hall C

Contributed Lectures
Chairperson: Ursula Röse

13:45 – 14:00 **A. J. Mordue**:
Mating behaviour & the sex pheromone of *Culicoides nubeculosus* (Diptera, Ceratopogonidae)

14:00 – 14:15 **Y. T. Qiu**:
Behavioural and electrophysiological studies on human emanations that attract the malaria mosquito, *Anopheles gambiae*

14:15 – 14:30 **R. Smallegange**:
Olfactometer studies on the effects of ammonia, lactic acid and a carboxylic acid on *Anpoheles gambiae* giles S.S.

14:30 – 14:45 **R. Newman**:
Aquatic herbivores and the chemical defense system of watercress

14:45 – 15:00 **T. Breithaupt**:
Chemical communication made visible in crayfish agonistic interactions

15:00 – 15:15 **P. Frade**:
Evidence that male tilapia use urine as chemical signal during reproduction

15:15 – 15:45 Coffee Break

Contributed Lectures
Chairperson: Victoria Soroker

15:45 – 16:00 **T. Schmitt**:
Cuckoos in wolves' clothing? Chemical mimicry in a cuckoo wasp of the European beewolf (Hymenoptera, Chrysididae and Sphecidae)

16:00 – 16:15 **M. Ayasse**:
Chemical ecology and reproductive biology of the social parasitic bumblebee, *Psithyrus norvegicus*

16:15 – 16:30 **H. Sasagawa**:
Co-evolution of plant and insect in Asia: Chemical mimicry of an oriental orchid and behavior of the Asian honey bees

16:30 – 16:45 **R. Beard**:
The chemistry of *Myrmecia gulosa*: A comparative study of the cuticle, post-pharyngeal, and Dufour's gland

16:45 – 17:00 **A. Hefetz**:
Motivation and nest volatiles affect nestmate recognition in the carpenter ant, *Camponotus fellah*

17:00 – 17:15 **M. Haverty**:
Reticulitermes species in California: Cuticular hydrocarbons and soldier defense secretions

Poster Session (Beer and Pretzel)

Tuesday, August 6

Zoology Department

Symposium on Aspects of Chemical Ecology in Molecular Biology

Organizer and Chair: Ian T. Baldwin

8:30 – 9:00 **D. Heckel**:
Cytochromes P450 and lepidopteran-plant interactions

9:00 – 9:25 **H. Vogel**:
Profiling plant-insect interactions

9:25 – 9:50 **J. Kroymann**:
Glucosinolates and insect resistance in *Arabidopsis thaliana*

9:50 – 10:15 **U. Wittstock**:
The power of plagiarism – glucosinolate hydrolysis in plants and specialist lepidopterans

10:15 – 10:45 Coffee Break

10:45 – 11:00 **J.-H. Kang**:
Developmental and wound-responsive regulation of threonine deaminase gene promoter from *Nicotiana attenuata*

11:00 – 11:15 **C. Schnee**:
Identification of maize terpene synthase genes involved in the indirect defense against lepitopteran herbivores

11:15 – 11:30 **D. Tholl**:
Terpene biosynthesis and emission from *Arabidopsis thaliana* flowers: New perspectives for investigations of floral scent biology and evolution

11:30 – 11:45 **R. Ozawa**:
Synergistic effects of exogenous spermine and jasmonic acid on the volatile production in lima bean leaves

11:45 – 12:00 **M. E. Hoballah**:
Petunia-pollinators interactions

12:00 – 12:15 **J. Kuhn**:
Selective thioglycosid-transport from gut to defence glands in chrysomelid leaf beetles

12:15 – 13:30 Lunch

Afternoon free

Wednesday, August 7

Zoology Department

Symposium on Symbiosis

Organizer and Chair: Thomas Hartmann

8:30 – 9:15 **D. Strack**:
Arbuscular mycorrhiza

9:15 – 9:45 **C. L. Schardl**:
Diversification of protective alkaloid profiles as relates to evolutionary processes in fungal endophytes of grasses

9:45 – 10:15 **B. F. Bowden**:
Soft coral-zooxanthellae symbiosis; the effect of coral bleaching on metabolite concentrations

10:15 – 10:45 Coffee Break

10:45 – 11:15 **K. Dettner**:
Symbiotic microorganisms in arthropods – their significance in chemically-mediated biotic interactions

11:15 – 11:30 **J. Piel**:
Biosynthesis of a beetle defense compound by a bacterial symbiont

11:30 – 11:45 **T. Fester**:
Possible role of carotenoids and apocarotenoids in the arbuscular mycorrhizal symbiosis

11:45 – 12:00 **M. Walter**:
Isoprenoid biosynthesis in mycorrhizal roots: Involvement of a second 1-deoxy-d-xylulose 5-phosphate synthase (DXS2)

12:00 – 12:15 **M. Spiering**:
Biochemical pathway studies on the loline alkaloids of the meadow fescue endophyte, *Neotyphodium uncinatum*

12:15 – 13:45 Lunch Break

Wednesday, August 7

Chemistry Department Hall B

Student Travel Awardees Presentation

Chairperson: Murray B. Isman

15:45 – 16:00 **P. Laurent**:
Biosynthetic studies of ladybird defensive alkaloids

16:00 – 16:15 **J. Allison**:
Kairomonal responses by woodborers to bark beetle pheromone blends and differential activity of individual components

16:15 – 16:30 **K. S. Han:**
Mechanism of sexual isolation between the two species of *Adoxophyes orana* and *A.* sp. in Korea

16:30 – 16:45 **A. Groot**:
Evolution of sexual communication in two closely related moth species

16:45 – 17:00 **T. Katzav-Gosansky**:
Dufour's glands secretion – A new honeybee queen signal

17:00 – 17:15 **A. Luxova**:
Studies on biosynthetic pathways of male's marking pheromone of bumblebee

17:15 – 17:30 **M. Stranden**:
The biological role of the plant volatile (-)-germacrene D in Heliothine moths: Electrophysiology and behaviour

17:45– 18:30 **Business Meeting**

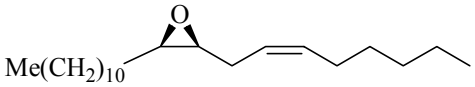
19:30 **Farewell Party / Banquet**

RECENT RESULTS IN PHEROMONE SYNTHESIS

Kenji Mori*

Fuji Flavor Co. Ltd., Insect Pheromone and Traps Division, Midorigaoka 3-5-8, Hamura-City, Tokyo 205-8503, Japan
email: kjk-mori@arion.ocn.ne.jp

(1) Synthesis of pheromone epoxides such as (6*Z*,9*S*,10*R*)-(+)-*cis*-9,10-epoxy-6-henicosene (**1**, a pheromone component of *Tiea anartoides*) and (3*Z*,6*R*,7*S*,9*R*,10*S*)-(–)-*cis*-6,7-*cis*-9,10-diepoxy-3-henicosene (leucomalure, **2**, pheromone of *Leucoma salicis*) was achieved by employing enzymatic resolution of epoxy alcohol **3** as the key-step.
(2) Synthesis of five candidates for the female pheromone of the screwworm fly (*Cochliomyia hominivorax*) was accomplished to give **4**–**8** as racemic and diastereomeric mixtures. Their bioassay by Dr. David A. Carlson (USDA) revealed **4** and **8** to be the pheromone. Acetate **4** was more bioactive than ketone **8**.



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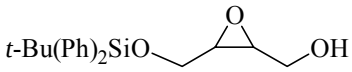

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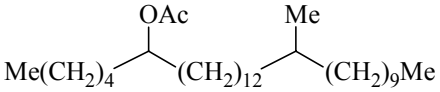

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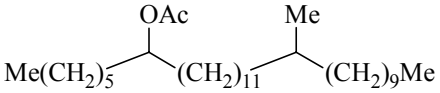

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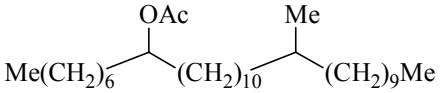

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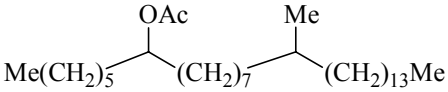

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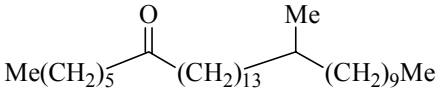

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SUITES OF NOVEL HYDROCARBONS FROM MELOLONTHINE SUGAR CANE SCARABS: STRUCTURE, SYNTHESIS AND STEREOCHEMISTRY.

William Kitching*

Department of Chemistry, The University of Queensland, Brisbane, 4072, Australia
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Suites of hydrocarbons, previously unknown as molecular classes from insects, have been characterised from several genera of Australian scarab beetles (sub-family, Melolonthinae). A suite of allenes are represented by the formula $CH_3(CH_2)_n$-CH=C=CH-$(CH_2)_7$-CH_3 with n = 11-15, 17 and 19 and all have $\Delta^{9,10}$-unsaturation. These structures have been confirmed by synthesis and spectroscopic and chromatographic comparisons. In the cases of $\Delta^{9,10}$-tricosadiene (n = 11) and $\Delta^{9,10}$-pentacosadiene (n = 13) predominating (R)-chirality (> 85% ee) was demonstrated by enantioselective gas chromatographic comparisons with separately synthesised non-racemic allenes of known chirality [1]. A more limited suite of two highly methyl branched cuticular hydrocarbons containing 28 and 27 carbon atoms has also been identified, largely by applications of high-field nmr spectroscopy and partial syntheses. These are 4,6,8,10,16,18-hexamethyldocosane and 4,6,8,10,16-pentamethyldocosane. The likely relative stereochemistry of these compounds will be assessed [2].

[1] Fletcher, M.T.; McGrath, M.J.; König, W.A.; Moore, C.J.; Cribb, B.W.; Allsopp, P.; Kitching, W. *Chem Commun*., 2001, 885-886.
[2] Fletcher, M.T.; Gallagher, P.O.; Chow, S.; Moore, C.J.; Cribb, B.W.; Allsopp, P.; Kitching, W. (Unpublished Results).

IDENTIFICATION AND SYNTHESIS OF CONSTITUENTS OF THE SEMIOCHEMICAL SECRETIONS OF SOUTH AFRICAN ANTELOPE SPECIES

Barend V. Burger

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Some of the South African antelope species have as many as five glandular structures known to produce semiochemical exudates. There are several theories concerning the purpose of some of the glandular secretions of these antelopes. Perhaps the most popular one is that secretions deposited on objects or on the ground are used for territorial marking, the object of which is supposed to be that the animal defines a territory which will be defended against intrusion by conspecific intruders. Although this theory is confirmed by the observation of the territorial behaviour of many small antelope, it does not seem to be tenable in other cases. For example, it seems more likely that the secretion produced by the interdigital or foot gland of the bontebok, *Damaliscus dorcas dorcas*, serves to provide conspecific animals with a sense of security or assurance that they are in familiar territory. On the other hand, there does not seem to be an acceptable theory concerning the semiochemical function of the dorsal secretion of the springbok, *Antidorcas marsupialis*. It is even possible that this secretion is not used for semiochemical communication at all, but rather that, in a certain sense, it serves as a defence mechanism against blood-sucking ticks.
Several hundred constituents of the exocrine secretions of a large number of South African antelope species have been identified over the past thirty years. The identification and synthesis of some of the more interesting compounds found in these secretions and the problems experienced in this type of research will be discussed.

SOME PAINLESS CHEMISTRY FROM STINGLES BEES

David Morgan

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The stingless bees are an ancient group of social hymenopterans that have received relatively little attention from chemical ecologists. Their organization, behaviour and chemistry varies considerably among the 50 genera and make interesting contrasts with those of honeybees and bumblebees.

The eggs of the stingless bee *Melipona bicolor* float upright on their larval food. The upper part of the egg has been shown to be covered with a monolayer of hydrocarbons and fatty acids that renders that part water repellant, while the bottom part is wettable

Virgin queens of *M. bicolor* have been found to produce wax from intersegmental abdominal glands like workers. The pure wax of a stingless bee has been analysed for the first time. The wax produced by quens and workersis very similar. It consists of alkenes, alkanes and acetyl and isobutyl esters, quite different from its cuticular hydrocarbons and from honeybee wax.

Foraging bees collect plant resin on their hind tibia for transport to the nest. They mix the resin with the relatively soft wax to build nest structures. Resin consists of mono-, sesqui-, di- and tri-terpenes. The compounds are characteristic of the preferred plants of the species. Collection times vary with the species.

Electroantennographic studies have shown that the workers of two species of *Frieseomelitta* respond to substances from their mandibular glands and the plant resin. Cuticular hydrocarbon mixtures in these species are surprisingly simple mixtures.

STEREOCHEMICAL ANALYSIS OF NATURAL VOLATILES BY ENANTIOSELECTIVE GAS CHROMATOGRAPHY

Wilfried A. König

Institut für Organische Chemie, Universität Hamburg, 20146 Hamburg, Germany

In current applications of enantioselective gas chromatography (GC) in the stereochemical analysis of natural compounds cyclodextrin (CD) derivatives are predominantly employed. These macrocyclic, "highly chiral" structures exhibit several advantages against earlier chiral stationary phases by their great substrate versatility, based on unique "host-guest" interactions (compounds with only one carbon atom to compounds with up to 28 carbon atoms have been resolved), facile accesibility and high thermal stability. The chemistry for selective substitution of the 3 different hydroxyl groups of the glucose units of the CDs was optimized and many different CD derivatives have been evaluated. It was observed that by regioselective alkylation or acylation the geometry and shape selectivity of a CD derivative may be modulated.
Since capillary GC is one of the most efficient separation methods the enantiomeric composition and the absolute configuration of a specific constituent of a complex natural mixture can usually be determined without tedious isolation, provided that the order of elution of the enantiomers is known. In difficult cases two-dimensional GC can be applied to improve the validity and accuracy of the result. To establish the order of elution enantioselective synthesis of a reference compound or its isolation from a natural source is sometimes inevitable, however, in other cases micro-preparative separation (mg-scale) of the enantiomers of a synthetic racemate in conjunction with polarimetry may be the method of choice. The absolute configuration of a volatile constituent may also be determined by chemical correlation with reference compounds of known configuration. This technique has successfully been employed in structure elucidations of terpenoid compounds. Hydrogenations, dehydrations, oxidations and rearrangement reactions may be carried out on a microgram scale for such correlations.

CLASSIFICATION OF TERPENOID BIOSYNTHESIS ACCORDING TO THE MEP- OR MVA-PATHWAY BY ISOTOPE RATIO MASS SPECTROSCOPY

Wilhelm Boland[1*], Andreas Jux[1], Gerd Gleixner[2]

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Plant volatile blends generally comprise terpenoids, aromatic compounds and fatty acid derived degradation- and transformation products. Mono- and diterpenoids are produced in the plastids from isopentenyl diphosphate (IDP) along the methylerythritol phosphate pathway (MEP-pathway), while sesquiterpenoids are assembled from IDP *via* the well-known mevalonic acid pathway (MVA-Pathway) in the cytosol. This classical allocation of pathways is not completely strict as shown by incorporation of precusors from the MEP-pathway into sequiterpenoids. On the other hand, administration of precursors can affect the natural balance of the cellular intermediates resulting in shifted mass fluxes through the complex interacting pathways. An alternative to precursor-based approaches is provided by analysis of the isotope ratio of relevant compounds, at natural abundance, using isotope ratio mass spectrometry (IR-MS). By linking a gas chromatograph, with on-line combustion of the eluting compounds to CO_2 und H_2O to an isotope ratio mass spectrometer (GC-C-IRMS), analysis of the isotopic signature of individual compounds of complex mixtures is possible. In the biosphere these signatures are related to the isotope effects of the reactions catalysed by enzymes involved in the physiological processes. Here we report that the different groups of terpenoid volatiles exhibit significant differences in their $^{12}C/^{13}C$ ratio, depending whether the universal building block IDP is produced predominantly from mevalonic acid or along the novel MEP pathway. Evidence will be given, that different types of elicitors (e.g. herbivory or fungal elicitors) may selectively induce either the MVA- or the MEP-pathway for production of certain terpenoids.

A. Jux, G. Gleixner & W. Boland. *Angew. Chem. Int. Ed.,* **2001,** *40*, 2091-2093.

NOVEL COMPOUNDS FROM THE CUTICLE OF *PIERIS* BUTTERFLIES SHOWING CHEMICAL DIMORPHISM

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It is well known that the outer insect cuticle is covered by a thin layer of lipids, which consists predominantely of hydrocarbons. We have investigated the composition of these lipids in the two pierids *Pieris brassicae* and *Pieris rapae* by GC-MS, using solvent extraction and SPME as sampling methods. Surprisingly, a strong difference between the composition of the lipids on the antennae and on other body parts could be observed, while differences between the sexes were minimal. Furthermore, careful analysis of the samples showed the presence of several long chain unknown components, which were identified by analysis of mass spectra, chemical derivatization, and syntheses of reference compounds. Some of these compounds were not known previously from insects. Thus, the unusual terminal conjugated diene 1,3-pentacosadiene was identified besides several oxygenated compounds. While monoalcohols have been identified previously from other species, several long chain alkanediols were also present. Furthermore, two types of ethers occur on the cuticle of the pierids, methyl ethers and 2,5-dialkyltetrahydrofurans (THFs). Methyl ethers have so far only been identified from spiders, but THFs are known from several lepidopteran species [1]. Most of the pierid THFs contain a unique, very short side chain, in contrast to other known compounds of this class.
Some of the identified compounds exhibit stereocenters, e. g. the diols and the THFs. The absolute configuration of these compounds is difficult to establish, because attempts to separate these rather large compounds on chiral GC phases failed. Therefore we have developed a method of oxidative degradation, which allowed determination of the absolute configuration, and also gets insight in the reaction of these compounds with molecular oxygene. A method has also been developed for the configuration determination of 1,3- and 1,4-diols, using chiral derivatization reagents. The results will be presented for the determination of the absolute configuration of 4,6-nonadecanediol, a constituent of sunflower pollen lipids.

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INTERNATIONAL SOCIETY OF CHEMICAL ECOLOGY MEDAL LECTURE

TO ATTACK OR NOT TO ATTACK? LIFE AS A CHEMICAL ECOLOGIST, OR HOW PESTS AVOID PLANTS AND ANIMALS THAT ARE UNSUITABLE AS HOSTS

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My membership of this Society continues to bring not only tremendous scientific fulfilment, through being part of an organisation devoted to furthering the standing and impact of chemical ecology, but also the enjoyment of belonging to such a friendly and vibrant scientific community. It is, therefore, with very great pleasure that I accept the medal of the Society, and with humility that I take my place on the list of distinguished recipients, many of whom have played a large part in my own scientific development. Indeed, I could easily spend the entire period allotted for the medal lecture in describing how all of these, and many other members of the Society, have contributed to my career and to my scientific progress. I will not do this since we are essentially a society of practical science, but I would not want, for one moment, to give the impression that my personal contribution to the activities of the Society, and to its science, would have been possible without learning from, and interacting with, others. I hope, however, that all those individuals who have impacted on my work will excuse me if I pay a specific tribute to the tremendous influence of, and scientific debt to whom I owe, my long-suffering friend and colleague Lester J. Wadhams, and our long standing association formed in the early 1980s. Without Lester, I would not be here, or, indeed, anywhere near where I am today.

Over the years, we have published a large number of papers in the Journal, many on pheromonal studies conducted collaboratively with eminent members of the Society. However, in order not to test, too much, the endurance of the attendees of this meeting, I will restrict myself to studies involving interactions between insects and their hosts, this being a subject which I am sure will be a major vehicle in demonstrating potential and real impact of Chemical Ecology, into the long term future and appropriately integrated with the rapidly developing technologies of molecular biology.

Contrary to views extant when we first came to the subject of Chemical Ecology, olfactory interactions relating to the location of plant and animal hosts now appear to be based principally on responses of highly specific olfactory neurons, as opposed to generalist molecular receptor systems. This has underpinned the demonstration that host location relies upon the detection of specific compounds, or mixtures thereof, representative of suitable host taxa or physiological states [1]. We also see that morphological features of the peripheral olfactory neural system may allow the organism to make an accurate determination of the relative proportions within mixtures of semiochemicals [2]. Furthermore, it is now evidenced, with a number of insect Orders, that the avoidance of unsuitable hosts, again either by taxa or physiological state, also involves recognition of compounds associated with specific olfactory neurons [3]. In addition to taxonomic differences that determine host suitability, the issue of stress causing increased release or induction of novel semiochemicals

is now seen to play a prominent role [4] and has, of course, been pioneered by a number of groups represented in the Society [5]. Understanding the mechanisms by which stress signals can impact on plants at the molecular level will also contribute to practical exploitation, and to elucidating the nature of plant/plant stress interactions and the unequivocal definition of phytopheromones. Studies on such interactions extend from effects of damaged plants on intact neighbours to signalling by intact plants with particular taxonomic, or even varietal, characteristics, aerially or through the rhizosphere [6], and where the damage is caused by pathogen development as well as herbivore feeding (personal communication, Olu Latunde-Dada).

With these advances, and the associated identification of host and non-host semiochemicals, has come the greater prospect of exploiting semiochemically mediated interactions in crop protection. Thus, push-pull or stimulo-deterrent diversionary strategies can be employed, in which colonisation of hosts is reduced by deployment of non-host semiochemicals, whilst at the same time pests are aggregated onto trap plants upon which attractant semiochemical release is maximised. Such push-pull strategies can be managed by use of slow release semiochemical formulations, and by the more economic approach of using plant stress related signals such as jasmonates and *cis*-jasmone [7]. Considerable success has recently been achieved in subsistence agriculture in Africa by use of living plants producing, directly, the push-pull effects to control colonisation by insect pests [8]. Although this is suitable for low-input agricultural systems, by understanding the underpinning neurophysiology and associated chemistry, the approach will be made sufficiently robust and sustainable for widespread development. The lessons learned from these practical developments show the potential of such approaches for high input agriculture, but with the delivery of push-pull effects through use of phytopheromones or mixed cultivar seeding regimes. We are set to make great headway in the protection of human and other animal hosts against pathogen vectoring arthropods, where we can already see the same principles applying as with insect/plant interactions [9].

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SEMI-SYNTHETIC PREPARATION OF SEX PHEROMONES FROM PLANTS TRANSFORMED WITH AN INSECT DESATURASE

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Sex pheromones of moths, fatty acid (FA) derived compounds, are produced by females to attract males. The pheromone biosyntheses involve desaturation by specific desaturases, chain shortening and/or elongation, and functional groups formation. Here we describe the first construction of a genetically modified plant (GMP) with the ability to produce moth sex pheromone precursors.
Plasmid pBI-DESTn bearing Pdest-Tn Δ^{11}Z encoding cabbage looper moth (*Trichoplusia ni*, Hübner, Lepidoptera, Noctuidae) acyl-CoA Δ^{11}-(Z)-desaturase was electroporated into *Agrobacterium tumefaciens* cells used for transformation of the *Nicotiana tabacum*. The transformants selected on the basis of kanamycin resistance were replanted on rooting-supporting medium (with kanamycin). Viable plantlets were examined for fatty acids content (in the form of corresponding fatty acid methyl esters -FAME- prepared by methanolysis) using gas chromatography and spectral methods. A comparison of FAME profiles of transformants (n=50) with those of control *N. tabacum* showed a substantial presence of the potential pheromone precursor, methyl (11Z)-hexadec-11-enoate (**11Z-16:Me**), in the case of several transformants (n=6). 11Z-16:Me is virtually absent in parental *N. tabacum*.
The 11Z-16:Me was isolated in bulk from a greenhouse cultivated transgenic tobacco and the corresponding acetate, alcohol and aldehyde, which are principal components of large number of sex pheromones, were semi-synthetically prepared from the *in planta* produced pheromone precursor. In several cases the attractiveness of the prepared blends was proved in field trials.
The possibility that the transgenic plant will spontaneously produce sex pheromone components was tested and the potential use of such plants in an integrated pest management will be discussed.

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LACEWING CHEMICAL ECOLOGY – A PUSH AND A PULL?

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The enantiomerically pure diastereoisomers (1*R*,4*S*,4a*R*,7*S*,7a*R*)- (**1**) and (1*R*,4*R*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**2**) were synthesised diastereoselectively from a renewable resource, (4a*S*,7*S*,7a*R*)-nepetalactone, isolated as the main constituent of the essential oil of the catmint plant *Nepeta cataria*.. The stereochemistry of the compounds was determined by NMR spectroscopy and X-ray crystallographic means and they were identified respectively as neomatatabiol and isoneomatatabiol, natural products from *Actinidia polygama* for which the lactol stereochemistry was previously incompletely defined. **1** was found to catch significant numbers of three species of lacewing in the field, in Korea, *Chrysopa cognata* and in the U.K., *Nineta vittata* and most notably *Peyerimhoffina gracilis*.. The catch of *P. gracilis* with **1** is of particular interest as this lacewing has only recently been recorded in the UK. Where sexed, the lacewings of all species trapped were found to be male, implying a possible pheromonal role for these or structurally related compounds [1]. Air entrainment of male *P. gracilis* captured in the field revealed the presence of a structurally related iridoid and a tridecene. The tridecene was structurally elucidated as (*Z*)-4-tridecene by GC-MS of microchemical reaction products and by Microprobe-NMR. Stereoselective synthesis, using alkyl borane chemistry, gave verification of the structure and as this tridecene was previously reported from *C. carnea* [2], a possible biological role is investigated. In addition, isotopic labelling experiments using synthetic polydeuterated iridoids have revealed the lacewing as a suitable system for examining the biochemical relationship between attractant **1** and naturally produced compounds.

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SEX ATTRACTANTS FOR LEPIDOPTEROUS SPECIES: PURE ENANTIOMERS OR RACEMATES OF EPOXYDIENES

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In the case of a few geometrid species it has been reported that females enantioselectively produce epoxypolyenes as their essential pheromone components. Conspecific males are attracted only towards that specific enantiomer and are inhibited by the opposite enantiomer, which in turn acts as an isolation mechanism between taxonomically closely related, sympatric species [1, 2].
In order to investigate whether enantioselective pheromonal channels based on some epoxydienes are unique or widespread among Lepidoptera, we synthesized the (S,R)- and (R,S)-enantiomers of 3,4-, 6,7- and 9,10- epoxides of (Z3,Z6,Z9)-heptadecatriene, (Z3,Z6,Z9)-nonadecatriene, and (Z3,Z6,Z9)-heneicosatriene, respectively, and tested them *per se*, or in 1:1 mixtures (racemic) in field bioassays conducted at various natural biotops in Hungary. Enantiomers of Z9-6,7-epoxynonadecene and Z6-9,10-epoxynonadecene were also included in the tests.
Eight geometrid and two hypaenid (Noctuidae) species were trapped in significant numbers. Five species were attracted selectively only towards a specific enantiomer but not to the racemic mixture. Two further species were also attracted towards a specific enantiomer, however, this enantiomer retained its attractivity also in racemic mixture. Three species were attracted to racemic mixtures, and not to any of the composing enantiomers *per se*.
Sex attractants were established for *Semiothisa notata* L. (Geometridae, Ennominae) as Z3Z9-6S,7R-epo-17Hy (racemate also active), for *Ennomus quercinaria* Hbn. (Geometridae, Ennominae) as racemic Z6-9,10-epo-19Hy, for *Horisme tersata* Den. et Schiff. (Geometridae, Larentiinae) as Z3Z9-6R,7S-19Hy; for *Hypaena tarsicrinalis* Knoch (Noctuidae, Hypaeninae) as Z3Z6-6S,7R-epo-19Hy, for *Plagodis pulveraria* L. (Geometridae, Ennominae) as racemic Z3Z9-6,7-epo-19Hy, for Asthena albulata Hfn. (Geometridae, Larentiinae) as Z3Z6-9S,10R-19Hy, for *Minoa murinata* Scop (Geometridae, Sterrhinae) as Z3Z6-9S,10R-epo-19Hy (racemate is also active), for *Xanthorhoe fluctuata* L. (Geometridae, Larentiinae) as Z3Z9-6S,7R-epo-21Hy, for *Horisme rostralis* L. (Lepidoptera, Hypaeninae) as Z3Z6-9R,10S-epo-21Hy, and for *Eupithecia vulgata* Haw. (Lepidoptera, Larentiinae) as racemic Z3Z6-9,10-epo-21Hy, respectively.
These results show that males of geometrid and hypaenid species can discriminate between enantiomers of epoxypolyenes and are attracted only to either a specific enantiomer, or need both enantiomers for attraction.

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THE CHEMICAL ECOLOGY OF THE EASTERN LARCH BEETLE, *DENDROCTONUS SIMPLEX* IN MINNESOTA

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The eastern larch beetle, *Dendroctonus simplex* LeConte (Coleoptera: Scolytidae), is a major pest of tamarack, *Larix laricina* (Du Roi) K. Koch, a North American deciduous conifer (Langor and Raske, 1989). This insect colonizes the phloem of both standing and fallen *L. laricina,* and aggregations can result in tree mortality. The insect and its host occur throughout the boreal forests of North America and we investigated the chemical ecology of *D. simplex* in Minnesota, near the southernmost extent of its range.

Semiochemical extracts of male and female *D. simplex* were prepared from three treatment groups of newly emerged insects (unfed, fed 48 hr on *L. laricina* phloem, treated with JHIII in acetone). Gas chromatographic-mass spectrometric(GC-MS) analyses of Porapak and abdominal extracts revealed that females produced frontalin, seudenol, and seudenone. Feeding on phloem appeared to stimulate production of all compounds. Females and males treated with JHIII and injected with ^{14}C-acetate resulted in the female-specific incorporation of ^{14}C into frontalin, demonstrating *de novo* synthesis of frontalin by this species.

Gas chromatographic-electroantennographic detection (GC-EAD) analyses demonstrated that both sexes responded to naturally occurring and synthetic frontalin, seudenol, and seudenone. Field studies in three widely separated stands of *L. laricina* revealed that racemic seudenol was highly attractive in combination with alpha-pinene, but the addition of naturally occurring (–)-frontalin to this two-component mixture significantly reduced the trap catch. Seudenone has not yet been tested in the field. Responses in this experiment were highly biased to males (3:1), suggesting that a male-produced pheromone component may await discovery. Moribund *L. laricina* colonized by *D. simplex* are also colonized by many other subcortical insects, and in our field studies we also trapped 8 spp. of Scolytidae, 10 spp. of Cerambycidae, 5 spp. of Buprestidae, 3 spp. of Siricidae, and 5 spp. of Cleridae. These studies revealed significant kairomonal responses by two species of checkered beetles (*Thanasimus dubius* and *T. undatulus,* Cleridae) and one species of roundheaded woodborer (*Phymatodes dimidiatus,* Cerambycidae) to the *D. simplex* pheromone components frontalin and seudenol, respectively.

PHEROMONE ECOLOGY OF THE POTATO APHID, *MACROSIPHUM EUPHORBIAE* UNDER LABORATORY AND FIELD CONDITIONS.

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Sex pheromones have considerable potential in management programmes of many insect pests but for their most effective use we require a solid understanding of the pheromone biology of the species we wish to control. As we are interested in the potential use of the potato aphid *Macrosiphum euphorbiae* sex pheromone (recently identified by the authors in collaboration with colleagues at the BBSRC Chemical Ecology group at Rothhamsted) we undertook a series of experiments under laboratory and field conditions to study female calling behaviour and the response of males to virgin calling females.
Females initiated calling at a younger age at 10°C than at 20°C, a similar pattern being observed between virgins placed in the field in early and late September. As they aged females called for significantly longer periods on successive days although this was more evident under controlled laboratory conditions than under fluctuating field conditions. In the wind tunnel wind speed up to 150 cm/sec had little effect on calling, and this also held true for prevailing wind conditions on clear days in the field. However, on rainy days females seldom called.
Males showed clear orientation when placed downwind of calling females in the wind tunnel but never exhibited upwind flight to the source. We therefore provided a „bridge“ which permitted males to walk to the source. Under these conditions males responded equally over distances from 40 to 100 cm but it was clear than wind speeds in excess of 120 cm/sec inhibited male responses. Under field conditions similar results were obtained although there was one major difference. In about 20% of the cases males actually flew to the source and upon closer examination of the data it was clear that they took flight when there was a lull in the wind. This „point and shoot“ strategy is not particularly surprising when one considers the limited ability of aphids to control flight in wind current and we believe that males will combine flight and walking behaviours to locate females.

SEMIOCHEMISTRY OF THE CONIFER FEEDING SAWFLY *ACANTHOLYDA ERYTHROCEPHALA* (L.) (HYMENOPTERA)

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The Pine False Web Worm (PFW) *Acantholyda erythrocephala,* is a serious pest defoliator of a variety of economically valuable *Pinus* species in the U.S. and Canada. Repeated annual defoliation by this insect can result in growth reduction and death of host trees; hence, there has been a long standing interest in developing alternative management strategies for monitoring and control of this insect. Chemical analysis of aerations and whole-body solvent washes obtained from adult PFW revealed two compounds specific to female sawflies. Conclusive identification for these compounds was obtained based on comparisons to mass spectra and gas chromatographic retention times of synthetic standards. Using coupled gas chromatographic-electroantennographic detection (GC-EAD), we found that one female specific compound was antennally active only in males. Additionally, a number of smaller aldehydes obtained from both sexes were found to elicit responses in male and female antenna. Subsequent field assays were conducted in Illinois and New York to determine if male sawflies were attracted various combinations of the two most abundant smaller aldehydes and the antennally active female specific compound. Results show that significantly larger numbers male sawflies were caught in traps baited with the female specific compound compared to traps containing the only smaller aldehydes, male or female solvent extracts, or blank controls. These results are the first to suggest that female pine false webworm produce a sex pheromone that functions as an attractant for males. Ongoing analysis is being conducted to determine if the smaller aldehydes common to both sexes play a role in ovipositional site selection or sex ratios of offspring.

DO GENERALIST PARASITOIDS USE SPECIFIC OR GENERAL CHEMICAL CUES FOR FORAGING?

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Although the use of chemical cues for host finding has been examined for many parasitoid species, there are almost no studies on the question how generalist parasitoids are able to find and recognize their various hosts. Therefore, the present study was initiated with the pteromalid wasp *Lariophagus distinguendus*, a parasitoid of larvae and pupae of at least 11 beetle species from 5 families that develop in seeds of different plants. We examined the hypothesis that general chemical cues released by all hosts are used by generalists for foraging [1]. This was done in bioassays on host finding and host recognition behaviour of naïve females of *L. distinguendus* combined with comparative chemical analyses.
Concerning host finding, it turned out that *L. distinguendus* females are attracted by volatiles from faeces of several host complexes. Chemical analyses of volatiles from larval faeces of the lesser grain borer *Rhyzoperta dominica* (F.) revealed the presence of dominicalure 1 and 2, the species specific aggregation pheromones of this species. A synthetic mixture of the pheromones was attractive to naïve *L. distinguendus*.
For host recognition, host faeces covering infested grains are used by *L. distinguendus* to recognize the presence of hosts. Chemical analysis revealed that faeces of several host complexes contain α-tocopherol, β-sitosterol, ergostenol, cholesterol, and β-tocopherol, β-tocotrienol, or γ-tocopherol and γ-tocotrienol. A mixture of these compounds stimulates host recognition behaviour in *L. distinguendus*.
Thus, in contrast to the initial hypothesis *L. distinguendus* innately uses specific chemical cues from at least one host for host finding and general chemical cues present in the faeces of several hosts for host recognition. It is discussed that *R. dominica* is the primary host of *L. distinguendus* and that the parasitoid has expanded its host range only later. This might have been enabled by the presence of general host recognition cues.

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DAILY PERIODICITY IN PRODUCTION OF PLANT VOLATILES AFFECTS DAILY RHYTHM OF HERBIVOROUS AND CARNIVOROUS INSECTS.

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A number of studies on the daily rhythm of insects have been carried out. Important exogenous factors that affect the daily rhythm of insects are light and temperature. Recently, it has been reported that the production of volatiles by plants also shows daily rhythm [1, 2, 3], suggesting that such a plant rhythm may also affect the daily rhythm of organisms on the plant. In this study, we investigated the effects of daily periodicity in the production of volatiles by corn plants on the daily rhythm of common armyworms (*Mythimna separata*) and their parasitoids (*Cotesia kariyai*).
M. separata larvae are nocturnal insects [4]. This daily periodicity was, however, not observed when reared with an artificial diet. We introduced volatiles emitted from corn plants under light conditions or those emitted from corn plants under dark conditions to the rearing area of *M. separata* larvae that was under light or dark conditions. Irrespective of the light-dark condition of the rearing area, the larvae infested the artificial diet only when volatiles emitted from the plants under dark conditions were present. These data suggest that the daily rhythm of *M. separata* larvae is affected by host-plant volatiles.
By contrast, the daily rhythm of *C. kariyai*, a parasitoid of *M. separata* larvae, is affected by light [4]. We discuss why this parasitoid searches for its host larvae in the daytime by focusing on the daily periodicity of volatile production by plants.

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NEEDLE MONOTERPENE CONTENTS OF ELEVEN PINE SPECIES AND RELATIVE SUSCEPTIBILITY TO THE ATTACK OF *THAUMETOPOEA PITYOCAMPA* (DEN. & SCHIFF.)

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Thaumetopoea pityocampa the winter pine processionary moth is the most destructive pine defoliator in the Mediterranean region, the caterpillars of which frequently cause economic damage to stands of both indigenous and exotic *Pinus* species.
However, the relative susceptibility of host trees varies widely geographically, in relation to different pine species and even within monospecific stands. Resistance/susceptibility might be due either/or to the presence of certain substances in the resin of the pines, or to the emission by pine needles and branches of some components of the essential oil volatiles, which might be responsible for the primary attraction of the females, that is the selection of a host tree for oviposition.
In our study the relative susceptibility to the attack by the processionary moth of eleven species, aged between 6 and 10 years and located in an experimental plot in central Portugal was studied. The contents of the pine needles volatile monoterpenes and their enantiomeric ratios were determined by high-resolution gas chromatography (HRGC) and mass spectrometry (GC-MS) after headspace solid phase microextraction (HS-SPME).
First results concerning the racemic monoterpene composition of the species studied point to the existence of a strong correlation between the level of attack by *T. pityocampa* and the ratio of β-pinene/limone contents found in the needles.
A similar study conducted in a *P. pinaster* stand, located in southern Portugal, detected a significant statistical relationship between the needle contents of limonene, β-pinene and the ratio of β-pinene/limonene and the number of larval nests by *T. pityocampa*.
Since chirality of pine monoterpenes is an important variable influencing host-plant /insect interactions, the chiral composition of the volatiles analysed for both stands was also studied. Results obtained for the multi-specific pine stand did not show a significant correlation between monoterpene chiral composition and the level of attack by *T. pityocampa*. However, for the monospecific *P. pinaster* stand, using a principal component analysis, a correlation was found between the enantiomeric ratios of α-pinene, β-pinene and limonene and the level of attack by the processionary moth. Additionally it was found that other factors, namely the distribution of a predatory voracious ant, could interfere with the process of host-tree colonization within a pine stand.

HOST-TREE FINDING IN A BARK BEETLE BY AVOIDANCE OF NONHOSTS

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The bark beetle, *Pitoygenes bidentatus*, locates host pine branches, *Pinus sylvestris*, usually by orienting to pheromone components, *cis*-verbenol and grandisol, produced by feeding males. Attraction to fresh hosts by "pioneers" has so far not been observed, although aged and diseased hosts may release attractive volatiles. Paired cylinders above funnel traps were fixed 6 m apart and rotated slowly by a gear motor at 2 rph. Both clear plastic cylinders were baited with pheromone components, but one cylinder enclosed a fine wire screen cage containing nonhost materials while the other had only an empty wire cage. The rotation of traps in all positions tends to even out the normal catch variation so fewer tests can be used with more powerful parametric statistics. The attraction of the small beetle (2 mm long) to pheromone components was significantly reduced by volatiles from a surprising number of nonhost trees and shrubs now common, or historically common, in the habitat. Plant materials of Norway spruce, birch, oak, blueberry, mountain ash, and grass produced odors that reduced responses of beetles even to attractive pheromone. Odors from several of the plant materials were collected on Porapak Q and analyzed by GCMS. Several monoterpenes and sesquiterpenes were found in the odors and some of these compounds, and ethanol, were found to repel the bark beetle during the final 1-2 meters of approach to pheromone components. These results show that much more of the olfactory landscape is perceived by bark beetles than originally envisioned. The extent and importance of this complex behavioral repertoire and nonhost avoidance is not fully elucidated but probably aids the beetle in finding appropriate host substrates and thus reduces risks during dispersal and host location.

ELETROPHYSIOLOGICAL RESPONSES OF FEMALE AND MALE *HYPSIPYLA GRANDELLA* TO *SWIETENIA MACROPHYLLA* ESSENTIAL OIL

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The mahogany shoot borers, *Hypsipyla grandella* (Zeller, 1848) (Lepidoptera: Pyralidae), are one of the most economically important Neotropical forest pest insects. It attacks precious wood from plants of the Meliaceae, as *Swietenia macrophylla* (Kim), which present fast growth, adaptability, good form and high value. However, efforts to establish large-scale homogenous plantations of this native Meliaceae have almost invariably failed due to larval attacks by the mahogany shoot borers. The damage consists of death of the young terminal shoots, considerably retarding growth, and results in the formation of numerous secondary shoots causing twisted and unthrifty trees.
Various strategies and considerable research effort for control of this pest have been developed but no practical method of control currently exists. In plantations, only silvicultural measures have been employed.
The aim of the present work was to determine selectivity and sensitivity of antennal receptors of both sexes of *H. grandella* to the essential oil of the terminal shoots, mature and senescent leaves of the *S. macrophylla* as well as to identify the compounds present in the essential oil. Comparative studies between the tree oils type were done to determine similarities in olfactory receptors by electroantennogram (EAG) technique.
The pupae of *H. grandella* used in the experiments originated from the Laboratory of Entomology of Faculdade de Ciências Agrárias do Pará, Belém - PA, Brazil. The insects were obtained from laboratory rearing, where they were maintained on mahogany foliage. The rearing of the *H. grandella* pupae was established in the Insect Bioassay Laboratory of Universidade Federal de São Carlos, Brazil. They were sexed at the pupal stage and for emergence of adults the pupae were placed individually in plastic vials. Male and female pupae were maintained in an incubation chamber under a light cycle of L12:D12 h; 60 ± 5% r.h. and 25 ± 1 °C. The antennae of 1-2 days old *H. grandella* males and females were used for the electroantennographic experiments (EAG). The antennal responses were amplified and recorded with a data acquisition controller and software EAG (Syntech).
The essential oils were tested using 10 antennae of female and male *H. grandella*. The mean normalized responses of the different oils (5 μL in hexane, on filter paper) were submitted to ANOVA for statistical analysis and compared using the Tukey test ($P < 0.05$).
The essential oils were extracted by steam distillation and their constitutions were determined by GC/MS.
The data of the EAG response dose of the mahogany essential oils of the terminal shoots, mature and senescent leaves, in three concentrations, revealed that females and males of *H. grandella* elicited a greater antennal response than those of control stimuli. Comparing the EAG responses between females and males antennae, for each essential oil, we verify that responses were significantly higher only with respect to the shoot essential oil, where the females elicited higher antennal response. These are similar to the results observed in the EAG experiments with the essential oils from *Toona ciliata* and *Cedrela odorata*.
Constitution of the essential oils were predominantly of sesquiterpenes, with γ-Muurolene, D-Germacrene, E and Z-methyl communate present in all of them and predominance of D-Germacrene.

CHEMICAL ORIENTATION IN COCKCHAFERS

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Cockchafers of the genus *Melolontha* cause severe economic losses in forestry, agriculture, and horticulture in several parts of Central Europe. The two most important species are the forest cockchafer, *Melolontha hippocastani* Fabr. and the European cockchafer, *M. melolontha* L. sharing a very similar biology. At dusk, males of both species perform a spectacular swarming flight for mate location. The males hover around the treetops of infested host trees and alight when reaching the vicinity of females that stay on the host trees and continue to feed. Our results demonstrate that males of both species use olfactory cues for mate location. By orientation towards damage-induced plant volatiles (green leaf alcohols), males locate feeding females. In field experiments *M. hippocastani* selectively responded towards (*Z*)-3-hexen-1-ol [1], whereas males of *M. melolontha* were additionally attracted by the other naturally occurring leaf alcohols (*E*)-2-hexen-1-ol and 1-hexanol [2]. In both species, beetle-derived quinones act as pheromones significantly enhancing the male response towards the green leaf alcohols and thus allow for discrimination between leaf damage caused by feeding females and unspecific leaf damage. The active pheromone compound identified in *M. hippocastani* is 1,4-benzoquinone [3], whereas males of *M. melolontha* use the methyl derivative toluquinone [4]. Males of both species additionally respond to phenol that was identified in female extracts from both *M. hippocastani* and *M. melolontha* [5].

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REPRODUCTIVE CUES OF GENERALIST AND SPECIALIST APHIDS

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The performance or reproductive output of phytophagous insects is often considered to be determined by the ability of the insect to ingest and assimilate plant nutritional components. Here we demonstrate that preliminary reproductive decisions of generalist and specialist morphs of the black bean aphid, *Aphis fabae*, are in fact made in response to factors located outside the nutritional tissue of the plant (the phloem sieve elements). Using electrical penetration graphs (EPG) and video monitoring we show that 1) The time to first parturition is shorter than the time to first registered phloem access and first phloem ingestion in the winged generalist and specialist morphs 2) A high proportion of individuals (45-70%) of both generalist and specialist morphs display first parturition without showing the EPG waveform indicative of phloem access (E1) and 3). A reduced time to first parturition and greater reproductive output over 24 h is displayed by, respectively, the winged generalist and specialist morphs on a sucrose solution when pre-treated with brief plant access. The results indicate that reproductive cues are detected in the peripheral plant tissues: the epidermis and/or mesophyll. We further present the results of artificial diet studies investigating the importance of plant nutritional components and secondary chemicals in the reproductive decisions made by the winged generalist and specialist morphs of this aphid.

LARVA EXPOSURE TO OVIPOSITION DETERRENTS ALTERS SUBSEQUENT OVIPOSITION BEHAVIOR IN GENERALIST AND SPECIALIST MOTHS

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A number of studies have demonstrated that feeding experience can change feeding preferences in a variety of phytophagous insects. The present study was undertaken to determine the effects of larval feeding experience on the subsequent oviposition behavior of the resulting moths. Larvae of the cabbage looper (*Trichoplusia ni*, Noctuidae) and the diamondback moth (*Plutella xylostella*, Plutellidae) were exposed to the phenylpropanoid allelochemical *trans*-anethole (at 100 ppm fwt in artificial diet) or the limonoid allelochemical toosendanin (10 ppm, sprayed to runoff on cabbage leaves). Both compounds had been shown to deter oviposition in naïve moths in previous choice tests. *Trans*-anethole is a volatile compound whereas toosendanin is not. Moths developing from "experienced" larvae (both sexes) were placed in oviposition cages containing one cabbage leaf sprayed with the respective oviposition deterrent and another sprayed with the carrier (methanol) alone. Our results consistently demonstrate that moths exposed to the deterrents as larvae are significantly less deterred than naïve moths. In the case of *trans*-anethole in *T. ni*, "experienced" moths laid significantly *more* eggs on the treated leaves than on the control. This phenomenon, analogous to habituation to feeding deterrents in lepidopteran larvae, resulted whether larvae were exposed to the oviposition deterrent throughout the larval stage, or during the final instar only.

OVIPOSITIONAL AND ELECTROPHYSIOLOGICAL RESPONSES OF THE DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA*) TO ISOTHIOCYANATES

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The diamondback moth, *Plutella xylostella*, a specialist on plants in the family Brassicaceae, is one of the most serious pests of cole crops on a worldwide basis. Recent work has shown that soaking of cabbage leaves in chloroform for 90 minutes provides an extract that serves as a potent oviposition stimulant for this insect. Analyses of chloroform extracts have led to the isolation and identification of two active compounds as sulforaphane and iberin (Renwick & Haribal, unpublished). These are isothiocyanates, likely products of myrosinase-catalyzed hydrolysis of glucosinolates that are present in all host plants of the insect.

Experiments were conducted to determine the extent to which other isothiocyanates might stimulate oviposition and to investigate the possible involvement of antennal receptors in the host recognition process. Behavioral tests confirmed earlier results that allylisothiocyanate has some activity as a stimulant. Other natural isothiocyanates and one synthetic representative were tested in bioassays to determine relative activity, using allylisothiocyanate as a standard for comparison. Those compounds with sulfur in the side chain (sulforaphane, iberin, iberverin) and a synthetic bicyclic ketoisothiocyanate proved to be highly active in behavioral assays. In contrast, short chain aliphatic isothiocyanates were relatively inactive.

Electrophysiological recordings (EAGs) from antennae of the moths showed a good response to the most behaviorally active representatives and a low response to inactive compounds. Thus a reasonable correlation between activity as oviposition stimulants and EAG responses appears to be emerging.

DEFENSE IN HERBIVORES OF GLUCOSINOLATE-CONTAINING PLANTS.

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Glucosinolates and their breakdown products are on the one hand an efficient defense against generalist herbivores and fungi, on the other hand they are used by specialists as important stimulants and attractants. The greyish-black larvae of the turnip sawfly *Athalia rosae* (Hymenoptera: Tenthredinidae) are able to sequester aliphatic and aromatic glucosinolates. These secondary metabolites are stored in the larval haemolymph which comes readily into contact with predators when larvae are attacked, because of a rather low integument resistance. While the ant *Myrmica rubra* was deterred by these haemolymph droplets, predatory bugs (*Podisus maculiventris*) fed on the sawfly larvae without harm. In the bugs, glucosinolates could be detected shortly after a meal, but disappeared after several hours. Thus, glucosinolates can be transferred even in the third trophic level but are not sequestered there. Larvae of the butterfly *Pieris rapae* are green and well camouflaged, while larvae of *P. brassicae* are gregarious and brightly coloured. The haemolymph of both larval species had no deterrent effect on *M. rubra*. However, in the caterpillars the integument is much stronger and haemolymph is not released as readily as in *A. rosae.* In both *Pieris* species no glucosinolates could be detected, although a sequestration of glucosinolates had been reported for these in a paper more than 25 years ago [1] which has been cited in many articles since. However, *P. brassicae* larvae regurgitate and this fluid was well deterrent against *M. rubra. P. maculiventris* was not disturbed by the regurgitate and fed on larvae of *P. brassicae.* In these bugs, no glucosinolates were detectable. The lizard *Anolis carolinensis*, used as a model „sit and wait“ predator, attacked larvae of *P. rapae* at highest and *A. rosae* at lowest frequency. While all attacked larvae of both *Pieris* species were eaten, one third of the *A. rosae* larvae were rejected after an attack which might be due to the glucosinolates.

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DEFENCE STRATEGY IN SAWFLY LARVAE BY INTEGUMENT AND HEMOLYMPH ADAPTATIONS AND HOST PLANT CHEMISTRY.

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A new type of defence strategy has been discovered in some sawfly species (Hymenoptera, Symphyta). Their larvae show an integument adaptation that we call “easy bleeding” and which is characterised by a low mechanical resistance of the integument. Moreover, hemolymph extracts from easy bleeders strongly act as a feeding deterrent for the ants *Myrmica rubra*. Both traits, integument resistance and hemolymph deterrence, were negatively correlated by considering 22 sawfly species. A similar negative correlation was also obtained by only taking into account the species of the sawfly tribe Phymatocerini (9 species studied). In this tribe, many species feed on toxic plants and several species are known to sequester the powerful bioactive compounds originating from the plant. Thus, we propose a “harmful hemolymph hypothesis” to explain the co-occurrence of the morphological and chemical traits, jointly acting as a chemical defence strategy, and we suspect hemolymph deterrence to be often due to sequestration of plant secondary metabolites.

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CHEMOSENSORY PROTEINS

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A feature common to olfaction in all land-dwelling animals is that odorant molecules can never directly contact the sensory neurons but have to pass an aqueous perineuronal compartment, e.g. the nasal mucus in vertebrates and the sensillar lymph in insects. Therefore, the very first step in the process of odor recognition in insects is the solubilization and transport of the hydrophobic odorants in the hydrophilic antennal lymph. In the last few years the landmark identification of the class of olfactory binding proteins (OBPs) from the moth antennae has enlarged the field of study with respect to the reception of odorant molecules [1]. The OBPs from moths are low molecular weight (14-15 kDa) and relatively acidic proteins, which are characterized by six conserved cysteines and a specific expression in the antennal lymph. Detection of contact chemical signals by insects is accomplished by chemosensory neurons housed also within lymphatic structures distributed mostly on the whole body. The finding that the contact chemosensory sensillae exhibit the common structure of olfactory sensillae leads to the concept that all different chemosensory modalities may be served by OBP-like proteins. A novel class of proteins, the ChemoSensory Proteins (CSPs), identified in a variety of different insect species may mediate the reception of chemical molecules in contact chemosensory sensillae [2].
The first members of the CSP family (called "OS-Ds") have been identified in *Drosophila melanogaster* and then in cockroaches [3,4]. The OS-Ds like other CSPs have physical properties similar to OBPs (size, acidity) but show a primary structure and tissue-distribution unlike olfactory proteins. The protein CSP exhibits only four cysteines and shows none of the hydrophobic domains specific to OBP. The CSPs are found on the whole body, mostly in the legs, in contrast to OBPs that are tissue-specifically expressed in the antennae. The time course expressions of CSP and OBP are also different. The CSP mRNAs are detectable two days not only before the OBP synthesis but also before the appearance of the olfactory structures. Overall, this demonstrates that CSP has no role in olfaction [5,6,7]. In an extension of the study of CSP, further experiments using reverse genetics, site-directed mutagenesis, immunocytochemistry, ligand binding assay and structure cristallography should allow testing of the specific role these proteins play in insect chemosensation [8].

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PHEROMONE BINDING PROTEINS CONTRIBUTE TO THE EXCITATION OF OLFACTORY RECEPTOR CELLS IN MOTHS.

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Pheromone binding proteins (PBPs) occur in mM concentrations in the extracellular sensillum lymph surrounding the sensitive processes of olfactory receptor cells in moths. They may solubilize and carry the odorants to the receptor cells, protect the odorants from enzymatic degradation, mediate the interaction between odorant and receptor molecule and, finally, deactivate the odorant. Biochemical studies[1] have shown, that the different PBPs coexpressed in the pheromone sensitive sensilla of the silkmoth *Antheraea polpyhemus* interact differently with various pheromone components. The X-ray structure of the PBP of *Bombyx mori*[2] suggests that the pheromone binds to an inner cavity of the 15 kD protein. According to NMR studies[3], it may be released due to negative charges at the receptor cell membrane. Recombinant PBPs of the silkmoths *A. polyphemus* and *B. mori* were expressed in *E. coli* and provided by J. Krieger (Univ. Stuttgart-Hohenheim). Receptor cells were stimulated *in situ* by superfusing with the pheromone-PBP complex in the various combinations of pheromones and PBPs. The responses of receptor cells depended on both the pheromone and the PBP. Pheromones artificially bound to particular PBPs elicited nerve impulses in receptor cell types which they do not activate under natural conditions. This suggests that the PBPs contribute to the activation of receptor molecules.

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SELECTIVITY OF PHEROMONE-BINDING PROTEINS IN THE GYPSY MOTH, *LYMANTRIA DISPAR*.

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The pheromone olfactory system of male gypsy moths (*L. dispar*) is able to discriminate the enantiomers of *cis* 7,8-epoxy-2-methyloctadecane (disparlure) [1] and of *cis* 7,8-aziridine-2-methyloctadecane [2]. Furthermore, (7Z) 2-methyloctadec-7-ene and (7Z) octadec-7-ene are behavioural antagonists in the gypsy moth [3]. The olfactory hairs specialize on the pheromone enantiomers: one type responds to *cis* (7R, 8S)-epoxy-2-methyloctadecane ((+) disparlure), while the other type specializes on the enantiomer ((-) disparlure) [4]. Both types of hairs respond weakly to the alkene [4]. The dendrite of the olfactory neurons project into the hollow sensory hair and are bathed by a concentrated solution of pheromone-binding protein (PBP) [5]. The gypsy moth has two PBPs: PBP1 and PBP2 [6,7]. The PBPs are thought to desorb the pheromone from the hair cuticle and transport the odorant to the receptors on the dendritic membrane. We have studied the selectivity of these PBPs towards the enantiomers of disparlure [8]. Using a new, faster binding assay we have evaluated more compounds, such as the aziridine enantiomers and various aza and oxa-substituted disparlure analogs. Binding data, including pH profiles [9] and homology modelling (against the *Bombyx mori* PBP crystal structure [10]) suggest that (-) disparlure and (7S,8R)-aziridine-2-methyloctadecane both could make a hydrogen bond to threonine 9 in PBP1. In PBP2, residue 9 is an alanine and there are no potential H-bonds to either enantiomer. This is consistent with our data, which indicates that PBP1 binds the (7S, 8R) enantiomers of the epoxide and aziridine more strongly than the (7R, 8S) enantiomers, while PBP2 is the reverse. PBP2 was consistently the less discerning of the two proteins. The PBPs not only recognize chirality, but they also recognize the presence of the 2-methyl group: (7R, 8S)-epoxyoctadecane and (7Z) octadec-7-ene are both bound more weakly than the 2-methyl branched homologues. If time permits, we shall discuss results from recent photocrosslinking experiments.

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ELEMENTS OF ODOR RECOGNITION IN MOTHS:
SENSORY NEURON MEMBRANE PROTEIN (SNMP) AND G PROTEIN

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In insects, seven transmembrane domain proteins coupled to G-protein-mediated second messenger cascade as olfactory receptors have been found to date only in *Drosophila* [1,2,3] and *Anopheles gambiae* [4]. Attempts to find similar receptor proteins in other insects have failed. Olfactory-specific proteins (SNMP for Sensory Neuron Membrane Protein) of two transmembrane domains uniquely expressed in olfactory neurons have been characterized in several moth species [5]. These proteins are homologous with the CD36 receptor family which predominately recognizes proteinaceous ligands. One could then not exclude a possible role as olfactory receptor, considering the probable interaction with odorant binding proteins carrying the odorant molecule to the receptor.
In this context we have cloned two SNMP homologues in noctuid moth species and studied their expression pattern in the antennae using *in situ* hybridization. Expression is restricted to the olfactory neurons of the antennae, suggesting a role in olfaction. However, lepidopteran olfactory receptors may belong to the G protein coupled receptor family, as it has been shown that G proteins are functionally active in signal transduction of different sensory systems, including olfaction. From the antennae of the moth *Mamestra brassicae*, we have identified a lepidopteran G protein α subunit belonging to the Gq family, through immunological detection followed by molecular cloning [6]. The expression pattern of the Gq subunit in adult antennae was studied by *in situ* hybridization: it is associated with the olfactory sensilla suggesting a specific role in olfaction. These data provide molecular evidence for a component of the phosphoinositide signaling pathway in moth antennae: this G protein α subunit may be involved in the olfaction transduction process through interaction with G protein-coupled receptors, stimulating the phospholipase C mediated second messenger pathway.

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MOLECULAR CODING OF BEHAVIORAL ANTAGONISTS BY OBP IN TWO NOCTUID SPECIES, *MAMESTRA BRASSICAE* AND *HELICOVERPA ZEA*.

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In *Mamestra brassicae* males, as in *Helicoverpa zea* [1], the neuron tuned to behavioral agonist(s) is co-compartmentalized with the neuron specifically tuned to behavioral antagonist(s) in long sensilla trichodea [2]. The presence of receptor neurons specifically responding to pheromone-like compounds that are not part of the conspecific pheromone is one of the mechanisms avoiding interspecific mating, then ensuring isolation between species. We have previously demonstrated that, in *M. brassicae*, the behavioral antagonist Z11-16:OH is specifically bound by MbraGOBP2 in long sensilla trichodea, which house the neuron responding to this compound [2]. As Z11-16:OH is also a behavioral antagonist in *H. zea* [3], we have searched its binding protein(s), using binding assay with male antennal extracts. The tritium labeled analog of Z11-16:OH is specifically bound by a protein of N-terminal TAEVM, typical of GOBP2 proteins. By molecular cloning, we obtained the full-length cDNA encoding for HzeaGOBP2 (GenBank, accession number AY017411). Primary sequences of MbraGOBP2 and HzeaGOBP2 share 93% identity, and a common function. The expression pattern of HzeaGOBP2 was studied by *in situ* hybridization. The labeling is associated with sensilla trichodea and seems co-localized with HzeaPBP expression, as in *M. brassicae*.
Neurons tuned to behavioral agonist and antagonist in noctuids are co-compartmentalized in the same sensilla. This has been proposed to optimize the spatio-temporal resolution of pheromone blends, beginning with the reception level [4]. Our data strongly support the hypothesis that pheromonal mixtures are discriminated as early as the binding between OBPs and pheromonal compounds occurs. More, the molecular coding of behavioral antagonist appears more specific than the coding of agonist compound.

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CLONING OF GENES ENCODING ODORANT BINDING PROTEINS AND CHEMOSENSORY PROTEINS IN INSECTS

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Odorant-Binding Proteins (OBPs) and their genes have been identified in many insect species and are encoded by multigene families usually expressed specifically in the antennae. OBPs are small soluble proteins with six conserved cysteines and they are responsible for transporting volatile molecules from the air/sensillum interface to the olfactory receptors. They can be classified as either General OBPs (GOBPs) or Pheromone BPs (PBPs) with the first group generally showing a higher degree of homology between species. In addition, another group of chemosensory proteins (CSPs) has been identified in a range of sensorial organs in many insect species. These have only four conserved cysteins and show no homology with the OBPs. The function of these proteins is unknown but their wider distribution and higher homology across species suggests a more general role. The presence of multiple OBPs and CSPs within a species suggests that they may show specificity of ligand binding and be involved in molecular recognition.
This presentation will discuss OBPs and CSPs in *Drosophila melanogaster* and the aphid, *Megoura viciae*. For *Drosophila* we have used the genome sequence database to identify more than thirty putative OBPs and eight of these have been cloned. For the aphid we have screened antennal cDNA libraries but failed to identify OBPs probably reflecting the lack of homology between species. However, we have cloned two full-length cDNAs encoding CSPs. The OBP and CSP cDNAs are now being expressed for ligand binding studies.

ROLE OF THE VOMERONASAL ORGAN IN THE DETECTION OF PIG APPEASING COMPOUNDS

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Chemical signals are crucial for social interactions in mammals. In particular, pigs use them to establish a stable hierarchy between conspecifics. In industrial husbandries, the social link is disturbed by several practices that induce stress. To reduce stress and improve animal welfare, studies have evaluated the role of olfaction in aggressive behaviours [1]. Recently, a mixture of appeasing compounds has been isolated from milking sows. But mechanisms underlying the detection of these compounds are still unclear, in particular, is the vomeronasal system involved in the detection of the pig-appeasing compounds? To address this question, we have used tritiated analogues of appeasing compounds to characterise their binding proteins in the pig vomeronasal organ (VNO). Three lipocalins from the VNO extracts bind the appeasing compounds with different affinities. In western-blot experiments, these proteins showed cross-reactivity with antisera raised against porcine odorant-binding protein (OBP) [2], salivary protein (SAL)[3] and von Ebner's gland protein (VEG) [4]. These proteins have been previously localised in the respiratory mucosa (OBP, SAL, VEG) [5], the saliva (SAL, VEG) [6, 4] and tears (VEG) [7]. To confirm this unsuspected site of production, we have isolated the cDNAs encoding these proteins from VNO extracted RNA. The three amino-acid sequences showed 100% identity with the OBP, SAL and VEG sequences already obtained from other porcine tissues. These data suggest that OBP, SAL and VEG are involved in the detection of appeasing compounds in the VNO. Heterologous expression is ongoing and will contribute to understand the role of each protein in the molecular recognition of these compounds.

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RESPONSES OF SINGLE OLFACTORY CELLS IN THE PALM WEEVIL *RYHNCHOPHORUS PALMARUM* (COLEOPTERA, CURCULIONIDAE) TO INSECT AND HOST PRODUCED VOLATILES

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Single sensillum recordings from 200 male and 135 female sensilla, revealed the presence of at least 9 types of antennal olfactory receptor neurons (ORNs). The palm weevil, *R. palmarum*, detects volatile chemicals using highly sensitive and specialized ORNs.. The most abundant ORNs are tuned to Rhynchophorol the main component of the male-produced pheromone. They show high specificity and sensitivity (10 pg response threshold). The latter do not respond to the plant odours tested. Seven types of ORNs responded to a 16-component synthetic blend that has proven to attract both sexes of *R. palmarum* in the field, in synergy with Rhynchophorol. ORNs responded specifically to one or two components of the blend but showed lower sensitivities (1 ng response threshold) compared to Rhynchophorol-tuned ORNs. The most abundant neurons responded to low molecular weight and high volatility compounds such as acetoin, esters (ethyl acetate, ethyl propionate), ketones (2-nonanone, 2-heptanone) and alcohols (ethanol). An ORN responding to guaiacol (2-hydroxy phenol) was characterised. Some neurons showed more complex pattern of responses, being activated by Rhynchophorol and plant volatiles, or activated by plant volatiles and inhibited by Rhynchophorol. Synergy between pheromone and plant volatiles has been shown to be critical to attraction of *R. palmarum* both in the field and in the laboratory (olfactometer). Our results indicated that interaction between plant odours and pheromone occurs at the peripheral level and indicates other mode of sensory encoding than the labelled line system described for the majority of insect species. Investigations aiming at a better understanding of the encoding of plant and conspecific signals, acting in synergy on the behaviour, are in progress.

CHEMICAL ECOLOGY OF INDUCED PLANT VOLATILES: FROM MECHANISMS TO FUNCTIONS.

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Inducible defences allow plants to be phenotypically plastic. Inducible indirect defence of plants by attracting carnivorous enemies of herbivorous arthropods can vary with plant species and genotype, with herbivore species or instar and potentially with other environmental conditions. So far, inducible indirect defence has mostly been studied for simple linear food chains. However, ultimately, ecologists should address inducible indirect defence in a food web context, where more than one organism (different herbivores and pathogens) may attack a plant and where a plant that emits herbivore-induced volatiles is surrounded by other plants that emit odours that can mix with the herbivore-induced volatiles from the attacked plant.
Evolutionary ecologists are interested in the costs and benefits of interactions between plants and their attackers. These may be investigated by comparing different plant genotypes. The best comparison is between plant individuals that differ in only a single or restricted number of known traits. Such genotypes are difficult to obtain by conventional methods. However, rapid progress in the study of mechanisms of plant-attacker interactions and in the field of molecular genetics and genomics provides new tools that can be exploited by ecologists. For instance, genomic knowledge on A*rabidopsis thaliana* and the availability of characterized mutants and transgenes that are altered in one or a restricted number of genes can be exploited to address functional aspects of inducible indirect defence.
Here I will present progress related to mechanisms of inducible indirect defence of plants and its importance for investigating the functional aspects of plant responses to herbivorous arthropods.

PLANT - PATHOGEN - HERBIVORE INTERACTIONS: ECOLOGICAL AND CHEMICAL ASPECTS

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Fungi and insects form two of the most numerous groups of living organisms in the world. As many individual species from each group utilise higher plants as a food source, it is not surprising that they should interact. The simplest type of interaction is a direct one: for example, the insect feeding upon, or acting as a vector for, the fungus. In some of these interactions the plant is little involved. However, changes in the host-plant are crucial in indirect interactions in which the insect and fungus interact mainly through one changing the host-plant in a manner that affects the other.

The effects of these indirect interactions can be significant for the participants. For example, some insects alter their choice of host-plants due to plant fungal infection, or suffer increased mortality due to feeding on fungal infected tissue. Likewise, the pattern of fungal infection may be modified by the presence of herbivory on the plant. These altered distributions of insects and fungi on their host can affect the amount of damage the plant receives, with implications for crops and also the use of insects and fungi for the biocontrol of weeds [1].

There is a variety of mechanisms that might explain these interactions. Some work has focussed on gross changes in host-plant chemical constituents which are induced by insect or fungus attack. For example, plant pathogen infection often leads to changes in leaf nitrogen, water and carbohydrate content, and insect herbivores are known to be sensitive to changes in these plant constituents [2]. More recently, attention has shifted to the investigation of defence mechanisms induced in plants by either insect or fungal attack. These include the systemic acquired resistance pathway which is mainly active against fungal attack and in which salicylic acid is important, and wound or herbivore induced resistance in which jasmonic acid is important. The possibility of 'cross talk' between these induced resistance pathways has also been recognised: a pathway induced by an insect might affect a fungus, or vice-versa [3].

These aspects of insect - fungus - plant interactions will be illustrated largely by reference to a model system we have studied. This includes the perennial weeds *Rumex obtusifolius* and *R. crispus*, the chrysomelid beetle *Gastrophysa viridula* and the rust fungus *Uromyces rumicis*. Infection of the plant by the rust retards development and increases mortality of the beetle, and herbivory by the beetle induces both local and systemic inhibition of rust infection.

I will suggest the need to consider a range of factors that could influence interactions between plants, insects and fungi; from the ecological (e.g. altered distribution of insects and fungi), through physiological factors (e.g. effects on plant growth, changes in carbohydrate and nitrogen concentrations) to molecular factors (e.g. interactions between induced resistance pathways). Progress in understanding these complex multispecies interactions will require integration of these very distinct disciplines [4].

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PLANT SURFACE WAXES AND INSECT ECOLOGY

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Primary aerial plant surfaces are covered with a layer of so-called <<epicuticular waxes>> (EW), comprised of long chain alkanes, alkanals, alkanols, alkanoic acids and their esters, and other classes of lipophilic compounds, including cyclic triterpenoids. As the first point of contact between insects and plants, EW potentially are important in plant defense and in host selection by herbivores. Indeed, there are numerous examples of wax extracts or individual EW components that function as allelochemicals, and these will be reviewed briefly. The focus of the talk will be the effects of EW on the ability of insects to attach to leaf surfaces. Crystallized EW or waxy blooms typically severely impair insect attachment. As a result, waxy blooms can function as defenses against some insect herbivores, but may have complex effects at the community level because individual species of herbivores and carnivores are differently affected by waxy blooms. For example, within the naturally occuring insect fauna associated with *Pisum sativum,* some species populations increase whereas others decrease in response to a reduction in waxy bloom conditioned by a single gene mutation affecting EW accumulation. Experiments show that the intensity of interactions among predatory insect species within this fauna are influenced by EW blooms. Even the infection potential of a fungal entomopathogen, *Pandora neoaphidis,* is affected by a reduction in waxy bloom on *P. sativum* because its life cycle includes conidial attachment to leaf surfaces. To gain a mechanistic understanding of how EW affects insect attachment, we have concentrated on predatory coccinellidss. Although not fully generalized in their habitat preferences, coccinellids are adapted to forage on a wide range of plant surfaces in search of prey. Using a centrifuge, we have measured attachment by *Hippodamia convergens* to a range of natural plant surfaces, extracted EW, and pure compounds presented as amorphous films or beds of crystals. The experiments confirm that wax crystals are responsible for greatly reducing insect attachment either to wax extracts or the pure compounds we have tested. However, the bioassays also show that *H. convergens* attachment differs to amorphous preparations of EW extracts differing in chemical composition or to pure compounds. For alkanols and alkanoic acids, we find evidence that chain length is inversely proportional to attachment potential for *H. convergens.* The reasons for this pattern are not clear, but are related to the wet adhesion attachment mechanism employed by this insect. Among four species of coccinellids tested, two predominantly arboreal species, *Harmonia axyridis* and *Adalia bipunctata,* acheive much higher attachment forces to glass and to plant surfaces than do three species that forage predominantly on forbes. The two arboreal species differ strongly in the degree to which their attachment is hampered by waxy blooms of *Pisum sativum*, illustrating diversity of adaptations for attachment to plant EW. The broadest implication of these results is that both the chemical composition and the degree of crystallization of EW, by affecting insect attachment to plant surfaces, influence plant defense and habitat specialization by insect herbivores and carnivores.

RECRUITMENT OF PARASITIC WASPS BY CATERPILLAR-INJURED PLANTS

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Herbivory causes many plants to emit a specific blend of volatiles. Parasitoids use the resulting odour to locate their herbivorous prey. We study ways to exploit this phenomenon in order to increase the attractiveness of crop plants to these beneficial insects. Our model plant is maize, which shows a particularly fast reaction to caterpillar attack. Within hours after attack, maize emits a blend of volatiles consisting mainly of terpenoids. This response is triggered by an elicitor in the oral secretion of the caterpillars. We have found considerable variation among maize genotypes that is partially linked to geographic origin of the maize lines (Europe vs. America). This variation allows us to test the potential of "creating" maize varieties that are particularly efficient in attracting parasitoids. A complicating, but fascinating factor is the learning ability of parasitoids, which largely determines their responsiveness to specific odour blends. To study this learning ability and to determine relative attractiveness of maize varieties, we have developed a six-arm olfactometer with which we can combine behavioural studies and collection of odours. Results from laboratory experiments with this apparatus and results from field tests with different maize lines will be presented and discussed in the context of breeding crop plants to enhance pest control.

PLANT-MEDIATED INTERACTIONS BETWEEN A PHYTOPATHOGENIC FUNGUS AND A LEAF BEETLE ON CHINESE CABBAGE.

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A very large number of insects and fungi are specialised on using plants as a source of nutrients. Therefore, it is not seldom that herbivorous insects and pathogenic fungi will attempt to exploit the same individual host plant. Direct but also indirect, plant-mediated interactions between both types of attackers may take place in such a case. Indirect interactions may be the result of alterations in plant metabolism caused by insect infestation or pathogen infection. Thus, previous attack by either type of antagonist may have a beneficial or detrimental impact on the other type [1, 2]. We have investigated such tripartite interactions between the host plant Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) and the two crucifer specialists *Phaedon cochleariae* (Coleoptera: Chrysomelidae) and *Alternaria brassicae* (Hyphomycetes, Dematiaceous). Both antagonist species attack the leaves of their host. Interactions between the phytopathogenic fungus and the herbivore were investigated on a local (both antagonists on the same leaf) and systemic scale (antagonists on different leaves). The phytopathogenic fungus was found to have a negative plant-mediated effect on larval performance and a deterrent effect on the adult's host selection behaviour. The effect was evident on a local but not on a systemic scale. Larvae fed with leaves infected by the plant pathogen were significantly more susceptible to infection by the entomopathogenic fungus *Metarhizium anisopliae*. In contrast, herbivory did not result in measurable effects on the growth of *Alternaria brassicae*. The ecological studies were supplemented by physiological assays to elucidate the possible mechanisms of the plant's response to insect attack and pathogen infection. Concentrations of components of the plant primary (water, C/N ratio, sucrose, total protein) and secondary metabolism (glucosinolates, anthocyanins, peroxidase) were assessed. These studies showed that apart from other, yet unknown, factors the observed increase in peroxidase activity could be held responsible for the adverse effects of fungus-infected leaves on the leaf beetle.

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THE ROLE OF STINK BUG-INDUCED HOST PLANT VOLATILES IN THE ATTRACTION OF THE EGG PARASITOID *TRISSOLCUS BASALIS*.

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The ability of parasitoids to locate and attack their host, and consequently their efficacy, is a result of successive searching behaviours that are regulated by physical, semiochemical, and biochemical factors. Once the host-habitat is reached, the host plant will continue to influence the searching process in several ways, both direct and indirect. Direct influences include the attractive or arrestment effects of host plant volatiles, named synomones, which are emitted when plants are injured by phytophagous insects, and which allow the parasitoids to restrict their search to an area where they are most likely to find their hosts. Moreover, because parasitization leads to death of the phytophagous insects, plants may profit from the presence and action of parasitoids. Synomones released as a response to the phytophagous insect attack could be produced or released at the site of the attack, as well as systemically by other parts of the plant, or the compound could be produced at the site of the attack, transported to other sites, and released far from the site of the attack. Here we report laboratory observations, conducted in Y-olfactometer with the aid of an automated image analysis system, on the case of the host-parasitoid association *Nezara viridula-Trissolcus basalis*. Egg parasitoid females search for and develop in the egg stage of their hosts. Searching for the host at the egg stage within the host habitat may involve specific strategies, mainly because the eggs are generally small compared to the total surface to be searched, and also because there is evolutionary pressure on eggs to be inconspicuous. As a general view these problems can be overcome by the wasps using specific and detectable cues originated from other host stages, such as pheromones produced by adult stages. Cues arising from the interaction of plant and host seem to play a minor role in the parasitoid-stink bug system due to an apparent minimal interaction between egg and plant. In the tritrophic system considered, leaves of *V. faba* damaged by feeding of *N. viridula* females tested versus undamaged leaves did not significantly attracted wasp females. The same situation was observed on the response of volatile from *N. viridula* egg masses laid on filter paper versus uninfested leaves. In contrast, damaged leaves carrying egg mass of *N. viridula* attracted *T. basalis*, and this attraction was observed for both plants brad bean and French bean indicating that, by egg deposition, *N. viridula* females induce on the leaves volatiles which attract *T. basalis* females. Finally, the egg deposition by *N. viridula* systemically induces volatile. The leaves without egg mass that were directly neighbored above the leaves with egg mass significantly prolonged the parasitoid residence time in the test arm of the olfactometer, and this attraction was observed for both the plant, Brad bean and French bean. Due to it is unlikely that host-induced synomones are produced as a result of damage to the plant during oviposition, but subsequent interaction of the egg "glue" with the plant tissues may induce changes in the plant chemistry.

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SOME EMERGING ISSUES CONCERNING 'MIXTURES', 'NOISE', AND FINE-GRAINED ODOR PLUME RESOLUTION INVOLVING PLANT-ODOR AND SEX PHEROMONE MIXTURES

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We have been investigating the degree to which olfactory receptor neurons (ORNs) of heliothine moths respond to mixtures of pheromone components and behavioral antagonists. For *Helicoverpa zea,* when we presented mixtures of (*Z*)-11-hexadecenal, the major pheromone component, plus the behavioral antagonist, (*Z*)-11-hexadecen-1-ol-acetate, there was a doubling of action potential frequency by the ORN responding to the major pheromone component during the initial phasic portion of the response, as well as significant tonic enhancement in firing of this ORN, compared with its response to (*Z*)-11-hexadecenal alone. We also found similar phasic and tonic enhancement in response to blends of another antagonist, (*Z*)-11-hexadecen-1-ol, and also in response to the secondary pheromone component, (*Z*)-9-hexadecenal.

Because the enhancement of firing of this ORN appeared to occur somewhat unspecifically in response to both antagonistic and agonistic pheromone-related compounds, we decided to investigate further just how specific this mixture interaction phenomenon was by expanding the testing of mixtures to include various plant-related odors combined with the major pheromone component. Surprisingly, we found even greater mixture enhancement of response of this ORN when different plant-related volatiles were presented than when pheromone-related mixtures were tested. For both pheromone-component-related mixtures and pheromone component/plant volatile mixtures, the enhancement of firing frequency was not as great when the odorants were puffed simultaneously from separate odor cartridges as when the mixture was housed within and puffed from a single odor cartridge.

These studies began with behavioral investigations involving pheromone components and behavioral antagonists. Our work showed that male moths have an incredibly fine-grained spatio-temporal odor resolution ability. *H. zea* males for instance can discriminate between perfectly mixed strands of pheromone plus antagonist from strands that are incompletely mixed but only with 1 mm separating the strands. Over the years, research with male moths involving overlapping, confluent pheromone plumes has yielded a variety of results, nearly always showing some degree of discrimination by the males as they emerge from the confluent portion of the plumes and continue flying upwind in one single plume or the other to the source. With but a few exceptions, it has been assumed that the response of the males to the confluent portion of the 'mixed' plumes is a response to a complex, mixed blend of the components comprising the two upwind odor sources. Recent recordings in our laboratory of confluent plumes using a 4-antenna array called the 'quadro-probe biosensor' have shown that what has been assumed to be a well-mixed blend of odor components is not very well mixed at all. Only a very few odor strands in these confluent plumes are perfectly mixed. These findings have important interpretations with regard to how chemical ecologists should interpret odors as 'mixtures' or as 'noise'.

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ELECTROANTENNOGRAM RESPONSES OF THREE PARASITIC WASPS TO INDUCED ODOUR OF MAIZE, COWPEA AND COTTON.

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Many plants can release a specific blend of odour after an attack by insects herbivores such as caterpillars. This induced odour has been shown to be very attractive to natural enemies of the herbivores. In maize for example, the odour released is composed of many different compounds such as green leaf volatiles, homo and mono terpenes, sesquiterpenes, alkaloid and acetates. If the attraction of parasitic wasps by the odour blend is well assessed, the relative perception and importance for attractiveness of the different compounds in the odour blend is not clear. In this study, we have recorded the antennae responses of *Cotesia marginiventris*, *Microplitis rufiventris* and *Campoletis sonorensis*., three parasitoids that attack *Spodoptera* caterpillars, to the induced odour of maize, cotton and cowpea. GC-EAD method was used to find which compounds in the three odour blends stimulated the antennae olfactory receptors. It appears that most of the major compounds in the different odour blend induced a response of the insects antennae olfactory receptors, but also some minor compounds. Variations in the intensity of the response appear to be due to the parasitoid species. *C. marginiventris* female wasps showed relatively higher sensitivity compared to *C. sonorensis*. The amount of stimuli delivered on the antennae also played a role in the intensity of the electrophysiological responses of wasps. These results are of great importance as a first step to determine which compounds in the induced odour of plants play a major role in the attraction of parasitoids to plants.

A COMPARISON OF NAÏVE AND CONDITIONED RESPONSES OF THREE ENDOPARASITOIDS OF LEPIDOPTERAN LARVAE TO HOST-INDUCED PLANT ODOURS

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Many parasitic wasps that exploit herbivores as their hosts make use of herbivore-induced plant odours to locate their victims. We study this phenomenon in maize plants and one of our objectives is to determine if we can increase the attractiveness of the plant to the wasps. Using a new six-arm olfactometer we compared the responses of the three larval endoparasitoids parasitoid, *Cotesia marginiventris*, *Microplitis rufiventris* and *Campoletis sonorensis*, to induced odours. First, we tested the attraction of the three parasitoids to the simultaneously presented induced odours of three plant species (maize, cowpea, and cotton), comparing naive females with females that were conditioned before each experiment by having them parasitise two larvae eating on one of the plants. The three wasps responded differently. Naive *C. marginiventris* choose equally among the odours of the three plants, while *M. rufiventris* tended to prefer the odour of maize. Analyses of volatiles that were collected during the bio-assays showed that the total quantity of produced volatiles was approximately 4 times higher for maize than for cotton, and about 5 times higher for cotton than for cowpea. After conditioning *C. marginiventris* always preferred the odour of the plant species that they had experienced, while all of the conditioned *M. rufiventris* showed an even stronger preference for maize odours. *C. sonorensis*' behaviour was intermediate**.**
In a subsequent experiment, we tried to determine which of the maize volatiles were most important for the attraction of the wasps. Naïve and experienced wasps were offered the choice between freshly damaged maize plants and plants with older damage. The results revealed that naive *C. marginiventris* are highly responsive to so-called "green-leafy volatiles" (typical for fresh damage) and become more responsive to specific herbivore-induced volatiles after experience. In contrast, naive *M. rufiventris* respond to the total plant-produced blend, while experience renders this wasp more responsive to volatiles that are directly associated with the herbivore or its by-products. To further determine the relative importance of specific maize compounds, additional experiments are used to compare the attractiveness of different maize lines with distinctly different odour profiles. The combined results from these studies may lead to strategies of optimising the use of induced plant signals in biological control.

SELECTIVE SAMPLING AND ANALYSIS OF EPICUTICULAR WAX CRYSTALS IN *NEPENTHES ALATA* PITCHERS.

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In the pitchers of the tropical carnivorous plant *Nepenthes alata* a broad zone of the inner surface is covered with a thick layer of epicuticular wax crystals. The ability of insects to walk on them is strongly affected by the chemical and physical properties and the micromorphology of this outermost wax layer. The crystals are slippery for most insects and prevent escape from the pitcher, in this way having an ecological function in the nutrient supply for the plant.
A reinvestigation with scanning electron microscopy revealed entire platelets protruding perpendicularly from the surface. We applied recently developed methods allowing the selective sampling of the crystals. To this end, various adhesives for the mechanical removal were used. Chemical analysis showed that in the crystals very-long-chain aldehydes are highly accumulated, in contrast to the intracuticular waxes. The significance for the mechanism of slipperiness for insects will be discussed.

CHEMICALLY MEDIATED HOST-FORAGING STRATEGIES IN EGG PARASITOIDS OF SPECIALIST AND GENERALIST HERBIVORES.

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This paper investigates whether differences in host plant specialisation in tritrophic systems are reflected by different infochemical use of parasitoids. While parasitoids specialised on monophagous herbivores should show innate responses to chemical stimuli from the host plant, parasitoids of polyphagous herbivores should involve learning processes during host foraging to cope with the variable odour environment of their hosts [1].
In the tritrophic system *Ulmus minor* - *Xanthogaleruca luteola* - *Oomyzus gallerucae* egg deposition of the monophagous elm leaf beetle induces the emission of plant volatiles [2]. These synomones are attractive for *O. gallerucae*, while the odour bouquet caused by feeding activity of the herbivore is not attractive for the egg parasitoid [3]. Here it will be shown that even naïve *X. luteola* without oviposition and odour experiences are able to orientate towards induced elm volatiles.
Contrarily naïve females of *Oomyzus galerucivorus*, an egg parasitoid of the polyphagous tansy leaf beetle, *Galeruca tanaceti*, are not attracted to volatiles of different host plants [4]. Instead, new results revealed that learning of host plant volatiles mediate host foraging in *O. galerucivorus*. Yarrow, *Achillea millefolium*, is a very common food plant of *G. tanaceti* in Germany. In the field tansy leaf beetle eggs were parasitised more heavily by *O. galerucivorus* in patches where yarrow was present compared to yarrow free patches. While naïve parasitoids avoid yarrow odours in the laboratory, yarrow experienced females are attracted to the host plant volatiles. It was investigated whether specific secondary compounds or general leaf volatiles were involved in the learning process. The evolutionary forces shaping the infochemical use during egg parasitoid foraging for specialist and generalist herbivores will be discussed.

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PHOSPHOLIPASE ACTIVITY TRIGGERS THE WOUND –ACTIVATED CHEMICAL DEFENSE IN THE DIATOM THALASSIOSIRA ROTULA.

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Diatoms are exceedingly abundant unicellular algae comprising the main constitutents of the phytoplankton. They are thus regarded as most important primary producers sustaining the marine food chain. Given this central importance it is surprising that ecological studies were almost exclusively focused on the transfer of matter and energy between different trophic levels without paying attention to species-specific chemical defense. Only in the recent years we understand that individual defense mechanisms against herbivorous predators exist in unicellular algae [1]. For example, the abundant diatom *Thalassiosira rotula* reacts upon wounding with the fast release of 2,4-decadienal and 2,4,7-decatrienal [2]. It has been shown that these aldehydes have antiproliferative properties, reducing the hatching success of copepod eggs.
We present details about the mechanism regulating the chemical defense in this unicellular algae. Only upon cell damage active phospholipases come into contact with lipids, releasing preferentially unsaturated fatty acids [3]. These serve as substrates for lipoxygenases and hydroperoxide lyases transforming the free fatty acids into the aggressive defensive metabolites. The mechanism of this enzymatic cascade has been elucidated using stable isotope- and fluorescent-labeled substrates. The latter allow the intracellular localization of the enzymatic activities involved and give first clues of how an efficient targeting of defensive metabolites can be achieved during the feeding process. After recognition of mechanical stimulants diatoms can set off their chemical defense. This defense on demand thus enables the algae to overcome the risk of potential dilution of their defensive metabolites in the surrounding water. By releasing high amounts of the aldehydes only when damaged by, e.g., feeding copepods the algae warrant that elevated concentrations of defensive metabolites are present in the vicinity of the herbivores.
We could show that not only the C10 aldehydes previously identified from *T. rotula* but, moreover, a structural diverse entity of α,β,γ,δ-unsaturated aldehydes are released from wounded diatoms. These aldehydes have been synthesized and tested for their antiproliferative potential. According to our findings the defensive mechanism initially identified in *T. rotula* is widely distributed but not universally found in diatoms. The observed species specific defenses are compared with the identified defensive metabolites and verified with in vitro assays for different diatom and dinoflagellate species.

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IMPACT OF HERBIVORY BY *ACENTRIA* (LEPIDOPTERA: PYRALIDAE) ON POLYPHENOLS IN THE FRESHWATER ANGIOSPERM *MYRIOPHYLLUM SPICATUM*

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Herbivory of the aquatic moth *Acentria ephemerella* on milfoil *(M. spicatum)* has become a model system for aquatic plant-herbivore interactions. *M. spicatum* is a highly competitive submersed macrophyte containing high concentrations of hydrolyzable tannins, up to 22% based on dry weight in apical shoots. The major polyphenol is tellimagrandin II, present in concentrations up to 5%. Tellimagrandin II has cyanobactericidal and algicidal activity, inhibiting exoenzymes and photosystem II of target organisms. This compound also inhibits various aquatic bacteria. Despite this high concentration of polyphenols, which are also potential herbivore deterrents, *Acentria* preferentially feeds on the apical meristems and can fully metamorphose on this plant, albeit at a slower rate. We were interested, whether herbivory influences polyphenolic allelochemicals in milfoil, both quantitatively and qualitatively. Our results show a slight increase in total polyphenols by about 20% in apical shoots or leaves. However, single polyphenols exhibited an up to five-fold increase after herbivory. Due to the antimicrobial nature of polyphenols we investigated whether changes in these allelochemicals would also improve the milfoils protection against potential pathogens entering wounds caused by herbivory.

PHYSIOLOGICAL ADAPTATIONS TO HOST PLANT ALKALOIDS IN THE LEAF BEETLE GENUS *LONGITARSUS*

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Several *Longitarsus* flea beetle species sequester pyrrolizidine alkaloids (PAs) acquired from their Asteraceae and Boraginaceae host plants. We carried out feeding and injection experiments using radioactively labeled PAs to investigate the physiological mechanisms of uptake, metabolism and storage of alkaloids in adult beetles. We examined seven *Longitarsus* species belonging to different phylogenetic clades in a comparative approach. All species that accepted PAs in a preceding food choice study showed the ability both to store PA *N*-oxides and to metabolize tertiary PAs into their *N*-oxides. Regardless whether the beetles' natural hostplants contain PAs or not these species revealed to possess an oxidizing enzyme. This oxygenase appears to be specific to PAs: [^{3}H]atropine and [^{14}C]nicotine, two alkaloids not related to PAs, were neither stored nor *N*-oxidized quantitatively by any of the tested species. In feeding experiments with two different PA types, beetle species that naturally feed on PA containing plants showed a specialization to their respective host type of PA. One species, *L. australis*, that behaviorally strictly avoids PAs, proved also physiologically not to be adapted to PAs. After injection of tertiary [^{14}C]senecionine beetles of this species neither *N*-oxidized nor stored the compounds, in contrast to *L. jacobaeae*, an adapted species, that received the same treatment. *L. jacobaeae* demonstrated the same efficiency in *N*-oxidation and storage when fed or injected with tertiary [^{14}C]senecionine.

CHANGES IN ALKALOIDS IN SENECIO JACOBAEA BY ARTIFICIAL ROOT HERBIVORY; EFFECTS ON THE ABOVEGROUND HERBIVORE MAMESTRA

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The effects of artificial rootherbivory on a lepidopteran aboveground herbivore (*Mamestra brassiceae)* were investigated in a controlled environment. Three genotypes of the biannual *Senecio jacobaea*, which differed in pyrrolizidine alkaloid concentrations, were subjected to artificial rootherbivory by removing half of their root system. After a recovery time of two weeks, three 1st instar caterpillars were placed per plant with ten replicates. Two weeks later the caterpillars were weighed and the plants were harvested for biomass measurements and chemical analyses.
Foliar herbivory did not affect vegetative plant biomass, except for the smallest genotype. Root herbivory decreased both roots and shoot biomass of the two bigger genotypes. Both aboveground and belowground herbivory significantly affected pyrrolizidine alkaloid concentrations and compositions, depending on the plant genotype. Foliar herbivory affected pyrrolizidine alkaloid concentrations in one genotype only. Plants with *Mamestra* were found to contain higher erucifoline concentrations in the shoot, but lower concentrations of senecionine in the roots. In two of the three genotypes, enhanced concentrations of seneciphylline were measured in the roots of artificial damaged plants. In one genotype, the artificial root damage increased jacobine concentrations aboveground. In spite of the observed differences in pyrrolizidine alkaloids, the weight of the *Mamestra* caterpillars did not differ on plants with and without root herbivory. In conclusion, above en belowground herbivory may lead to significant changes in secondary metabolites concentration. We were not able to demonstrate whether this affects the aboveground herbivore *Mamestra*.

DIVERSITY IN PLANT SECONDARY METABOLITES: SELECTION OR NEUTRALITY?

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It is still poorly understood why plant species have evolved such an enormous diversity of structurally related secondary metabolites. The evolution of plant secondary metabolites is often thought to be driven by insect herbivores and under this coevolutionary model it is expected that related compounds differ in their effects on herbivores. Here we focus on the diversity of pyrrolizidine alkaloids (PAs) in *Senecio* species. Within one *Senecio* species, several PAs of the same structural type can be found. We studied the effects of different PAs, from a number of structural groups, on a range of herbivores. The specialist *Tyria jacobaeae* was attracted to PAs for oviposition but this was not very specific. PAs from non-host plant species also stimulated oviposition by this specialist herbivore. Larval performance of *T. jacobaeae* was not affected by the PA composition of its host plant. In diet experiments most PAs were toxic or deterrent to the generalist herbivores. There were differences in effects between the PAs. However, PAs of the same structural type did not differ in their effect. We found no indications that structurally related PAs differ in effects on herbivores, therefore it seems unlikely that herbivores are the driving force behind the evolution of the diversity of PAs. Alternatively, we propose that the evolution of the diversity of PAs in *Senecio* species is selectively neutral.

CHEMICAL DEFENSIVE STRATEGIES IN GALERUCINE LARVAE (COLEOPTERA, CHRYSOMELIDAE)

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Chemical defence of larvae of the leaf beetle subfamily Galerucinae has intensively been studied for species of the tribes Galerucini and Luperini. Active chemicals in the hemolymph of Galerucini are *de novo* produced anthraquinones and anthrones. Several Luperini larvae sequester curcubitacins from their foodplants.
The defensive strategy of larvae of the galerucine taxon Sermylini differs from the other tribes by the segmental release of liquid at disturbance. When attacked, larvae of the leaf beetle species *Agelastica alni* and *Sermylassa halensis* (Sermylini) discharge a fluid from paired openings located dorsolaterally at abdominal segments 1-8 in *A. alni* and additionally at the thorax in *S. halensis.* The fluid coagulates rapidly and defends the larvae against small predators such as ants or coccinellid larvae by sticking together the mouthparts and antennae of the enemy. The discharged exudates contain hemocytes. The protein patterns of the exudates in both species match the ones of conspecific hemolymph as revealed by SDS-PAGE. The defensive activity of the hemolymph of both species is not only due to its stickiness. Our bioassays strongly indicate the presence of chemicals that act as feeding deterrents, since non-sticky, aqueous dilutions of the exudates significantly deter the ant *Myrmica rubra* from feeding. Heating, drying, treating with proteinase K, and exclusion of molecules > 3kD did not inactivate the feeding deterrent quality of the defensive fluids. Exudates were fractionated by HPLC and active fractions are currently analysed by GC-MS.
The internal structures of the paired openings were studied by scanning electron microscopy and light microscopy, respectively. Larvae of. *A. alni* possess sac-like cuticular invaginations, each linked to a glandular cell by a short cuticular canal [1]. These features are lacking in *S. halensis.* However, in this species larvae show numerous cuticular fibrils around the openings, which are part of the muscular mechanism to close the bleeding site. Up to now, the function of the glandular cells in *A. alni* is unknown. The glandular secretion might play a role for the alarm behaviour of the gregariously living *A. alni* larvae. When exposed to volatiles of the defensive fluid, larvae within a group try to escape from the group. In contrast, the solitary larvae of *S. halensis* just drop from the plant, when disturbed.

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PHAGOSTIMULATION OF DIABROTICINA BEETLES (CHRYSOMELIDAE: GALERUCINAE) BY POLLEN EXTRACTS AND AMINO ACIDS

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Studies on host plant associations of Diabroticina beetles have focussed on the importance of plant secondary compounds as factors determining host plant specificity. Adults of all species, however, are pollen feeders and their host associations display the full range from specialists to highly polyphagous generalists. To date, phagostimulation through pollen extracts and amino acids have been investigated only for one species, the western corn rootworm (*Diabrotica virgifera virgifera*). The objective of this study is to determine if primary metabolites, such as amino acids, play a role in host specificity of Diabroticina beetles. Here, we present data of a comparative study on seven species of Mexican Diabroticina beetles. Pollen was collected from three host plants in the state of Veracruz, Mexico (*Zea mays*, cultivated and wild *Cucurbita* spp.). Bioassays were conducted, offering pollen extracts and amino acids to adult beetles. Phagostimulation by pollen extracts, six individual amino acids (L-alanine, β-alanine, proline, GABA, serine, asparagine), and amino acid mixtures was compared for male and female insects. Results were interpreted based on a recently developed hypothesis of the evolution of host plant breadth in these beetles [1].

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ATTRACTING VOLTILES FOR THE COLORADO POTATO BEETLE

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Chemicals responsible for attraction of the Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) to its host plant and conspecifics have long been sought, since McIndoo in 1926 [1] demonstrated the general attractiveness of potato plant volatiles to adult CPB.

Only recently, I could identify several attracting volatiles that were emitted from potato plants and stimulated isolated antennae of CPB and its predators in coupled gas chromatography / electroantennogram preparations [2,3]. Six artificial mixtures of these components attracted adult CBP in laboratory behavioral tests. One of these artificial blends is attractive not only to adults of both sexes but also to larval forms [4], the first plant attractant for larval CPB. Thus, these blends may serve as attractants for food plants for all stages as well as for oviposition sites for females.

Further, together with colleagues, we also succeeded to finally identify a male-produced aggregation pheromone for the beetle [5]. Since male beetles normally release only minor amounts of the pheromone, collection and chemical identification of it was only enabled after its release was enhanced nearly 200 fold by topical application of JH III and antennectomy. The pheromone surprisingly is (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol, representing a novel structure for an insect pheromone. Antennal receptors of both sexes respond selectively to the (S)-enantiomer; in laboratory behavioral bioassays, the (S)-enantiomer attracts both sexes while its antipode is inactive or might even be inhibitory. This investigation provides the first identification of a pheromone for the CPB. Since it is produced by the males, our discovery is in contrast to the existing paradigm of a female-produced pheromone for this insect. Its role in the ecology of the beetle, however, has yet to be fully clarified. Furthermore, our findings show a positive influence of JH III on production and/or release of the pheromone and imply a control of pheromone release by antennal sensory input via a negative feedback loop, a new mechanism, only mentioned so far for the cotton boll weevil, *Anthonomus grandis* [6].

The discovery of both insect and plant produced attractants for larval and adult CPB provides tools for use in alternative management strategies for pestiferous populations. Based on the results presented here and others from recent studies in my lab, the chemical basis for conspecific interactions and host plant selection is discussed.

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MATING BEHAVIOUR & THE SEX PHEROMONE OF *CULICOIDES NUBECULOSUS* (DIPTERA, CERATOPOGONIDAE)

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The mating behaviour of the farmyard midge *Culicoides nubeculosus* has been well described and involves both swarming behaviour of males, with copulation following females entering the swarm, and reduced or 'truncated' patterns in which mating occurs on the substrate without swarming [1]. The presence of female derived sex pheromone has been established and peak production occurs at 24-48h post-emergence [2]. A blood meal must be taken immediately following mating in autogenous species such as this.
The chemical ecology and behaviour of *C. nubeculosus* has been studied in the laboratory in relation to the production and identification of the female-produced sex pheromone and the role of host blood volatiles in stimulating mating or feeding behaviour. Results show that sheep blood odours and an extract of volatiles collected by air entrainment of emerging adults, significantly increased the number of mating behaviour displays, and specific grooming behaviour of both males and females.
Air entrainment of emerging mixed groups of male and female adults, followed by GC-MS analysis and comparison with an authentic sample, showed the presence of n-heptadecane as a major component. Y-tube bioassays demonstrated a significant directional response of virgin and mated males (24-48h post emergence) to physiologically relevant levels of n-heptadecane but not to n-hexadecane or n-octadecane.
The main conclusions of this study were that that n-heptadecane is a major component of the female-produced sex pheromone and that blood volatiles play a significant role in stimulation of mating and feeding behaviour depending upon the physiological state of the female.

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BEHAVIOURAL AND ELECTROPHYSIOLOGICAL STUDIES ON HUMAN EMANATIONS THAT ATTRACT THE MALARIA MOSQUITO *ANOPHELES GAMBIAE*

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The highly anthropophilic mosquito *Anopheles gambiae* is one of the most important malaria vectors in tropical Africa. This nocturnally active mosquito is guided to its human host predominantly by chemical cues discharged from the human body [1] [2]. Single components or blends of kairomones thus far were never as attractive as a complete human blend presented as a human hand, entire body or as a skin extract [3][4]. Therefore we do not yet know the key chemical compounds and their correct ratio on which mosquitoes rely for locating human hosts. The understanding of the chemical ecology of mosquito-human interactions will eventually enable the design of more effective mosquito traps that can be applied in mosquito surveillance and, possibly, in population management as part of the control tactic of this malaria mosquito.
Human emanations that attract *Aedes aegypti* (L.) can be transferred to glass marbles by rubbing marbles in human hands[5][6][7]. We studied the attractiveness of human emanations collected on glass marbles to *An. gambiae* in a dual-port olfactometer. One glass bead handled in a hand for 10 minutes (1.6 cm in diameter) caused attraction of *An. gambiae*. The attractive effect of the emanations disappeared after 4 hrs. The rubbed beads lost their attractiveness upon freezing during one or more weeks. Human individuals differ in attractiveness for mosquitoes. It is possible to rank individuals for their mosquito attractiveness by testing their skin emanation on glass marbles. Comparison of the emanations released by glass beads rubbed by human individuals with different attractiveness might provide us with indication of potential mosquito attractants and/or repellents. Emanations of rubbed glass marbles elicited EAG responses. Responses to emanations from rubbed marbles handled by volunteers differing in attractiveness differed significantly in amplitude such that differences in composition are suggested. A coupled GC-EAD method was used successfully to study the olfactory sensitivity of *An. gambiae* in response to complex odour profiles.

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OLFACTOMETER STUDIES ON THE EFFECTS OF AMMONIA, LACTIC ACID AND A CARBOXYLIC ACID ON *ANPOHELES GAMBIAE* GILES S.S.

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Female mosquitoes need a blood meal for egg production. In addition to physical and visual factors they are guided to their blood host by olfactory cues. Anthropophilic species like the malaria mosquito *Anopheles gambiae* Giles s.s. and the yellow fever mosquito *Aedes aegypti* L. are attracted to odours emanating from the human body [6]. Lactic acid, ammonia and fatty acids with chain lengths of C1-C3, C5-C8 or C13-C18 have been identified as attractants for *Ae. aegypti* females. A blend of lactic acid, ammonia and two fatty acids proved to be almost but not as attractive as an extract of human skin residues [1, 3, 4]. Females of *An. gambiae* s.s. were equally found to be attracted to the human sweat components ammonia and lactic acid when tested alone [2] and to a synthetic mixture of 12 short-chain aliphatic carboxylic acids that are present in foot odour [5]. However, the bioassays used to study these effects were different. Here we describe the results of experiments with *An. gambiae* s.s. that were done in the same bioassay (Y-tube olfactometer) as was used to obtain the results with *Ae. aegypti*.
After 1 minute of stimulation with ammonia equal or lower numbers of *An. gambiae* females were trapped in the test arm as in the control arm (water) of the Y-tube olfactometer. However, it was clear from direct observation that ammonia activated the mosquitoes. Lactic acid attracted significantly more mosquitoes than water. The carboxylic acid showed no attractiveness. Adding ammonia or the carboxylic acid to lactic acid did not increase the attractiveness of lactic acid, whereas the combination of lactic acid, ammonia and the carboxylic acid was more attractive than lactic acid alone, suggesting that the three compounds combined cause a synergistic effect. However, the combination of these three chemicals appeared to be less attractive than a natural odour source (human finger). The latter indicates that more volatile components than these three compounds may be involved in the host-seeking behaviour of *An. gambiae* s.s.
The experiments showed that lactic acid, ammonia, and carboxylic acids play an important role in the host-seeking behaviour of *An. gambiae* s.s. females. In addition, they revealed that other volatile components are involved as well, because the synthetic blend was less attractive than the emanations from a human finger. From the results it was also clear that, although *An. gambiae* was observed to make a clear choice based on the odours offered, the fact that the mosquitoes did not stay in the catching device because of enhanced activity makes this Y-tube a rather unsatisfactory bioassay device for this mosquito species. Unlike for *Ae. aegypti*, which does not express the enhanced activity patterns. Therefore, the Y-tube should not be used for direct behavioural comparison studies between *An. gambiae* and *Ae. aegypti*.

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AQUATIC HERBIVORES AND THE CHEMICAL DEFENSE SYSTEM OF WATERCRESS.

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The watercress (*Nasturtium officinale*) glucosinolate-myrosinase system is a deterrent to feeding by generalist aquatic herbivores. The hydrolysis product of the predominant glucosinolate, 2-phenylethyl isothiocyanate, is the main feeding deterrent in this species. In North American, where watercress is exotic, many stream invertebrates (the limnephilid caddisflies, *Limnephilus* sp. and *Hesperophylax*, snails and *Gammarus pseudolimnaeus*) will not feed on live, chemically-defended watercress, although they readily consume undefended senescent watercress even though it is lower in nitrogen. European herbivores have had much longer evolutionary contact with watercress and may have adapted to watercress defenses. We showed recently that two English snails, *Gammarus pulex* and one caddisfly were deterred from feeding on fresh, but not senescent or heat-deactivated tissue, similar to North American herbivores. In contrast, the limnephilid caddisfly *Limnephilus lunatus* preferred fresh green to senescent tissue and consumed similar amounts of fresh and senescent tissue. Thus, at least one English herbivore appears adapted to watercress defense. Recent studies with a German population of watercress (from Leutra, eastern Thuringia) suggest less feeding on fresh green tissue by both *Gammarus pulex* and *Limnephilus lunatus* compared to their English counterparts. However, this German population contains a broader array of glucosinolates than previously reported for watercress. Eight glucosinolates were detected. Although 2-phenylethyl glucosinolate is often a major component, 2-OH-2-phenylethyl glucosinolate is typically the most abundant glucosinolate and 7-methylsufinylheptyl glucosinolate is also abundant. Analysis of wild and cultivated *N. officinale* from a German watercress farm (near Erfurt) show that this watercress does not contain 2-OH-2-phenylethyl glucosinolate and suggests that the Leutra population either possess a previously undescribed watercress glucosinolate profile, or that it represents the closely related *N. microphyllum* or an *N. officinale* X *N. microphyllum* hybrid. Ongoing studies of these populations and their herbivores may provide interesting insights into the role of glucosinolates in defense against aquatic herbivores and the patterns of herbivore counteradaptation.

CHEMICAL COMMUNICATION MADE VISIBLE IN CRAYFISH AGONISTIC INTERACTIONS

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Chemical communication is a widespread phenomenon in aquatic animals but is difficult to investigate because the signals are transparent in water. Here, a technique is presented for the visualisation of urinary signals in aquatic animals. Using this technique the behavioural context of urine release in social interactions of blindfolded crayfish *Astacus leptodactylus* could be investigated including the receiver's response to the chemical signal. Crayfish were blindfolded to prevent reactions to the visible cloud of stained urine. The study revealed an important function of urinary chemical signals in the dominance system of the crayfish [1]. Previous studies [2, 3] have yielded contradictory results about the significance of pheromones in crayfish agonistic behaviour. The results of our study now give conclusive evidence for the significance of chemical communication in crayfish: Detailed analysis of 30-minute dyadic interactions showed that (a) urine release is largely limited to aggressive behaviours, (b) release rates co-vary with varying aggressive level, (c) the receiver responds to aggressive actions (e.g. threat displays, pushing, claw-lock) of the opponent only when the actions are accompanied by urine release; aggressive actions alone do not intimidate the receiver, (d) crayfish control the transmission of urinary signals using gill currents and currents generated by fan organs [4].
Together, these results indicate that chemical signals are necessary in crayfish to establish dominance. Urine signals appear to mediate "honest" information about the fighting motivation/ability of the signaller. Individual recognition does not seem to be involved in crayfish dominance hierarchies since a subordinate crayfish is deterred equally well by a familiar and an unfamiliar opponent.
The study proves that visualisation of urine signals can provide a powerful tool to analyse the social context and function of chemical communication.

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EVIDENCE THAT MALE TILAPIA USE URINE AS CHEMICAL SIGNAL DURING REPRODUCTION

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Despite their variety of reproductive stategies and complex social hierarchies, little is known about the possible role(s) of chemical communication in cichlids. The current study investigated whether chemical communication occurs during courtship of Mozambique tilapia, *Oreochromis mossambicus*. The urination frequency of males in the presence of pre- and post-ovulatory females was visualized by injection of a dye, and the olfactory potency of male body fluids was assessed by the electro-olfactogram. In the presence of post-ovulatory females, male frequency of urination did not change significantly, but they sometimes exhibited some aspects of courtship behaviour. However, in the presence of pre-ovulatory females, the frequency of urination rose dramatically from 3,5 to 46.4 hr-1 ($P< 0.01$), and the males exhibited the full range of courtship behaviours (nest-building, shivering, dark colouration, etc.). Urine pulse duration did not change in either treatment.
Females had high olfactory sensitivity to water previously occupied by sexually mature males and male body fluids (urine, bile and faeces). Considering the likely rates of release of these fluids, it is the urine that provides the most potent odorant. Crude chemical fractionation implicated a sulphated steroid (or steroids) in the urine as the biologically active component. These results suggest that males not only are able to discriminate between pre- and post-ovulatory females, but are using their urine as a chemical signal during courtship.

CUCKOOS IN WOLVES' CLOTHING? CHEMICAL MIMICRY IN A CUCKOO WASP OF THE EUROPEAN BEEWOLF (HYMENOPTERA, CHRYSIDIDAE AND SPHECIDAE)

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Host-parasite interactions are among the most important biotic relationships. Host species should evolve mechanisms to detect their enemies and employ counterstrategies. Parasites, in turn, should evade detection to maximise their success. Females of the European beewolf (*Philanthus triangulum*, Hymenoptera, Sphecidae) hunt honeybees as food for their progeny. The brood cells containing the paralysed bees are the target of a highly specialised cuckoo wasp (*Hedychrum rutilans*, Hymenoptera, Chrysididae). Female cuckoo wasps enter beewolf nests to oviposit on paralysed bees. Cuckoo wasp larvae kill and feed on beewolf larvae and the bees and can be a major cause of mortality. Observations suggest that beewolves attack the cuckoo wasps in front of their nest but do not recognise these parasitoids when they encounter them in the nest. Since insects heavily rely on chemical senses we hypothesised that the failure to detect this crucial enemy is the result of chemical cloaking. Cuckoo wasps might either mimic their beewolf host or the paralysed honeybees that are temporarily couched in the nest burrow. Cuticles of honeybees, beewolf females and males, and cuckoo wasps of populations from Würzburg were extracted and analysed by GC-MS. There was little similarity between cuckoo wasps and honeybees. However, there was a considerable similarity between beewolf females and cuckoo wasps that was even larger than between beewolf females and conspecific males. The occurrence of isomeric forms of certain compounds on the cuticle of the cuckoo wasps but absence on beewolf females suggests that cuckoo wasps synthesise the cuticular compounds rather than sequester them from their host. Thus, this study provides evidence that the specialised cuckoo wasp *H. rutilans* exhibits chemical mimicry of the odour of its the European beewolf *P. triangulum*.

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CHEMICAL ECOLOGY AND REPRODUCTIVE BIOLOGY OF THE SOCIAL PARASITIC BUMBLEBEE *PSITHYRUS NORVEGICUS*

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The bumblebee species *Psithyrus norvegicus* is an obligate social parasite of *Bombus hypnorum*. *Psithyrus* females have to successfully invade the host nest and to remain in the host colony without being killed. There are indications that *Psithyrus* females may use allomones to defend themselves against attacking host workers. After entering a nest *Psithyrus* females often kill the host queen. In these cases she must be able to control the ovarian development of the host workers in order to maintain dominance in the nest as the queen would normally do. It is unclear if she uses chemical or physical means. The following questions were adressed: 1) Do host nest invading *Psithyrus* females use allomones to defend themselves against attacking host individuals? 2) Do *Psithyrus* females use pheromonal and/or behavioral mechanism to maintain the reproductive dominance and inhibit reproduction in workers?
In gas chromatography analysis coupled with electroantennography (GC-EAD) using *Psithyrus* scent we found eleven compounds that triggered electroantennographic responses in antennae of the host workers. In a bioassay a synthetic mixture of these compounds, as well as the main compound dodecyl acetate showed a strong repellent effect on host workers. Therefore *Psithyrus* females use dodecyl acetate to repel attacking host workers during nest usurpation and afterwards during colony development.
To investigate the use of pheromonal and/or behavioral mechanism for regulation of worker reproduction behavioral observations and experiments were performed with groups of individually marked callow workers. *Psithyrus* females never showed aggressive behavior against host workers. Furthermore, we found that workers that were reared together with *Psithyrus* females or the host queen as well as workers that were only under influence of semiochemichals produced by the host queen or the parasite showed significantly smaller ovaries as compared to a control. Therefore, the host queen as well as the *Psithyrus* female inhibits worker ovarian development via semiochemicals.

CO-EVOLUTION OF PLANT AND INSECT IN ASIA: CHEMICAL MIMICRY OF AN ORIENTAL ORCHID AND BEHAVIOR OF THE ASIAN HONEY BEES.

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Flowers of an oriental orchid, *Cymbidium floribundum* Lindl. (Kin-ryou-hen in Japanese), blooms in spring and specifically attracts the Japanese honey bee (*Apis cerana japonica* Rad.) for pollination. The European honey bee (*Apis mellifera* L.) is not attracted The flowers do not have nectors and pollinia will attach onto the back of the thorax when visited by the bees. So, the pollination occurs successfully but there seems no reward for bees. Moreover, the flowers attract not only workers but also drones and sometimes whole swarning colonies of *Apis cerana japonica*. Because the orchid is native in southern China and was brought into Japan about two to three hundreds of years ago by human activity, this intimate relationship seems originally established between the orchid and the Chinese honey bee (*Apis cerana cerana*). This project was intended to reveal and to understand the molecular basis of this co-evolutionary process between the honeybee and the orchid.
We have identified the semiochemicals that elicit social behaviors and communications in the above two honey bee species along with the attractants in the flower aroma, honey bee pheromone gland components by GC/EAD, GC/MS and by bioassays.
Results showed that (1) Semiochemicals in the honeybee exocrine glands were species specific. Components of the aggregation pheromone from Nasonov glands were totally different between *Apis cerana* and *Apis mellifera*. Worker mandibular gland components were not different in nurse bees but quite different in foragers between the two honey bee species. In GC/EAD analysis, drone mandibular gland extracts elicited a single EAD response against drone anntena. 9-ODA was common major mandibular gland components in both species.
(2) Flower aroma contained the same components that can be found in honeybee aggregation pheromone, especially those found in the Japanese honeybee, *Apis cerana japonica*, in large quantity. (3) Flower aroma did not contain honey bee queen mandibular substances. (4) Presence of drone aggregation pheromone was suggested to exist in both the mandibular gland of drone *Apis cerana japonica* and the flower aroma. (5) The Chinese *Cymbidium floribundum*, probably the origin of Japanese one, strongly attracted the Asian honeybee (*Apis cerana cerana*) with the flower aroma by having several common components in the aggregation pheromone of *Apis cerana cerana*.
Future works will reveal: (1) the reason for the specific attraction of drone, queen, and the swarming colony of the Japanese honeybee. (2) the details of chemical mimicry (pollination strategy) by the oriental orchid and its molecular basis.(3) the symbiotic, co-evolutionary relationships between the oriental orchid and the Asian honeybee.

THE CHEMISTRY OF *MYRMECIA GULOSA*: A COMPARATIVE STUDY OF THE CUTICLE, POST-PHARYNGEAL AND DUFOUR'S GLAND.

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In *M. gulosa*, all workers are daughters of a lone queen. They lay exclusively trophic eggs that are fed to all nestmates. The loss of the queen (through death or removal), initiates agonistic interactions and reproductive egg-laying in some workers. The chemistry of queens, reproductive and non-reproductive workers was studied qualitatively through GC-MS. Dufours glands were similar in content between the groups, the major components being (Z8)-heptadecene and geranylcitronellol. Minor components included eicosenal, dienes, and alkanes. In contrast, post-pharyngeal gland contents (PPG) and cuticular hydrocarbon (CHC) differed between reproductive (queen and workers) and non-reproductive individuals. The former contained alkanes, alkenes and various methyl alkanes, whereas the latter lacked the alkenes. As in several other ant species, PPG composition closely matches CHC profiles of individuals. Alkenes (Z9)-pentacosene, (Z9)-heptacosene; methyl alkanes 3-methylpentacosane, 3-Methylheptacosane and several monomethyl centrally branched pentacosanes form the major differences between the groups. The possibility of these compounds acting as part of a fertility signal will be discussed.

MOTIVATION AND NEST VOLATILES AFFECT NESTMATE RECOGNITION IN THE CARPENTER ANT *CAMPONOTUS FELLAH*

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The behavior of/towards isolated ants when returned to the nest depend largely on the time of isolation. The behavior toward ants that were isolated for upto 14 days is largely amicable and they are apparently still considered as nestmates. These ants show elevated solicitation for trophallaxis irrespective of satiety. Differential aggression is also apparent when the ants are isolated within the nest by placing them in a small dish equipped with a single or double mesh screen. After 3 weeks, single-mesh-ants behaved and were treated as ordinary nestmates, whereas aggression towards the double-mesh-ants was intermediary between nestmates and completely isolated ants. This suggested that nest odors affected either the recognition system or the behavior of the isolated ants. To test this hypothesis we isolated individual ants to prevent any possible contact or vision with nestmates, but allowed airflow from the nest to pass through the ant cages. Aggression tests revealed an intermediary aggression towards these ants, similar to the double mesh experiment. The origin of these volatiles, queen, worker or combined, and the manner by which they affect nestmate recognition are currently investigated.
Colonies deprived from their queens gradually become more permissive. If confronted with ants from nests with which they had odor flow, they treated them in an amicable manner. Queenright ants under the same condition, on the other hand, remained hostile to the alien ants despite this odor habituation. Being a few months queenless made these colonies completely permissive to the point that alien colonies merged without any hostile interactions. During the merging of the queenless colonies high incidence of trophallaxis between the alien ants were observed resulting in a congruent cuticular hydrocarbon profiles. The respective queenright colonies, on the other hand, remained completely insulated and as predicted kept their distinct cuticular hydrocarbon profiles.

RETICULITERMES SPECIES IN CALIFORNIA: CUTICULAR HYDROCARBONS AND SOLDIER DEFENSE SECRETIONS

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Cuticular hydrocarbon mixtures of *Reticulitermes* samples from disparate locations in northern and southern California were characterized. Literature records indicate that there are two extant species of *Reticulitermes* in California: *Reticulitermes hesperus* Banks and *R. tibialis* Banks. In northern California (west of the crest of the Sierra Nevada and north of San Luis Obispo) we have identified 5 cuticular hydrocarbon phenotypes: CA-A, CA-A', CA-B, CA-C, and CA-D. CA-A, CA-A', and CA-C are characterized by an abundance of internally branched monomethylalkanes, whereas, CA-B and CA-D have abundant 5-methyl- and 5,17-dimethylalkanes. CA-A, CA-A', and CA-D are essentially sympatric in the San Francisco Bay Area, but CA-D tends to be restricted to areas near the coast in northern California, whereas CA-A and CA- A' can be found in the inland areas as far north as Mt. Shasta. CA-B and CA-C have only been collected in the Sierra Nevada foothills. In southern California (the coastal area from Santa Barbara south to Oceanside) we have found 2 cuticular hydrocarbon phenotypes: SCA-A and SCA-B. SCA-A is characterized by an abundance of internally branched monomethylalkanes, while SCA-B has abundant 5-methyl- and 5,17-dimethylalkanes. Throughout the range that we have sampled, SCA-A and SCA-B appear to be sympatric with neither phenotype being predominant in any area. The distributions of the northern and southern California populations appear to be parapatric, but the potential zones of overlap have not yet been thoroughly sampled. Characterization of the soldier defense secretion mixtures indicates a one-to-one correspondence of cuticular hydrocarbon phenotypes with soldier defense secretion phenotypes. Even though CA-A/CA-A' are similar to SCA-A in the composition of their cuticular hydrocarbon mixtures, the soldier defense secretions confirm that CA-A/CA-A' ≠ SCA-A; the soldier defense secretions of CA-A/CA-A' contain predominantly γ-cadinene (>90%), whereas SCA-A produces a predominance of germacrene A with smaller amounts of γ-cadinene (<30%). Likewise, CA-D is similar to SCA-B in the composition of their cuticular hydrocarbon mixtures, the soldier defense secretions confirm that CA-D ≠ SCA-B; the soldier defense secretions of CA-D contain an abundance of geranyl linalool, germacrene A, and γ-cadinene, whereas SCA-B produces germacrene A, γ-cadinene, but smaller amounts of geranyl linalool. Collections from the type locality of *R. hesperus* produced a phenotype very similar to CA-A' in both cuticular hydrocarbon and soldier defense chemistry, therefore we infer that this cuticular hydrocarbon phenotype represents *R. hesperus*.

CYTOCHROMES P450 AND LEPIDOPTERAN-PLANT INTERACTIONS.

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The heliothines (genera *Heliothis* and *Helicoverpa*) comprise the most destructive complex of agricultural insect pest species in the world. Their significance is largely due to their ability to rapidly overcome chemical insecticides by developing resistance, in which cytochromes p450 play a major role. This feature makes the heliothines a useful model system for investigating the role of p450s in plant-insect interactions. This system has additional advantages: in addition to the polyphagous, host-plant generalists that are agricultural pests, the group contains host-plant specialists that are not pests. In at least two cases, a generalist and a specialist are closely enough related that they can be crossed to produce fertile progeny. This enables a genetic approach to studying plant-insect interactions in general, and the role of interspecific differences in detoxicative mechanisms in particular. We describe our approaches to developing genetic tools of sufficient power and resolution to systematically investigate the role of all members of the p450 gene family in this regard.

PROFILING PLANT-INSECT INTERACTIONS

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In the long course of plant-insect and plant-pathogen coevolution, plants have developed sophisticated mechanisms to ward off pathogen and insect attack. In addition to preformed chemical and physical barriers, plants have evolved induced protection mechanisms. Wounding by attacking herbivores induces the activation of many wound-responsive genes both locally and systemically. Due to the complexity of the different induction systems, types of response and putative crosstalk between defense signaling pathways there is a multitude of different genes being induced or downregulated.
To gain a better understanding of the complex interactions between the different signaling pathways and genes involved, we are utilizing *Arabidopsis thaliana* and related species of the genus Arabis as excellent model systems to study plant defenses against insects such as *Plutella xylostella* (Diamondback Moth), *Trichoplusia ni* or *Spodoptera littoralis*.
To identify defense-related genes and to dissect the pathways involved in the induction of these genes we employ mutant screens, QTL mapping, positional cloning of candidate genes and expression profiling.
The expression profiles of induced genes both in Arabidopsis and Arabis are monitored by membrane based macroarray approaches. A simultaneous analysis of the expression of a large subset of genes putatively involved in plant defense responses is prerequisite to the understanding of the underlying complex mechanisms of signal transduction and gene expression regulation.
On the other hand, crucifer specialist insects have evolved mechanisms to evade the host plants´ defenses. We will show how Diamondback Moth, a world-wide distributed crucifer pest, circumvents the preformed defense barrier posed by the glucosinolate-myrosinase sytem.

GLUCOSINOLATES AND INSECT RESISTANCE IN *ARABIDOPSIS THALIANA*.

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Glucosinolates (mustard oil glucosides) constitute a major component of plant secondary metabolites in Arabidopsis and other Brassicaceae. Glucosinolates and their breakdown products (isothiocyanates, nitriles, thiocyanates, epithiocyanates, etc.) contribute to plant defense against various pests, mediate feeding behavior of herbivores, and influence oviposition preferences of insects. They also determine quality and flavor of important crop plants such as mustard, canola, cauliflower or broccoli.
Arabidopsis thaliana ecotypes vary in both composition and quantity of glucosinolates. A major locus controlling variation in glucosinolate profiles is *Elong*. This genomic region harbors several *MAM* genes encoding methylthioalkylmalate synthases that determine the side chain length of glucosinolates [1]. Comparative sequencing reveals that this locus is highly variable among ecotypes, and gene rearrangements are frequent. We demonstrate that *Elong* controls resistance to a generalist insect, but not to a specialist insect adapted to crucifers, and investigate the ecological factors responsible for variation.

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THE POWER OF PLAGIARISM – GLUCOSINOLATE HYDROLYSIS IN PLANTS AND SPECIALIST LEPIDOPTERANS.

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The glucosinolate-myrosinase system is believed to protect plants of the Brassicaceae family, including crops such as oil seed rape and cabbage as well as the model plant Arabidopsis, from herbivory by generalist insects. Intact glucosinolates are non-toxic, but upon tissue damage they come into contact with myrosinases and are hydrolyzed into unstable aglycones which rearrange into a variety of biologically active compounds many of which have hot and bitter tastes. At physiological pH, the major hydrolysis products are isothiocyanates and nitriles. Isothiocyanates have been shown to be highly toxic to insects.
The nature of the hydrolysis products depends on the structure of the glucosinolate side chain, but also on the plant species and the reaction conditions, and has an influence on herbivory by generalist insects. For example, larvae of the generalist lepidopteran *Trichoplusia ni* avoid *Arabidopsis thaliana* ecotypes that produce mainly isothiocyanates upon glucosinolate hydrolysis, preferring to feed instead on ecotypes that produce nitriles. Mapping of the genetic locus controlling hydrolysis product formation led us to the identification of an *A. thaliana* epithiospecifier protein whose presence results in the formation of nitriles instead of isothiocyanates upon myrosinase-catalyzed hydrolysis of glucosinolates.
Numerous insects have adapted to the glucosinolate-myrosinase defense system and are able to use species of the Brassicaceae as their major or sole host plants. However, few studies have tried to elucidate the biochemical mechanisms that underlie this high degree of specialization. Larvae of the cabbage white butterflies, *Pieris rapae* and *P. brassicae*, belong to the most serious pests of crops of the Brassicaceae family. We have investigated the fate of glucosinolates in the body of *P. rapae* and *P. brassicae* larvae and identified an enzyme activity in the larval gut that seems to be responsible for the ability of these larvae to feed on glucosinolate containing plants. Further characterization of the protein, which shares functional similarities with plant enzymes involved in glucosinolate hydrolysis, and elucidation of the genetic basis of this adaptation will give new insights into the evolution of plant-insect interactions.

DEVELOPMENTAL AND WOUND-RESPONSIVE REGULATION OF THREONINE DEAMINASE GENE PROMOTER FROM *NICOTIANA ATTENUATA.*

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Threonine deaminase (TD), which catalyzes the first commited step in the biosynthesis of isoleucine, was cloned from *Nicotiana attenuata* after a differential display revealed that it's transcripts were rapidly induced by wounding and herbivore attack [1, 2]. To study the expression pattern of TD in various condition, a genomic DNA fragment containing 5' upstream region and the full open reading frame of TD was isolated. A series of TD promoter fragments were fused to the GUS reporter gene and used to develop transgenic *Nicotiana attenuata* plants. Promoter analysis showed that GUS activity was seen in callus, cotyledone, young sink leaf, flower bud, sepal and the tip of flower under control condition, suggesting that TD is developmentally regulated. When leaf was wounded, it showed strongly induced GUS activity. To see which regions are responsible for developmental and wound induction, a series of deleted promoter were analysed. When -1902 ~ -100 bp upstream regions from the ATG translational start sequence were tested during developmental stage and upon wounding, longer than -360 bp showed strong GUS activity, -297 bp showed weak GUS activity, -210 bp showed very weak GUS activity and -100 bp showed no GUS activity. These results indicate that -297 ~ -210 bp regions may be responsible for basal TD induction and -360 ~ -297 bp regions may be responsible for full induction of TD. Plant cis-acting regulatory DNA element database (PLACE and PlantCARE) predicted that -360 ~ -297 bp regions contain putative ABRE (ABA responsive), EIRE (elicitor responsive) and WRKY (pathogen and wounding responsive) binding motif and -297 ~ -210 bp regions contain P-box (gibberellin responsive) and AuxRR-core (auxin responsive) binding motif.

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IDENTIFICATION OF MAIZE TERPENE SYNTHASE GENES INVOLVED IN THE INDIRECT DEFENSE AGAINST LEPIDOPTERAN HERBIVORES

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Secondary plant metabolites provide protection against pathogens and herbivores not only by acting as phytoalexins, toxins and feeding deterrents (direct defense), but also by attracting natural enemies of the herbivores (indirect defense). Several volatile metabolites are thought to have a role in indirect defense by serving as a signal in tritrophic interactions between plants, herbivores and parasitoids.
Upon attack by lepidopteran larvae, maize (*Zea mays*, L.) emits a mixture of volatiles that is dominated by mono- and sesquiterpenes. This blend attracts parasitic wasps that use the lepidopteran larvae as their hosts [1]. In order to understand the function of volatile emission and its regulation by herbivore damage, we are investigating the molecular genetics and biochemistry of terpene biosynthesis. A key step in terpene biosynthesis is catalyzed by the enzyme class of terpene synthases which convert acyclic prenyl diphosphates to a wide variety of basic terpene skeletons. To extend our understanding of terpene biosynthesis to maize, we have isolated a group of terpene synthase genes that are part of a large and diverse gene family in this plant. Heterologous expression of the genes in *E. coli* resulted in functional terpene synthases that accepted geranyl diphosphate as well as farnesyl diphosphate to form monoterpenes and sesquiterpenes, respectively. Each enzyme forms a characteristic blend of products that consists of three to forty different terpenes *in vitro*. Most of these enzymatic products are constituents of the terpene blend that is emitted by maize plants after herbivore damage. The terpene synthase genes *tps1* and *tps2* encode enzymes that form different enantiomers of the sesquiterpene alcohol (*E*)-nerolidol, which is not emitted by most maize cultivars. We demonstrated that (*E*)-nerolidol is a pathway intermediate in the formation of the homoterpene *(E)*-4,8-dimethyl-1,3,7-nonatriene (DMNT), one of the major components of the herbivore-induced maize volatile blend [2]. Transcript levels of *tps1* and *tps2* as well as (*E*)-nerolidol synthase activity increase after damage by lepidopteran larvae but not after mechanical damage to the leaves, suggesting a tight regulation of gene expression by herbivory.
In an attempt to study the functional role of individual terpene synthase genes in the indirect defense against herbivores, we are characterizing maize lines with transposon insertions in terpene synthase genes. These ‚knock out' mutants will enable us to determine the contribution of each gene to herbivore-induced volatile production and parasitoid attraction.

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TERPENE BIOSYNTHESIS AND EMISSION FROM *ARABIDOPSIS THALIANA* FLOWERS: NEW PERSPECTIVES FOR INVESTIGATIONS OF FLORAL SCENT BIOLOGY AND EVOLUTION

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Arabidopsis thaliana has been used as a model system for numerous fundamental investigations in plant morphology, physiology and metabolism. However, nothing is known so far about the detection of floral scent from Arabidopsis, either by analytical methods or the human nose.

Terpenoids are known to be important compounds in the floral scent of many plant species [1]. By applying highly sensitive volatile collection systems we were able to measure terpene emission from flowers of Arabidopsis plants. Besides small amounts of monoterpenes like myrcene, linalool and limonene, over 20 different sesquiterpene hydrocarbons were found to be released from the floral tissue. Investigations of floral scent from different ecotypes revealed qualitative as well as quantitative variation.

Since sequencing of the entire Arabidopsis genome has been completed, 30 genes have been identified with homology to known plant terpene synthases (*TPS*). These encode enzymes that catalyze the formation of the basic skeletons of plant terpenes from linear diphosphate precursors. Arabidopsis *TPS* genes are located on all 5 chromosomes and often have a clustered organization. RT-PCR analysis showed that more than half of the Arabidopsis *TPS* genes are constitutively expressed in floral tissue. In an ongoing investigation to characterize all flower expressed *TPS* genes, we have cloned and functionally expressed 5 of them. Two function predominantly as monoterpene synthases, whereas the other three encode sesquiterpene synthases. One sesquiterpene synthase catalyzes the formation of (-)-*E*-β-caryophyllene which is also the predominant compound in the scent of Arabidopsis flowers.

The discovery of Arabidopsis floral scent opens up questions about its significance for outcrossing events that occur at low levels in natural Arabidopsis populations [2,3] and could help mitigate inbreeding depression in this mainly selfing plant. Comparative biochemical and molecular analyses with selected Arabidopsis mutants and perennial, outcrossing Arabidopsis relatives like *Arabidopsis lyrata* or *Arabis* sp. will provide further insights into the functional significance of floral scent biosynthesis in *A. thaliana* and its evolution.

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SYNERGISTIC EFFECTS OF EXOGENOUS SPERMINE AND JASMONIC ACID ON THE VOLATILE PRODUCTION IN LIMA BEAN LEAVES

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Plants have developed a multitude of defense mechanisms against herbivores. One class of defense mechanism is referred to as 'induced indirect defense'. An example of induced indirect defense is to emit specific blends of volatiles in response to herbivore damage (herbivore-induced plant volatiles: HIPVs), which attract carnivorous natural enemies of the herbivores. Jasmonic acid (JA), salicylic acid and ethylene are endogenous-signaling small molecules involved in several induced responses of plants to being wounded and infected. It has been reported that treatments of JA [1-3], JA and salicylate [3], and JA and a precursor of ethylene [4] on lima bean leaves induce the production of volatiles that are similar to a blend of HIPVs.

Polyamines (PAs) are basic small molecules that are ubiquitous in all plant cells. They have been shown to bind strongly to nucleic acids and proteins. While evidence has accumulated that PAs are involved in resistance to microbial attack and environmental stress in plants, the effects of PAs in response to herbivory are still unknown. In this study, we investigated whether exogenous polyamines affect the biosynthesis of induced volatiles in lima bean leaves.

Lima bean leaves exogenously treated with putrecine, spermidine or spermine (SPM), did not induce volatiles. However, SPM, together with a low concentration of JA, induced the production of (*Z*)-3-hexenyl acetate and seven terpenoids, which were not induced by the low concentration of JA alone. Lipoxygenase (LOX) and alcohol dehydrogenase (ADH) are involved in the production of (*Z*)-3-hexenyl acetate, and farnesyl diphosphate synthetase (FPS) is involved in the production of volatile terpenoids. In our preliminary experiments, expressions of genes for LOX, ADH and FPS were induced in lima bean leaves 24 h after treatment with SPM and JA, while the JA treatment alone induced only the expression of the LOX gene. SPM alone induced the expression of none of these genes. These data suggest that exogenous SPM and JA synergistically enhance the biosynthesis of induced volatiles in lima bean leaves by inducing gene expressions involved in their biosynthesis.

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PETUNIA-POLLINATORS INTERACTION.

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Within the genus *Petunia*, different species rely on different pollinators: *P.integrifolia spp*. are pollinated by bees, while *P.axillaris spp*. are pollinated by hawkmoths [1]. These differences in reproductive biology are expressed in sharply contrasting floral pollination syndromes, which are suites of characters that have evolved in adaptation to the pollinators. The main goal of our project is to unravel the molecular genetic basis for the evolution of these pollination syndromes and to understand their ecological significance in natural populations. One of the questions we would like to answer is: can the presence of a specific gene be responsible for the attraction of specific pollinators? If so, this would mean that speciation of *Petunia* may occur trough very simple genetic modification. Our first step was to determine which flower characteristics are important to attract pollinators. In a greenhouse we offered bumblebees and hawkmoths the choice among plants of two subspecies of *P. integrifolia* and two subspecies of *P. axillaris*. *Bombus terrestris* landed significantly more on *P. integrifolia* and *Manduca sexta* approached and fed significantly more from flowers of *P. axillaris* subspecies. We decided to focus on floral scent and collected and analysed odour emissions of flowers of different species, subspecies and ecotypes, which showed very high variability in quantity and quality of the volatile blend. Furthermore, we observed a difference in the circadian rhythm between species that correspond with the activity of their respective pollinators. We crossed *P. axillaris parodii* and *P. hybrida* W138 (transposon strain) and we analysed the odours of several BC1F2 * W138. The most evident differences among the odour blends of these plants were found to be the proportions of the compounds emitted and their circadian rhythmicity. We tested preferences of *M. sexta* for these plants and carried out other experiments to identify which compounds are the most attractive for these pollinators. The next step is to screen BC1F4 plants (almost homozygous) for differences in emission of single compounds and subsequent cloning of the genes responsible for the production of these compounds. Furthermore, studies in Uruguay should permit us to identify if differences in odour blends and other pollination syndromes among *Petunia* ecotypes result in pollination by different insects. If we confirm this hypothesis the minor differences among ecotypes could be signs of incipient speciation.

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SELECTIVE THIOGLYCOSID-TRANSPORT FROM GUT TO DEFENCE GLANDS IN CHRYSOMELID LEAF BEETLES

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Chemical defenses are an important aspect of the interaction between chrysomelid leaf beetles and their host plants. Phylogenetically derived taxa such as *Chrysomela populi* sequester salicin, a glycosidically bound aromatic compounds found in their obligate hosts (*Salix* spp.). The larvae convert salicin to the defense compound salicylaldehyde. Less derived chrysomelids, for example *Hydrotassa marginella*, produce iridoids such as chrysomelidial or plagiodial *de novo,* without depending on plant-derived precursors. On the other hand, it has recently been demonstrated, that these *de novo* producing larvae are also functionally able to sequester glycosides of terpenoids which are involved in iridoid biosynthesis [1]. Although "sequestration" of plant-derived precursors is, thus, a rather widespread phenomenon, virtually nothing is known about the molecular basis of the uptake of glycosides.
Thioglycosides like thiosalicin combine a unique structural similarity to natural substrates with an exceptional chemical and biological stability when exposed to hydrolytic enzymes and thus, can be used to study transport phenomena. Uptake experiments using aromatic and terpenoid thioglycosides clearly demonstrate that both the derived, sequestering leaf beetle larvae (*C. populi*), as well as the *de novo* producing species (*H. marginella*) possess highly selective transport mechanisms for certain glycosidically bound secondary plant compounds. Accordingly transport systems should play a critical role in the evolution of new chemical defenses following a shift in host plants.

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ARBUSCULAR MYCORRHIZA

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Most terrestrial plants live in mutualistic symbioses with root-colonizing fungi called mycorrhizas. Several types of mycorrhizal symbiosis exist, defined by plant/fungus combination and the symbiotic structure. The endotrophic arbuscular mycorrhiza (AM) may have co-evolved with land plants and persists in most extant angiosperms. The other major mycorrhizal type, the ectotrophic ectomycorrhiza, evolved as a more recent symbiosis of woody trees and shrubs. The key feature of the arbuscular mycorrhiza is the arbuscule, a highly branched haustorium-like structure within root cortical cells, responsible for the supply of water and mineral nutrients to the plants and fungal uptake of plant carbohydrates.
Screening for mycorrhiza-specific metabolites in plant roots inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* has resulted in the isolation of two distinct groups of isoprenoid compounds [1-4], cleavage products of xanthophylls. They comprise glycosylated C_{13} cyclohexenone derivatives and an acyclic C_{14} polyene compound named mycorradicin, which is a major component of the so-called "yellow pigment" of most arbuscular mycorrhizal roots. These roots show strongly elevated transcript levels of two pivotal enzymes of the non-mevalonate methylerythritol phosphate (MEP) pathway, 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) [3]. Screening for DXS clones in mycorrhizal root cDNA libraries, two distantly related *DXS* cDNAs were identified. The possible importance of DXS1 in many housekeeping functions (plant growth and development) and presumed ecological functions of DXS2 in the biosynthesis of secondary isoprenoids (carotenoids and apocarotenoids in mycorrhizal roots) is discussed [5].
Carotenoid profiling of mycorrhizal roots revealed mycorrhiza-specific accumulation of □-carotene and inhibition of phytoene desaturase (PDS) activity by norflurazon resulted in the accumulation of considerable amounts of phytoene in mycorrhizal roots. Plants transformed with a *PDS* promoter-*GUS* construct showed a cell-specific activity in root cells containing arbuscules [6]. The plastids of these root cells show a dramatic reorganization. They form large network-like compartments covering the arbuscules [7]. Upon disintegration of arbuscules a concomitant disintegration of the plastid networks and accumulation of "yellow pigment"-containing droplets was observed.
Regarding the possible role of carotenoids and apocarotenoids in arbuscular mycorrhizal roots the current hypothesis is that strongly intensified metabolism in arbuscule-harboring root cells produces reactive oxygen species (ROS) which might act in these cells as second messengers mediating carotenoid biosynthesis. The accumulated carotenoids could provide efficient protection against oxidative stress (ROS scavenging).

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DIVERSIFICATION OF PROTECTIVE ALKALOID PROFILES AS RELATES TO EVOLUTIONARY PROCESSES IN FUNGAL ENDOPHYTES OF GRASSES

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Among the many fungal symbionts of plants, the highest degree of specialization and developmental coordination is exhibited by *Epichloë* (sexual) and *Neotyphodium* (asexual) species, which are endophytes of grasses. These endophytes are excellent models for the evolution of symbiosis because they span the continuum from mutualistic to antagonistic symbionts, and because much is known about mechanisms by which they benefit their hosts. Specifically, four distinct classes of endophyte alkaloids are associated with anti-herbivore activities and, thus, bioprotection of the host plants. These include two classes of indole alkaloids, namely ergotoxins and indolediterpenes, which have anti-vertebrate and anti-insect activities. The other two classes are more specifically anti-insect: lolines (1-aminopyrrolizidines) are insecticidal, and the pyrrolopyrazine, peramine, is an insect deterrent. Interestingly, there is considerable variation in the alkaloid profiles among the endophytes. Furthermore, the profiles and levels of alkaloid expression appear to relate to the degree of mutualism that typifies the endophyte. Those that are completely non-pathogenic often produce multiple alkaloid classes in easily detectable amounts. Those that are relatively pathogenic rarely produce detectable levels of the bioprotective alkaloids. Phylogenetic analysis of housekeeping genes indicates that most asexual, mutualistic endophytes are interspecific hybrids with multiple sexual species in their pedigrees. Phylogenetic analysis of the *dmaW* gene for the first step in ergot alkaloid biosynthesis fails to reflect the phylogenies of housekeeping genes. Therefore, it is conceivable that the endophytes have exchanged secondary metabolism genes by horizontal transfer following interspecific hybridization. This hypothesis is under further investigation for ergot alkaloid synthesis genes and for a cluster of genes associated with loline alkaloid biosynthesis. Evidence will be also presented that interspecific hybridization can cause metabolic diversification, which may in turn provide a selective advantage to the hybrids. Selection on metabolic capabilities and bioprotective effects may help account for the abundance and diversity of interspecific hybrids among the mutualistic endophytes.

SOFT CORAL-ZOOXANTHELLAE SYMBIOSIS; THE EFFECT OF CORAL BLEACHING ON METABOLITE LEVELS

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In an attempt to understand how molecular changes are linked to biochemical responses, biochemical changes were monitored during natural and induced bleaching for the soft corals *Lobophytum compactum* and *Sinularia flexibilis*. High water temperatures and increased radiance were used to induce bleaching, and these parameters were found to have a synergistic effect on bleaching severity.

Concentrations of photo-protective mycosporine-like amino acids (MAA's) in the soft corals studied were found to correlate with seasonal water temperature and solar irradiance [1]. Concentrations of MAA's peaked immediately prior to spawning in female colonies (reflecting photoprotection for the buoyant eggs and resultant larvae), and in summer when exposure to solar irradiance and high water temperatures is at a maximum.

Biochemical responses to bleaching included short-term changes to secondary metabolite concentrations and ratios, possibly as a strategy to prevent fouling by opportunistic microorganisms [2]. No correlation between bleaching and predation was observed; predation levels remained low.

Surviving corals from a bleaching event recovered their zooxanthellae within 4 months, but protein, lipid, MAA and carotenoid levels were affected for a much longer period, and this had a pronounced effect on mean egg diameter and fecundity for female colonies.

To demonstrate the role played by zooxanthellae in production of coral terpenoid defenses, asymbiotic larvae of the soft coral *Lobophytum compactum* were reared and infected with zooxanthellae cultured from disparate marine sources [3]. The success of the established symbiotic association was assessed by polyp growth, and an investigation of terpenoid metabolites from 3 month old polyps revealed no significant variation in the nature of the metabolite composition from polyps with algal partners from disparate marine sources. Only the terpenoid chemistry characteristic of the parent colonies was detected by high resolution mass spectrometry of extracts from individual polyps. This result was interpreted as solid evidence to support the proposal that terpenoid defenses are synthesised by corals, not their algal partners.

Some preliminary investigations of the host-symbiotic association for a sponge genus that produces an array of secondary metabolites will also be discussed with reference to published work by other researchers.

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SYMBIONTIC MICROORGANISMS IN ARTHROPODS – THEIR SIGNIFICANCE IN CHEMICALLY MEDIATED BIOTIC INTERACTIONS

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There is presented a survey on new ecto- and endosymbiontic interactions of microorganisms with selected arthropods. Bacteria which can be isolated from gut compartments are especially regarded.
At first isolation of aerobic microorganisms from arthropod guts and the microbial diversity in various hosts is described. Moreover these bacteria and fungi are characterized and their abilities to biosynthesize various biologically active natural compounds in the laboratory are reported. The microbiological diversity of gut communities and especially of actinomycetes is described according to the nutritional requirements of the arthropod hosts.
From the autochthonous bacterial flora of selected water beetles, *Bacillus*-strains were isolated and tested for their steroid transforming ability. This may be important since various zoosterols represent natural precursors of dytiscid defensive steroids.
Finally from gut compartments of *Spodoptera*-larvae, bacterial strains are isolated and characterized. Their possible involvement in biosynthesis of N-Acylaminoacids in interactions between plants, herbivores and their predators is discussed. Moreover microorganisms from various developmental stages, food and other compartments of these herbivores are characterized.

BIOSYNTHESIS OF A BEETLE DEFENSE COMPOUND BY A BACTERIAL SYMBIONT

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Paederus beetles are efficiently protected against predators by their hemolymph toxin pederin, the only complex polyketide known from insects and a highly active cytotoxic agent. Natural products with similar structures and activities are also known from various marine sponges. As for many other drug candidates from invertebrates, considerable evidence exists that the true producers are as yet uncultured microbial symbionts. Genes encoding the biosynthesis of bacterial secondary metabolites are usually clustered, which simplifies the manipulation of complete biosynthesis pathways. Large amounts of such rare substances could therefore be generated by the cloning of symbiont genes and their heterologous expression in a culturable bacterium. We have chosen pederin as model system to test this approach and to obtain more insight into the putative symbiosis. These studies led to the isolation of a large modular polyketide synthase gene cluster from total DNA of *Paederus fuscipes* that is only present in beetles with high pederin content. Sequencing of this cluster and adjacent regions revealed that the system belongs to a bacterium with high similarity to *Pseudomonas aeruginosa*, whose presence in the beetles is also supported by 16S rRNA data [1]. The gene cluster is architecturally in perfect agreement with the structure of pederin and contains a number of features that reveal unexpected aspects of pederin biosynthesis. This is the first direct proof for the participation of bacterial symbionts in the biosynthesis of pharmacologically valuable invertebrate “metabolites” and opens up new possibilities for the generation of long-term and ecologically friendly supplies of such drug candidates.

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POSSIBLE ROLE OF CAROTENOIDS AND APOCAROTENOIDS IN THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

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A large number of various plants produces considerable amounts of apocarotenoids during the establishment of the arbuscular mycorrhizal symbiosis. In *Zea mays* these compounds are deposited as a complex mixture of various esters in cytosolic or vacuolar lipophilic droplets [1]. This deposition occurs mainly during the degradation of the short-lived symbiotic plant-fungus interfaces, the arbuscules. In *Z. mays* and *Medicago truncatula* the accumulation of the apocarotenoids mentioned is accompanied by the accumulation of small amounts of various carotenoids, most notably of zeta-carotene and by a general stimulation of carotenoid biosynthesis. This stimulation might be induced by reactive oxygen species, which have been reported to stimulate carotenoid biosynthesis [2]. The accumulation of hydrogen peroxide close to disintegrating arbuscules [3] is supporting such a connection. This hypothesis is currently investigated by analysing carotenoids and apocarotenoids from arbuscular mycorrhizal plants with changed contents of reactive oxygen species and by investigating the extent of oxidative cell damage in arbuscular mycorrhizal roots which were not able to perform carotenoid biosynthesis.

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ISOPRENOID BIOSYNTHESIS IN MYCORRHIZAL ROOTS: INVOLVEMENT OF A SECOND 1-DEOXY-D-XYLULOSE 5-PHOSPHATE SYNTHASE (DXS2)

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Various cyclic and acyclic apocarotenoids accumulate in mycorrhizal roots of many plants as a part of the so-called yellow pigment. We have recently proposed biogenesis of both classes of these apocarotenoid compounds from a common carotenoid (xanthophyll) precursor based on their concomitant accumulation and the fact that a carotenoid cleavage at the 9,10 (9',10') position would create the core structures of both compounds [1].
Isopentenyl diphosphate (IPP) for carotenoid biosynthesis in plastids is produced by the non-mevalonate pathway (2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway) located within this compartment. 1-Deoxy-D-xylulose 5-phosphate synthase (DXS) catalyzes the first step of this pathway. We have shown that DXS transcript levels are strongly elevated in mycorrhizal roots of various cereals, correlated with the accumulation of the apocarotenoids. A more detailed analysis of this phenomenon in the model legume *Medicago truncatula* revealed the existence of two distantly related *DXS* genes, which are preferentially expressed in shoot tissues (*DXS1*) or in mycorrhizal roots (*DXS2*). Mining from public databases and the use of specific probes derived from this information confirmed the existence and differential regulation of two *DXS* genes also in tomato and maize. The preliminary picture of regulatory profiles of *DXS1* and *DXS2* from this work and from literature reports shows *DXS1* to be expressed in many above-ground tissues, probably linked to primary photosynthetic functions, but not in non-mycorrhizal or mycorrhizal roots. *DXS2*-type genes are expressed in mycorrhizal roots and in essential oil (monoterpene)-producing leaf trichomes, which can both be placed in the context of ecological interactions [2]. Evolutionary aspects of the *DXS* genes are being studied by analysis of exon-intron structures and promoter functions.

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BIOCHEMICAL PATHWAY STUDIES ON THE LOLINE ALKALOIDS OF THE MEADOW FESCUE ENDOPHYTE, *NEOTYPHODIUM UNCINATUM*.

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The insecticidal loline alkaloids (1-aminopyrrolizidines) are produced by fungal endophytes of several cool-season grasses (subfamily Poöideae). Reliable production of *N*-formylloline (NFL) and *N*-acetylnorloline (up to a total of 1000 μg/ml) in cultures of the meadow fescue endophyte, *Neotyphodium uncinatum*, has facilitated studies on the loline alkaloid biosynthetic pathway. Based on loline alkaloid structures, it was previously suggested that lolines are polyamine products. However, our precursor feeding experiments, employing GC-MS and NMR analyses, indicated that lolines share common precursors with, but are not derived from, polyamines. Labels from 5-[^{13}C]-ornithine and 1,2-[^{13}C]-ornithine were incorporated into specific positions of the B-ring in the pyrrolizidine-ring structure of NFL, but the pattern of incorporation differed from what would be expected if a polyamine intermediate was involved. Feeding studies with 4-[^{13}C] and [^{15}N]-aspartic acid gave enrichment in the expected position of the pyrrolizidine A-ring (at C-3) and the 1-amine, respectively. Universally [^{13}C]-labeled methionine did not incorporate in the pyrrolizidine ring system, but labeled the 1-amino methyl and 1-amino formyl groups. These studies demonstrated the origins of the carbon atoms in NFL: the B ring is derived from ornithine, the A ring is from aspartic acid, and the *N*-methyl and *N*-formyl carbons are from the *S*-methyl carbon of methionine.

INDUCTION OF JASMONATE BIOSYNTHESIS IN ARBUSCULAR MYCORRHIZAL BARLEY ROOTS.

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During the interaction of arbuscular mycorrhizal fungi with plants, phytohormones, such as jasmonic acid (JA), are believed to play an essential role for the establishment of the mycorrhizal symbiosis. Colonization of barley (Hordeum vulgare L. cv. Salome) roots by an arbuscular mycorrhizal fungus, *Glomus intraradices* Schenck & Smith, leads to elevated levels of endogenous JA. This rise in JA is accompanied by the expression of genes coding for an enzyme of JA biosynthesis (allene oxide synthase) and of a jasmonate-induced protein (JIP23). *In situ* hybridization and immunocytochemical analysis revealed the cell-specific occurrence of the expression of both genes within arbuscule-containing root cortex cells. This concomitant gene expression indicates that jasmonates are generated and act within arbuscule-containing cells. By use of a near-synchronous mycorrhization, analysis of temporal expression patterns showed the occurrence of transcript accumulation four to six days after the appearance of the first arbuscules. For that reason, it is suggested that the endogenous rise in jasmonates might be related to the fully established symbiosis rather than to the recognition of interacting partners or to the onset of interaction. A model is proposed, in which the induction of JA biosynthesis in colonized roots is linked to the stronger sink function of mycorrhizal roots compared to non-mycorrhizal roots.

THE GRASS ENDOPHYTE GENE FOR THE FIRST STEP IN ERGOT ALKALOID BIOSYNTHESIS

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The endophytic *Neotyphodium* spp grow in aerial tissues of grass hosts where they cause no symptoms and exhibit no signs; yet, often they help protect against insects, nematodes and vertebrate herbivores, increase drought tolerance, and improve plant growth and nutrient acquisition. Several endophyte alkaloids are variously active against insects and/or vertebrates. Among these are ergot alkaloids, including lysergic acid and its peptine derivative, ergovaline. Ergovaline is closely related to ergotamine, which is produced by the infamous ergot fungus *(Claviceps purpurea),* and is a potent mycotoxin (but a useful pharmaceutical at low doses). Ergovaline is thought to be responsible for toxicoses and reproductive problems in cattle and horses that ingest certain endophyte-infected fescues and ryegrasses. However, ergot alkaloids are also toxic to insects, so it is possible that they enhance biological protection against invertebrate herbivores. The *dmaW* gene, encoding the probable first step in ergot alkaloid biosynthesis (dimethylallyltryptophan synthase), was cloned from *Claviceps fusiformis* and *C. purpurea*, then from the tall fescue endophyte, *N. coenophialum,* and a *Neotyphodium* sp. (Lp1) from perennial ryegrass. There were two *dmaW* genes in *N. coenophialum*, but only one in Lp1. Marker-exchange mutagenesis of *dmaW* in Lp1 eliminated production of ergovaline as well as simpler ergot alkaloids, confirming the role of the *dmaW* gene. Mutagenesis of toxin-production genes in this manner presents opportunities to develop forage grass cultivars with protective endophytes that lack anti-livestock activities. The effects that knocking out ergot alkaloid production may have on other endophyte-enhanced fitness traits, such as protection from insects, can also be tested.

DETECTION OF VOLATILE COMPOUNDS INDICATIVE OF FOOD DETERIORATION.

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Volatile molecules indicative of food spoilage are present at an early stage. Conventional analytical techniques are generally inadequate and human olfactory perception is too insensitive and unreliable to predict incipient food degradation accurately. Biosensors are thus widely sought by the food processing and retail industries for early warning of loss of quality and safety.

Insects have evolved exquisitely sensitive olfactory sensors on their antennae and can perceive, with great specificity, very low concentrations of airborne volatiles, e.g. odours from over-ripe fruit or rotting meat. Electrophysiological recordings demonstrated that the fruit fly *Drosophila melanogaster* has olfactory cells which can discriminate between a healthy fruit and one which, although visually perfect, will begin to deteriorate within a few days. Similar specificity and sensitivity were found for the blowfly *Calliphora vomitoria* with volatiles from fish meat, either fresh or in an early state of deterioration through interrupted cold storage.

A biosensor incorporating preparations from insect antennae could comprise either a live insect or, in the longer term, molecular recognition proteins. The ability of insects to detect a wide range of volatile compounds could be exploited to provide biosensors relevant to the food industry and, indeed, having many other industrial applications.

BIOASSAY METHODS FOR PLANT VOLATILES AS ATTRACTANTS FOR *HELICOVERPA* MOTHS.

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The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is the key pest of cotton and many other summer crops in Australia. Due to problems with insecticide resistance as well as increasing costs of insecticides and environmental concerns, alternative methods such as semiochemicals are needed to complement the integrated approach to management of this pest. Our project investigates attractants based on plant volatiles in combination with a feeding stimulant and small amount of toxicant, as an attract-and-kill system for male and female moths.

We employed various laboratory and field techniques to evaluate plants, single plant volatile compounds and volatile blends for attractiveness to *H. armigera* moths. We have screened about 40 plants, which were both hosts and non-hosts of *H. armigera* using a two-choice olfactometer system in the laboratory. Volatile compounds that were in common in the most attractive plants were tested for moth attractiveness singly or as blends in the olfactometer. We also used electroantennography to test whether the antennal responses of female moths to single volatiles and blends correlated with attractiveness observed in the olfactometer.

Chemical blends that were found to be attractive in the olfactometer were then tested as lures in AgriSense® canister traps in the field. Feeding experiments using laboratory-reared moths in cages were conducted to see if moths would ingest these blends mixed with feeding stimulant and toxicant. This was followed by field wind tunnel experiments to test attractant formulations under field-like conditions using captive moths which were released in the 4m-long tunnels before sunset. Dead moths in the tunnels were counted the following morning. Finally, open field trials were done by spraying vegetative sweet corn and beans with the attract-and-kill formulations late in the afternoon. Effectiveness of these formulations was determined by the number of dead moths found on the treated and adjacent rows of plants over several days after spraying.

The advantages and disadvantages of these field and laboratory methods for bioassaying attractants for noctuid moths, based on plant volatiles, will be discussed.

THE ROLE OF PINE DITERPENE RESIN ACIDS IN CAVITY TREE SELECTION BY THE RED-COCKADED WOODPECKER

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The Red-cockaded Woodpecker (*Picoides borealis*) is an endangered, cooperatively breeding woodpecker that nests almost exclusively in living pine trees. Nest cavity excavation may take up to 6 years, with the breeding male selecting nest trees based on a number of characteristics including: sapwood thickness, resin yield, tree age and size. If available, woodpeckers will also nest in artificial cavity inserts installed in trees by biologists [1].
When nests are established in either natural or artificial cavities, the woodpeckers make and maintain resin wells around the cavity hole that ooze resin for approximately 5 years. The resulting resin sheet inhibits nest predation by rat snakes [2].
To further understand this unique interaction, we tested two hypotheses. (1.) If woodpeckers select trees for excavation based on specific resin chemistries, then the naturally selected cavity trees should have a different chemical profile than either artificially selected cavity or control trees. (2.) If woodpecker resin well activity alone induces changes in resin chemistry, then the active artificial cavity trees should have a different chemical profile than the controls.
The resin chemistry of loblolly (*Pinus taeda,* n=36) and shortleaf (*P. echinata*, n=16) pines from 13 active naturally excavated cavity trees, 13 active artificial cavity trees and 26 similar control trees was analyzed using gas chromatography. Monoterpene and diterpene methyl ester peaks were identified using GC-MS.
Isopimarate, levopimarate and palustrate were difficult to separate chromatographically and were quantified as a single peak. Mean concentrations of this methyl ester mix from the naturally selected cavity trees was approximately 20% greater than controls and 22% greater than trees with artificial cavity inserts ($F_{2, 91} = 3.50$; $P = 0.034$). None of eight other resin components differed among treatments ($P>0.05$). Resin composition from trees with active resin wells did not differ from controls. Likewise, mean concentrations of diterpene methyl esters from the artificial cavity trees were not different from control trees.
These data suggest that woodpecker resin well activity alone does not induce changes in the composition or concentration of xylem pine resins. The Red-cockaded Woodpecker, however, may select pines with specific resin properties for nest cavity excavation.

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CHEMICAL ATTRACTION OF NUISANCE SPECIES OF YELLOWJACKET WASPS (HYMENOPTERA: VESPIDAE).

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Although yellowjackets are highly beneficial insects because they prey on a variety of pest insects, when disturbed by man or animals they can inflict painful, life-threatening stings. Therefore, it is desirable to eliminate nuisance species of yellowjackets from areas of human activity. A very effective chemical attractant (heptyl butyrate) is available for *Vespula pensylvanica* which is the main nuisance species in the western U.S. Neither heptyl butyrate nor related compounds are effective attractants for eastern U.S. species of yellowjacket wasps. Acetic acid with isobutanol (or similar alcohols) more effectively attracts yellowjackets and paper wasps common in the eastern U.S. (Landolt, 2000, U.S. Patent Serial No. 6,083,498), but this blend also attracts substantial numbers of wasps that are beneficial and not usually harmful to man or animals. Simple blends of volatile chemicals will be reported which act synergistically with the aforementioned patented wasp attractant to greatly increase attraction of queens and workers of *V. vulgaris* spp. group yellowjackets (*V. maculifrons*, *V. germanica*, *V. vulgaris* and *V. flavopilosa*) in the eastern U.S. An attract and kill trapping system using the combined lures will be described for suppression of nuisance yellowjackets.

BIOSYNTHETIC STUDIES OF LADYBIRD DEFENSIVE ALKALOIDS.

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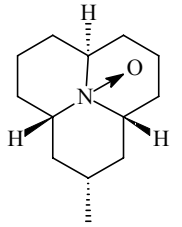
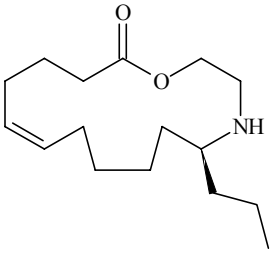
When molested or disturbed, ladybirds fall into thanatosis and exude a yellowish fluid from their tibio-femoral articulations, the so-called "reflex bleeding" [1], [2]. It is now well established that many coccinellids owe their protection, at least in part, to the presence of repellent and, in some cases, toxic alkaloids in this fluid [1], [2]. Over 50 alkaloids have been isolated and characterised from ladybirds up to now, including perhydroazaphenalenes (e.g. coccinelline **(1)**), azamacrolides (e.g. epilachnene **(2)**) or homotropanes (e.g. adaline **(3)**) [1], [2].



(1)



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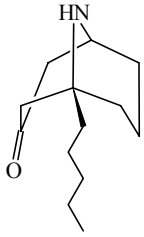

(3)

Although these alkaloids have been the subject of many biological and synthetic studies, only a few investigations have focused on the elucidation of the pathways through which they are biosynthesised. In *Coccinella septempunctata*, coccinelline **(1)** was shown to be labelled after the beetles had been fed with [1-^{14}C] and [2-^{14}C]acetate. Results of degradation experiments on the labelled coccinelline were in agreement with a polyacetate origin for that compound [3]. More recently, radioactive adaline **(3)** was obtained after feeding the same labelled precursors to *Adalia bipunctata*. Chemical degradation of this sample led also to the conclusion that adaline must derive from the condensation of seven acetate units [4]. However, it was not established if coccinelline **(1)** and adaline **(3)** are biosynthesised through a fatty acid or a polyketide pathway. Moreover, no informations were available concerning the origin of the nitrogen atom and the anatomical localisation of alkaloid biosynthesis in the beetles.
We will present the results of in vitro incubation assays using ladybird tissues, which have enabled us to demonstrate that these two alkaloids are most likely biosynthesised through a fatty acid rather than a polyketide pathway, that glutamine is the preferred source of the nitrogen atom, and that the alkaloid biosynthesis takes place in the insect fat body [5].

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KAIROMONAL RESPONSES BY WOODBORERS TO BARK BEETLE PHEROMONE BLENDS AND DIFFERENTIAL ACTIVITY OF INDIVIDUAL COMPONENTS

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Male and female *Monochamus scutellatus* (Say), *M. clamator* (LeConte) and *M. obtusus* Casey are able to detect bark beetle pheromone components electrophysiologically. Traps baited with a blend of bark beetle pheromone components (frontalin, ispenol, ipsdienol, and MCH) in combination with host volatiles attracted significant numbers of *M. clamator*, *M.. obtusus*, *M. notatus* (Drury) and *M. scutellatus*. Traps baited with host volatiles in combination with a second blend composed of *exo*- and *endo*-brevicomin, *cis*- and *trans*-verbenol, and verbenone caught no more beetles than unbaited traps or traps baited with the host blend alone. Traps baited with the first pheromone blend alone or both pheromone blends together captured significantly more *M. scutellatus* and *M. clamator* than unbaited traps, demonstrating a response to bark beetle pheromones in the absence of host volatiles. When components from both pheromone blends were tested individually only traps baited with ipsenol and/or ipsdienol together with the host volatiles ethanol and α-pinene caught significantly more male and female *M. scutellatus* and *M. clamator* than traps baited with host volatiles alone. Ipsenol and ipsdienol are aggregation pheromones of secondary bark beetles in the genus *Ips* DeGeer while the remaining components are pheromones of primary bark beetles in the genus *Dendroctonus* Erichson. The former should be the most reliable indicator of suitable host material because most *Ips* spp. attack weakened or moribund trees or trees already successfully under attack by primary bark beetles, while most *Dendroctonus* spp. Attack healthy or stressed trees and attacks are not always successful. Consequently *Ips* pheromones may be more persistant in space and time than those of *Dendroctonus* spp. In two successive years in an operational mass-trapping program, traps baited with ethanol, α-pinene, and ipsenol captured twice as many beetles as traps baited with host volatiles alone. These results suggest that operational monitoring or mass-trapping programs could be improved significantly by the inclusion of ipsenol in baits at a minimal cost.

MECHANISM OF SEXUAL ISOLATION BETWEEN THE TWO SPECIES OF *ADOXOPHYES ORANA* AND *A*. SP. IN KOREA.

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Adoxophyes orana and *Adoxophyes* sp. have not been clearly distinguished yet as a separate species in Korea. But recently, through more information about sex pheromone and ecology of *Adoxophyes*, it may be separated into two *Adoxophyes* species. *A. orana* attacked apple, peach and pear trees mainly in the central and southern part of South Korea and *A*. sp. attacked on tea and pear trees in southern part of Korea. Their distributional regions overlap each other in the southern part of Korea. The mechanism of sexual isolation between *A. orana* and *A*.. sp. may be due to differences in characters, such as sex pheromone composition. GC-MS analysis on sex pheromone gland extracts of *A. orana* showed the unique sex pheromone composition, different from that of her neighboring countries, 95:5 ratio between *Z*11-14:Ac and *Z*9-14:Ac but 69:31:3:42 ratio between *Z*11-14:Ac, *Z*9-14:Ac, 10me-12:Ac and *E*11-14:Ac in *A*. sp. In behavioral and field trapping assays, *A. orana* males were attracted mainly to 95:5 ratio between *Z*11-14:Ac and *Z*9-14:Ac, but *A.* sp. to various ratios of the two components. A component, 10me-12:Ac, enhanced the attractiveness of *A*. sp. dramatically when added to blend of *Z*11-14:Ac and *Z*9-14:Ac, but not in *A orana*. Another component *E*11-14:Ac, detected in *A*. sp. seemed to play an antagonistic role in male attraction of *A. orana*. The diel rhythms of activities in mating and male response to pheromone trap of *A. orana* and *A.* sp. also were different, most *A. orana* mated before lights-on but *A*. sp. started to mate immediately after lights-on under 16L:8D photo regime at 26. And the concentrations of sex pheromone in both species were peaked at their own mating period. In mating choice experiments in laboratory, both species showed tendencies to the conspecific mating, although, heterospecific mating between males of *A*. sp and females of *A. orana,* took place. The sexual isolation between the two species dues not seem to be complete yet, because the progenies obtained from heterospecific mating successfully reproduced. However, in addition to variation of sex pheromone composition, apparent differences in esterase isozymes pattern and mitochondrial DNA sequences between the two species imply the isolation of these two species is almost normal in nature. So it might be a good case for studies on speciation and evolution.

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EVOLUTION OF SEXUAL COMMUNICATION IN TWO CLOSELY RELATED MOTH SPECIES

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There has been an ongoing debate among evolutionary biologists about the processes that enable evolution of complex traits, where the intermediate states between lack of the trait and presence of the perfected trait are selected against. A good example of a complex trait is sexual communication in insects. Within a population, females with atypical sex pheromone blends have been shown to be less attractive to males than females with the population's common blend. Similarly, rare males that respond to atypical pheromone blends are expected to be at a disadvantage in finding mates. On a simple theoretical level, this type of sexual communication system is expected to be evolutionarily constrained or static because an individual with a mutation leading to an altered blend or response will be selected against, when rare. However, the diversity of sex pheromones used by lepidopterans for sexual communication stands as evidence that evolution has not been stymied. Either our understanding of mating success is incomplete or moth population structure enables evolution through adaptive valleys.

In this project we examine whether the evolution of sexual communication in two moth species may be the result of Fisherian selection or a combination of genetic drift and selection, in a process resembling that of the three phase, shifting balance theory proposed around 1930 by Sewall Wright. These alternatives are examined by moving sexual communication genes (through repeated backcrossing) between two closely related moth species that can be hybridized, i.e., *Heliothis virescens* (Hv) and *H. subflexa* (Hs). Molecular markers are used to map quantitative trait loci (QTL) that control the relative concentration of a number of female pheromone components and male responses that differ between the two species. First results will be shown of mating fitness differences between the parental species and backcross individuals, where we have determined whether backcross females attract males similarly as the parental females, and whether the mating success of backcross males is comparable to that of the parental males. These fitness estimates will be used in setting parameters of general and specific evolutionary models to determine population structures that would be needed to enable the fixation of these traits.

DUFOUR'S GLAND SECRETION - A NEW HONEYBEE QUEEN SIGNAL

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Caste specific pheromones are known to function in many colonial activities in honeybees. We recently discovered that Dufour's gland, a gland associated with the sting apparatus, exhibits such caste specificity. Queen glands are more copious and exhibit higher chemical diversity than worker glands. While queenright worker glands contain only odd n-alkanes, queen glands also possess long-chain esters. However, glandular expression is plastic since queenless egg-laying workers produce the major queen-characteristic esters. The amount of secretion in egg laying workers is correlated to the degree of ovarian development. Moreover, the esters are preferentially expressed over the hydrocarbons, contributing largely to the increase in the secretionary volume.

Dufour's gland secretion may function as a component of a complex queen signal that, along with the other sources control, directly or indirectly, colonial activities. To investigate any possible releaser effect of Dufour's gland components we conducted a bioassay for assessing worker attraction to the secretion.. The bioassay demonstrated that queen, but not queenright worker secretion is attractive to workers and that they form a retinue around the source. Moreover queen like secretion of queenless egg-laying workers also elicited significant attraction. Of the glandular components the esters, but not the hydrocarbons, were responsible for the retinue-eliciting behavior.

Notwithstanding the retinue-eliciting function of Dufour's gland secretion it may also serve other functions in the complex queen-worker interactions. The maintenance of reproductive dominance by a single queen in the honeybee colony is more complex than a one-pheromone one-signal system. It can, for example, signal the workers about queen quality - queen fecundity. Supercedure of an old, less fecund queen is common in honeybees as in other eusocial Hymenoptera. Queen signal occurrence in queenright workers, on the other hand, may disclose their potentiality as egg-layers and therefore induce aggression towards them by nestmates.

STUDIES ON BIOSYNTHETIC PATHWAYS OF MALE`S MARKING PHEROMONE OF BUMBLEBEE

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Marking pheromone of a bumblebee male (MMP) is produced by the cephalic part of the male's labial gland. During the premating behaviour, patrolling males scent-mark their territories to attract conspecific females for mating. Each bumblebee species produces a specific blend of compounds. The gland secretions contain mostly two structural classes: presumably fatty acids derived straight chain compounds and diverse terpenoids. Based on analyses of diverse MMP it was suggested [1] that strait chain compounds are produced from saturated fatty acids by the action of specific glandular enzymes. Despite a relatively large number of papers dealing with biosynthesis of lepidopteran pheromones, there are no experimental data available on biosynthetic pathways of the pheromones formation in bumblebees.

Biosynthetic pathways of the formation of MMP of three bumblebee species, *Bombus terrestris*, *B. lapidarius* and *B. lucorum*, were studied using deuterium labelled fatty acids (LFA) precursors. The solutions of LFA were injected either into the head capsules and/or into abdomens of bumblebees and labeling of the MMP followed using GC-MS.

In *Bombus terrestris*, producing predominantly terpenoids, there is ethyl dodecanoate in high amounts in labial gland. When the LFA ($[^2H_{31}]$-hexadecanoic acid (**1**), $[^2H_{27}]$-tetradecanoic acid, $[^2H_{23}]$-dodecanoic acid) were incubated for 1-2 days the corresponding labeled ethyl esters were detected in the labial gland. In *B. lucorum* **1** was also esterified to the corresponding ethyl ester, however in *B. lapidarius*, that MMP did not contain ethyl esters, no esterification took place.

After application of **1** the corresponding deuterium-labelled pheromone analogues were detected in the labial gland extracts of males of both species. In *B. lucorum*, ethyl $[^2H_{29}]$-hexadec-9-enoate was identified. In *B. lapidarius*, $[^2H_{31}]$-hexadecan-1-ol and $[^2H_{29}]$-(*Z*)-hexadec-9-en-1-ol were found in the labial gland. Furthermore, the deuterated precursor was predominantly built into triglycerides of the fat bodies of males.

These results indicate that common lipids found in the body are most probably used as a pool material for semiochemicals and they are transformed by glandular specific enzymes into MMP components of the male's labial glands.

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THE BIOLOGICAL ROLE OF THE PLANT VOLATILE (-)-GERMACRENE D IN HELIOTHINE MOTHS. ELECTROPHYSIOLOGY AND BEHAVIOUR.

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The complexity of the volatile mixtures released by plants has made it difficult to identify the components that are biologically relevant odorants for various organisms. In our laboratory we have collected naturally produced plant volatiles by headspace procedures. These mixtures have been tested by the use of gas chromatography linked to electrophysiological recordings from single cells (GC-SCR) with different capillary GC-columns.
Recordings from the females of the polyphagous American tobacco budworm moth *Heliothis virescens* have demonstrated a major type of olfactory receptor neurones (ORNs) on the antennae, responding primarily to the sesquiterpene germacrene D [1] and weakly to a few structurally similar sesquiterpenes. No responses were obtained to other compounds in the various mixtures of host and non-host plants. The same ORN type with has been identified in the polyphagous cotton bollworm moth *Helicoverpa armigera* and the oligophagous oriental tobacco budworm moth *Helicoverpa assulta*. All of the germacrene D ORNs showed the same enantioselectivity; (-)-germacrene D having ten times stronger effect than the (+)-enantiomer [2].
The behavioural significance of this plant odorant was tested in a two-choice wind-tunnel. The experiments showed that (-)-germacrene D is attractive to mated *H. virescens* females [3]. They preferred the host tobacco with dispensers releasing (-)-germacrene D and laid more eggs on these plants as compared to tobacco plants with control dispensers.
In addition to the (-)-germacrene D ORNs, 16 other functional types of ORNs detecting plant volatiles have been identified, showing narrow tuning and no overlap of the molecular receptive range [1,2,4]. According to the 62 ordinary glomeruli present in the antennal lobes of these species, one may expect that more ORN types exist on the antennae [5]. Altogether the results of this study have demonstrated that the plant ORNs are as sensitive and selective as the pheromone receptor neurones. The large portion of the ORNs on the antennae tuned to (-)-germacrene D implies the importance of this attractive plant volatile to the moths. However, it is likely that the other ORN types also contribute with information about more plant volatiles influencing the behaviour of the moths.

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Poster Abstracts

NOVEL TERPENOID AND ACETOGENIC MACROLIDES FROM THE SCENT GLANDS OF TROPICAL BUTTERFLIES

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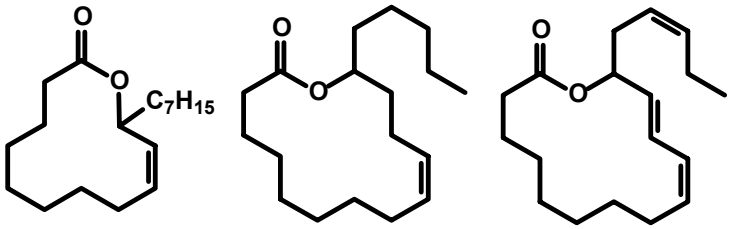
The hairpencil extract of the african danaine butterfly *Amauris niavius* contains a complex mixture of hydrocarbons, aromatic compounds, terpenoids, and fatty acids. Meinwald et al. identified danaidone and 3,4-dimethoxy acetophenone as the main haipencil components in 1974 [1]. A GC/MS analysis of haipencil extracts showed the presence of at least two so far unknown sesquiterpenes. Isolation, high resolutionNMR experiments and chemical derivatizations revealed that these compounds are macrolides derived from farnesol. The structures of these compounds, which we call niaviolide and epoxyniaviolide, were established by total synthesis. Additionally, the absolute configuration of epoxyniaviolide was determined by its asymmetric synthesis and GC experiments on a chiral stationary phase.



1 **2** **3**

The scent organs of *Heliconius cydno* contain long chain fatty acid esters and their derived macrolides. The major component was isolated by Miyakado et al. and identified as (9*Z*,11*E*,13*R*)-9,11-octadecadien-13-olide [2]. Our investigation by GC/MS revealed the presence of other macrolides as well, with one or three unsaturations. Hydrogenation confirmed the ring sizes of the original macrolides to be 12- or 14-membered. Fragmentation pattern and the assumption that those macrolides are derived from oxidised oleic or linolenic acids lead to several structural proposals. Based on these investigations octadec-9-en-11-olide **1**, octadec-9-en-13-olide **2** and 9,11,15-octadecatrien-13-olide **3** were synthesised and their structures were proved. The absolute configuration of the macrolides was determined by stereoselctive synthesis and chiral GC.

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NOVEL HYDROCARBONS AND KETONES IN THE PARACLOACAL GLAND SECRETIONS OF ALLIGATORS

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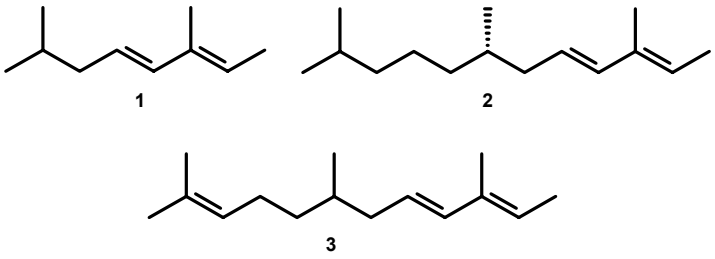
All crocodilians possess a pair of paracloacal glands, which are integrated in the cloacal walls. The secretions produced in these exocrine organs, which are assumed to contain pheromones, may play a role in mate finding or marking of nest sites. Earlier analyses showed that the glandular secretion are made up of a complex mixture of lipophilic compounds which composition may vary with the animals age and sex [1].



We have investigated the glandular secretions of the alligatorids *Paleosuchus palpebrosus* and *Alligator sinensis* by gas chromatography/mass spectrometry (GC/MS). Besides several known esters of terpenoids several unknown terpene hydrocarbons were present. The mass spectra suggested structures containing a rare trisubstituted 2,4-diene system. The monoterpene (2*E*,4*E*)-3,7-dimethyl-2,4-octadiene (**1**) and the sesquiterpenes (2*E*,4*E*,7*S*)-3,7,11-trimethyl-2,4-dodecadiene (**2**) have not been reported from other natural sources so far, while (2*E*,4*E*)-3,7,11-trimethyl-2,4,10-dodecatriene (**3**, caparratriene) is known from a tropical tree. All compounds were synthesized to verify the proposed structures and for the elucidation of their absolute configuration.
Furthermore, the GC/MS analysis of the paracloacal gland secretion from *Caiman crocodilus* revealed the presence of a family of new acyclic ketones. We will report here on their structure and synthesis.

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EXPLOITING PLANT SECONDARY METABOLISM IN INSECT PHEROMONE PRODUCTION

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Semiochemicals, particularly pheromones, can be used with great success as components of insect pest management strategies. However, in many cases, difficulties in achieving cheap and efficient synthesis starting from fine chemicals have obstructed commercialisation, particularly in resource-poor countries. Higher plants offer tremendous potential as cheap and renewable resources for the production of semiochemicals through the wide array of secondary metabolites that they can generate. This potential is demonstrated through the subsequent development of plant-based alternative production and delivery routes for insect pheromones identified earlier at IACR-Rothamsted:
Aphid sex pheromone cyclopentanoids, (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*, 7a*R*)-nepetalactol, from *Nepeta* spp. (Lamiaceae), for commercial production of aphid parasitoid kairomones [1].
The *Culex* spp. mosquito oviposition pheromone, (5*R*,6*S*)-6-acetoxy-5-hexadecanolide, *via* conversion of a Δ^5-fatty acid in the seed oil of the summer cypress plant, *Kochia scoparia* (Chenopodiaceae) [2].
A male-produced sex pheromone of the sandfly, *Lutzomyia longipalpis*, 9-methylgermacrene-B, *via* conversion of a sesquiterpenoid from the cranesbill plant, *Geranium macrorrhizum* (Geraniaceae).

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE 4 STREOISOMERS OF 4-METHYL-3-HEPTANOL, THE MAJOR PHEROMONE COMPONENT OF *SCOLYTUS AMYGDALI*

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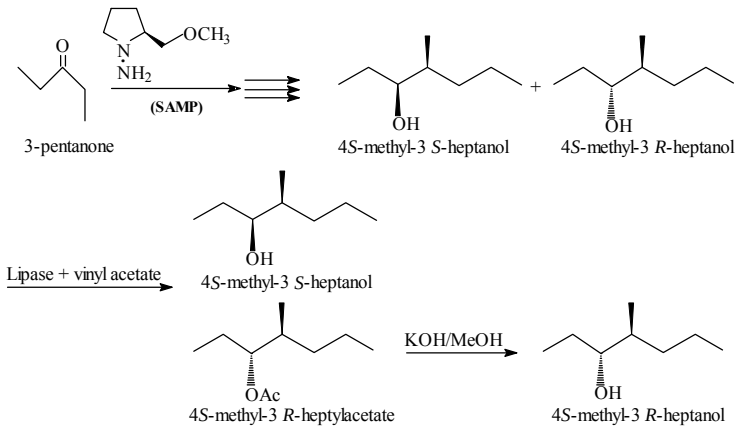
Four EAG-active compounds have been recently identified in airborne collections of the aggregation pheromone of *Scolytus amygdali*. The major compounds of the aggregation pheromone were tentatively identified as 4*S*-methyl-3*S*-heptanol (4*S*M,3*S*HP) and 4*S*-methyl-3*S*-hexanol (4*S*M,3*S*HX). The main component, 4*S*M,3*S*HP, was attractive alone, while 4*S*M,3*S*HX displayed synergistic activity. Highest beetle captures were obtained with a 2 : 1 mixture of 4*S*M,3*S*HP and 4*S*M,3*S*HX. The same mixture containing racemic 4M,3HP gave lower trap captures [1].

To confirm the configuration of the main component of the aggregation pheromone we synthesized the four steroisomers of 4M,3HP and evaluated their biological activity in the orchard.

The starting materials for the two pairs of diastereoisomeric 4M,3HP were 4*S*-methyl-3-heptanone and 4*R*-methyl-3-heptanone which were prepared from 3-pentanone and the SAMP and RAMP reagents respectively [2,3]. Reduction of 4*S*-methyl-3-heptanone with $LiAlH_4$ produced the diastereomeric pair of 4*S*-methyl-3*S*-heptanol and 4*S*-methyl-3*R*-heptanol. Transesterification of this pair with vinyl acetate catalyzed by Lipase enzyme afforded a mixture of 4*S*-methyl-3*S*-heptanol and 4*S*-methyl-3*R*-heptylacetate which were readily separated by chromatography. The ester was hydrolyzed to 4*S*-methyl-3*R*-heptanol. The same procedure was employed to obtain 4*R*-methyl-3*R*-heptanol and 4*R*-methyl-3*S*-heptanol (Scheme 1).



Scheme 1

Traps baited with a 2 : 1 mixture of 4*S*M,3*S*HP and 4*S*M,3*S*HX caught 76 beetles per trap per week, while traps loaded with baits where 4*S*M,3*S*HP was replaced with the one of the other three streoisomers of 4M,3HP, caught 5.3-8.9 beetles per trap per week. These findings prove that 4*S*M,3*S*HP is indeed the natural component of the pheromone of *S. amygdali*.

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SYNTHESIS OF (2*R*)- AND (2*S*)- 2-METHYL-1,6-DIOXASPIRO[4.5]DECANE: A COMPONENT OF THE PHEROMONE SYSTEM OF *PARAVESPULA VULGARIS*

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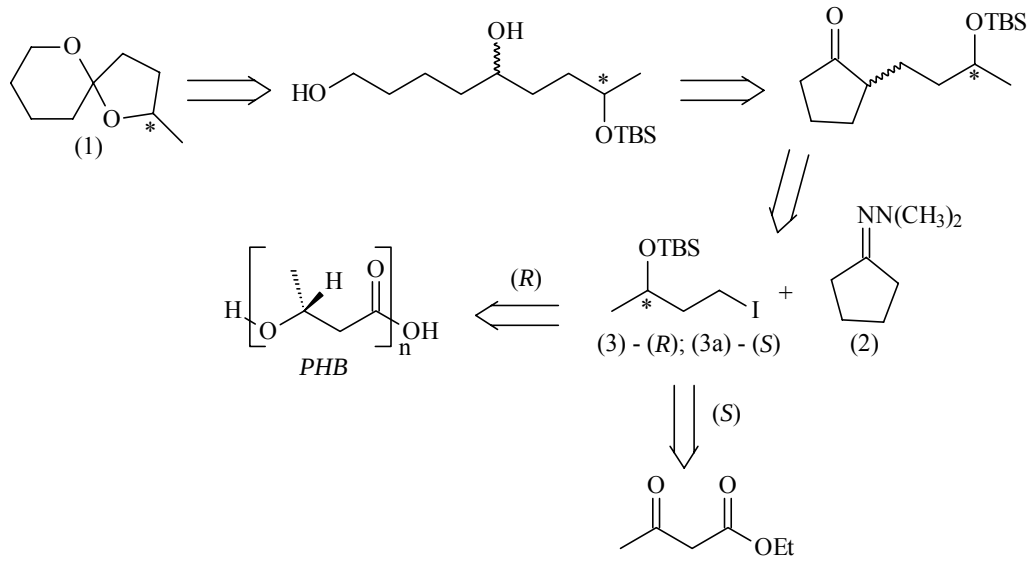
The compound 2-methyl-1,6-dioxaspiro[4.5]decane **(1)** was identified by Francke and co-workers[1] as a component of the pheromone system of the North America wasp *Paravespula vulgaris*.. This work describe a stereoselective synthesis of two isomers of compound **(1)**, employing the alkylation of hydrazone **(2)**, derived from cyclopentanone, with the chiral iodides **(3)** and **(3a)**, as the key reaction.



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SYNTHESIS OF ISOROBINAL, A CYCLIC MONOTERPENOID FROM AN ACARID MITE, *RHIZOGLYPHUS* SP.

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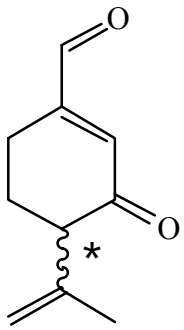
Isorobinal [4-isopropenyl-3-oxo-1-cyclohexene-1-carbaldehyde] with positive rotation has been identified as a novel cyclic monoterpenoid from an acarid mite *Rhizoglyphus* sp. [1] The compound is widely distributed not only among genus *Rhizoglyphus* but also sporadically among other Astigmata. Its biological activity is obscure at present. Furthermore, no attempts have ever been made to determine the absolute configuration of the natural product in each species. Herein we report the simple syntheses of both enantiomers to determine the stereochemistry.



Isorobinal

(*R*) and (*S*)-isorobinal were synthesized in 7 steps starting each from (*S*)- and (*R*)-perillyl alcohol commercially available, respectively. Both products, $[\alpha]_D$ –45° (*c* = 1.3, $CHCl_3$) for (*R*)-isorobinal, and $[\alpha]_D$ +51° (*c* = 1.0, $CHCl_3$) for (*S*)-isorobinal, were enantiomerically pure [(*R*)-isomer: 88% *ee*, (*S*)-isomer: > 95% *ee*] as measured by an HPLC on a chiral column. From the result, the absolute configuration of isorobinal from *Rhizoglyphus* sp. was concluded to be (*S*). Chromatographic behavior of both enantiomers on the present chiral column will enable us to determine the absolute configuration of isorobinal of the other mite origin in future.

[1] Sakata, T. and Kuwahara, Y.; *Naturwissenschaften*, **83**; (1996), 427

CITRAL-DERIVED SYNTHETIC ISOPRENOID FURANONES AS APHID ANTIFEEDANTS.

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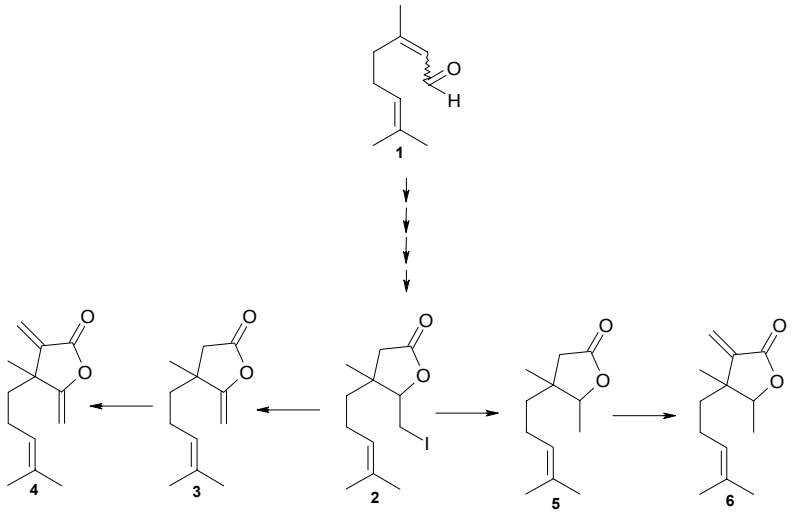
Insect behaviour-modifying chemicals, such as repellents, oviposition inhibitors, and antifeedants have attracted a lot of attention as crop protection agents that, at least in part, might replace conventional insecticides. The most important discoveries included terpenoids of plant origin. From practical point of view, the synthetic analogues of natural compounds are more accessible for application.

The furanone terpenoids (**2**-**6**) were obtained in five or six step synthesis from citral (**1**). γ-Methylene lactone 3 was obtained by dehydrohalogenation of iodolactone **2** with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene). Lactone **5** was the product of reductive (n-Bu_3SnH) dehalogenation of the same iodolactone. α-Methylene lactones **4** and **6** were synthesized by decarboxylative methylenation of lactones **3** and **5** respectively.



The biological tests for antifeedant activity of studied compounds required a three-step approach due to the specificity of aphid feeding. Aphids lack external contact chemoreceptors: the taste organ is located in the hypopharynx and the ingestion of phloem sap is crucial for the recognition and acceptance of the host plant.

Tested compounds were applied to adaxial surface of a leaf as a 0.1 % ethanolic solution, 0.01ml/cm^2 of the leaf.

1. Aphid settling – the half-leaf test: compounds were applied on one half of the leaf, the other side of the midrib acted as a control. Aphids settled on each side of the midrib were counted at 15', 30', 1h, 2h, and 24h intervals (8 replicates, 20 apterous adult aphids/replicate).
2. Aphid behaviour - direct observation: the behaviour of freely moving aphids was recorded for 15 min (16 aphids/compound).
3. Aphid probing - electronic recording of probing (**E**lectrical **P**enetration **G**raph technique): aphid behaviour during probing was monitored for 8 hours continuously (16 aphids/compound).

Antifeedant activity was shown by citral-derived synthetic isoprenoid furanones: **4, 5**, and **6.**

MECHANISM OF SEXUAL ISOLATION BETWEEN THE TWO SPECIES OF *ADOXOPHYES ORANA* AND *A*. SP. IN KOREA.

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Adoxophyes orana and *Adoxophyes* sp. have not been clearly distinguished yet as a separate species in Korea. But recently, through more information about sex pheromone and ecology of *Adoxophyes*, it may be separated into two *Adoxophyes* species. *A. orana* attacked apple, peach and pear trees mainly in the central and southern part of South Korea and *A.* sp. attacked on tea and pear trees in southern part of Korea. Their distributional regions overlap each other in the southern part of Korea. The mechanism of sexual isolation between *A. orana* and *A.*. sp. may be due to differences in characters, such as sex pheromone composition. GC-MS analysis on sex pheromone gland extracts of *A. orana* showed the unique sex pheromone composition, different from that of her neighboring countries, 95:5 ratio between *Z*11-14:Ac and *Z*9-14:Ac but 69:31:3:42 ratio between *Z*11-14:Ac, *Z*9-14:Ac, 10me-12:Ac and *E*11-14:Ac in *A.*. sp. In behavioral and field trapping assays, *A. orana* males were attracted mainly to 95:5 ratio between *Z*11-14:Ac and *Z*9-14:Ac, but *A.* sp. to various ratios of the two components. A component, 10me-12:Ac, enhanced the attractiveness of *A.* sp. dramatically when added to blend of *Z*11-14:Ac and *Z*9-14:Ac, but not in *A orana*. Another component *E*11-14:Ac, detected in *A.* sp. seemed to play an antagonistic role in male attraction of *A. orana*. The diel rhythms of activities in mating and male response to pheromone trap of *A. orana* and *A.* sp. also were different, most *A. orana* mated before lights-on but *A.* sp. started to mate immediately after lights-on under 16L:8D photo regime at 26□.. And the concentrations of sex pheromone in both species were peaked at their own mating period. In mating choice experiments in laboratory, both species showed tendencies to the conspecific mating, although, heterospecific mating between males of *A.* sp and females of *A. orana,* took place. The sexual isolation between the two species dues not seem to be complete yet, because the progenies obtained from heterospecific mating successfully reproduced. However, in addition to variation of sex pheromone composition, apparent differences in esterase isozymes pattern and mitochondrial DNA sequences between the two species imply the isolation of these two species is almost normal in nature. So it might be a good case for studies on speciation and evolution.

[1] Guerin, P.M.; H. Arn; H.R. Buser and P.J. Charmillot. J. Chem. Ecol., **12**; (1986), 763-772

[2] Sato, R. and Y. Tamaki. Appl. Entomol. Zool. **12**; (1977), 50-59

BIOLOGICAL ACTIVITY OF TWO NEW LEPIDOPTERAN SEX PHEROMONES

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Branched chain alkenes and esters of a long-chain acid and a short-chain alcohol has rarely been reported as female sex pheromone components of order Lepidoptera. Such type of chemical structures has been identified recently as main female sex pheromone components of the herald moth *Scoliopteryx libatrix* L. (Noctuidae) [1] and bagworm moth *Megalophanes viciella* Denis & Schiffermüller (Psychidae) [2] - (6Z, 13)-methylheneicosene and 1-methylethyl octanoate respectively. Electrophysiological and field tests on *S. libartix* males have shown that the active component in the natural female sex pheromone is (6Z, 13S)-methylheneicosene [1].
Biological activity of the main sex pheromone components of the both species were confirmed by additional laboratory and field investigations.
When four possible isomers of 6, 13-methylheneicosene (ZS, ZR, ES and ER) were tested individualy in a wind tunnel, the most active isomer to *S. libatrix* males was (6Z, 13S)-methylheneicosene, followed by the ZR and ES isomers. The lowest behavioural reaction showed the ER isomer. These wind tunnel bioassay results correlate with the results from field tests with sticky traps in Bulgaria.
The main sex pheromone component of *M. viciella*, 1-methylethyl octanoate (MEO), and both enantiomers of four other esters of fatty acid, identified as sex pheromones for two psychid moths (*Thyridopteryx ephemeraeformis* and *Oiketicus kirbyi*), were tested electroantennographically (EAG) on antenna of conspecific males. The highest mean EAG response of the male antenna was registred to MEO. Significant EAG activity elicited the two enantiomers of 1-methylbutyl octanoate and 1-methylbutyl nonanoate. R and S enantiomers of the four esters investigated did not influence the attractiveness of the main sex pheromone component in field tests when applied as a 10 % and 30 % additives to it. The only exception was (R)-1-methylbutyl octanoate, which reduced significantly the catches of *M. viciella* males.

[1] Francke, W., E. Plass, N. Zimmermann, H. Tietgen, S. Franke, T. Tolasch, M. Subchev, T. Toshova, J. A. Pickett, L. J. Wadhams, C. M. Woodcock; J. Chem. Ecol., 2000, 26, 5: 1135-1149.
[2] Subchev, M.,T. Toshova, L. Stanimirova, Gh. Stan, G. Embacher, W. Francke, A. Reckziegel, T. Ferreira, E. Priesner; J. Chem. Ecol., 2000, 26, 2: 487-495.

SEX PHEROMONE SYSTEM IN PSYCHIDAE AND PROCRIDINAE (ZYGAENIDAE) MOTHS

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In most lepidopterans that use pheromones for finding the sexual partner, females have a sex pheromone gland located in the intersegmental membrane between the 8th and 9th abdominal segments and display a specific calling posture for exposing the gland. The usual pheromone compounds in these cases are acetate esters, alcohols, and aldehydes and hydrocarbons with 10-22 carbon atoms per chain.

In representatives of both Psychidae and Procridinae (Zygaenidae) investigated so far, the sex pheromone gland is found in an unusual location for lepidopterans. In *Megalophanes viciella* (Psychidae) the pheromone gland cells were found on the terga of the 2nd and 3rd thoracic and 1st abdominal segments of females. A similar location was found in *Theresimima ampellophaga* (Procridinae) where the pheromone gland cells are located on the terga of the 3-5th abdominal segments in females. However in another psychid moth, *Thyridopteryx ephemeraeformis*, the ventral thorax and the 1st abdominal segments in females were reported to be a site for pheromone synthesis.

In the bagworm females investigated so far there is no a specific calling behaviour and posture because they do not leave the pupa after eclosion. The representative of Procridinae, *T. ampellophaga*, display a calling posture characterized by a dorsally curved abdomen, that coincides well with the site of the pheromone gland.

In both groups the pheromone compounds identified so far are long-chain fatty acid esters. For example the main female sex pheromone component of the psychids *T. ephemaeriformis* and *M. viciella* are 1-methylbutyl decanoate and 1-methylethyl octanoate, respectively, and the main female sex pheromone components of the Procridinae species *Harrisina metallica* (= *H. brillians*) and *T. ampellophaga* are (2S)-butyl (7Z)-tetradecenoate and (2R)-butyl (7Z)-tetradecenoate, respectively.

RIGHT STEREOISOMERS FOR SEX PHEROMONE COMPONENTS OF THE APPLE LEAFMINER, *LYONETIA PRUNIFOLIELLA* IN KOREA.

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Sex pheromone of *Lionetia prunifoliella* (Lepidoptera: Lyonetiidae) was reported to be composed of three components, 10,14-dimethyloctadec-1-ene (10me14me-1-ene-18Hy), 5,9-dimethyloctadecane (5me9me-18Hy) and 5,9-dimethylheptadecane (5me9me-17Hy) by Gries *et al*.. (1997. J. Chem. Ecol. 23: 1119-1130) for the American population. Each component has four different stereoisomers, but their stereochemistry has not been clarified yet. This study was initiated to find out the attractivity of 4 stereoisomers for each component by synthesizing all 12 of them and examining their activity through EAG measurements and field trapping experiments in Korea. Among the four stereoisomers for the major component, 10me14me-1-ene-18Hy, the 10*S*,14*S*-isomer only elicited the strong electrophysiological response on *L. prunifoliella* male. Among 4 stereoisomers for each of the other two minor components of 5me9me-18Hy and 5me9me-17Hy, the 5*S*,9*S*-isomers also elicited the electrophysiological response. Other stereoisomers of the three components were electrophysiologically inactive.
In field trappings, the racemic mixture of 10me14me-1-ene-18Hy, among the three components, could attract *L. prunifoliella* males strongly and the binary or tertiary combinations with racemic mixtures of the other two components were not better in attraction with the 10me14me-1-ene-18Hy only. Among 4 stereoisomers of 10me14me-1-ene-18Hy, the 10*S*,14*S*-isomer only attract *L. prunifoliella* males, as shown in EAG test, and the attractivity of the other 3 isomers was not observed. All stereoisomers of each minor component as well as its racemic component did not seem to give any additional effect on male attraction in Korea.

UNUSUAL ACETYLENIC SEX PHEROMONE OF THE GRAPE LEAFFOLDER, *DESMIA FUNERALIS* (LEPIDOPTERA: PYRALIDAE).

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The female-produced sex pheromone of grape leaffolder, *Desmia funeralis* Hübner (Lepidoptera: Pyralidae), has been identified as a blend of (*Z*,*Z*)-11,13-hexadecadienal, 11-hexadecynal, and (*Z*)-11-hexadecenal. The first two components were essential for attraction of male moths, whereas the third compound was not essential, but increased trap catches approximately twofold when added in appropriate doses to the optimum blend of the other two components. In field tests, male moths were trapped equally well in traps baited with pheromone doses of 0.2 to > 6 mg, and lures remained attractive for at least 5 wk.

MALE SEX PHEROMONE OF ENDOCLITA EXCRESCENS AND RELATED COMPOUNDS: STRUCTURES AND BIOLOGICAL ACTIVITIES.

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Behavioural analysis of Endoclita excrescens mating flight in the dusk indicated that a flying female was attracted to the calling of flying male, the visual cues from a distance and also the male scent from a short distance [1,2]. The touch in flight by a female to a male is a sign that the female recognised the conspecific male.
The male moth has brush organ on its hind legs. The GC-EAD analyses showed that components in brush organ extract evoked the electrophysiological responses to conspecific female antenna. The mass spectrum of main EAD active component was same as that of 1,3,8-trimethyl-2,9-dioxabicyclo[3.3.1]non-7-ene, provided by Professor W. Francke, University of Hamburg. It was reported as one component of scent gland secretion of Hepialus hecta [3]. The stereochemistry of it obtained from Endoclita excrescens was determined to be (1R, 3S, 5S) from the comparison of GC data on chiral capillary column with those of synthetic stereoisomers, provided by Professor K. Mori, Science University of Tokyo.
The hind leg extract and also the synthetic EAD active compound induced the touching behaviour to females in dust mating flights at biological assay experiments in field cage.
These results concluded that (1R, 3S, 5S)-1,3,8-trimethyl-2,9-dioxabicyclo[3.3.1]non-7-ene is the male sex pheromone of Endoclita excrescens.

[1] Kan, E. *et al.*, Appl. Entomol. Zool., **37**:147-153 (2002)
[2] Kan, E. *et al.*, Appl. Entomol. Zool., **37**:163-169 (2002)
[3] Schulz, S. *et al.*, J. Chem.Ecol., **16**:3511-3521 (1990)

RESPONSE OF CURRANT SHOOT BORER *LAMPRONIA CAPITELLA* CL. (LEPIDOPTERA: PRODOXIDAE) TO (Z,Z)-DIENIC PHEROMONE COMPONENTS

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The currant shoot borer, *Lampronia capitella* Cl. (Lepidoptera: Prodoxidae), is one of the major pests of currants in Northern and Central Europe. Its larvae infest the buds in early spring, causing the death of developing shoots. In pheromone gland extracts of calling *L. capitella* female three EAG-active dienic compounds were identified by Löfstedt et al. (2002): (Z,Z)-9,11-tetradecadien-1-ol (I), (Z,Z)-9,11-tetradecadienal (II), and (Z,Z)-9,11-tetradecadien-1-yl acetate (III). To elucidate the role of identified components in pheromone communication of *L. capitata*, field experiments in Estonia, at Rõngu, have been conducted in 2000 – 2002 on red currant plantation. Glued-bottoms deltatraps Atrakon A were used. Substances, synthesized by stereospecific reduction of triple bond and purified by medium-pressure column chromatography, consisted of >98.5% of (Z,Z)- isomer. Stabilized by BHA solutions of substances, used for impregnation of dispensers, had the content of (E,Z)-isomer of 2.3 – 2.6 %. The isomeric purity of compounds did not change during the further storage or the impregnation process. In silicon-based Miniket dispensers, used in described here experiments, no isomerization of the compounds proceeded. All the other tested dispensers, red rubber septa from Thomas Scientific, Illinois, included, caused rapid isomerization and decomposition of compounds (I) – (III).

Field screening demonstrated that (I) alone had rather high attractivity, up to 50% of those for the optimal blend. (II), added to (I) in amounts of 10% (loaded amount of (I) was 100 micrograms), or in amounts of 2.5% (loaded amount of (I) was 400 micrograms), caused about two-times elevation in traps catches. Higher content of (II) did not influence on the trap catch. Addition of (III) to the mixture of (I) and (II), in case of 0.1-mg dose of (I), caused a rise of attractivity on 30% ($P>0.95$), in case of dose of (I) of 0.4 mg, having 2.5% addition of (II), had no effect on attractivity. Role of (III) is not quite clear. (III) alone or mixture of (II) and (III), did not attract *L. capitella* males. In experiments of 2001, 0.1 mg of (III), added to 0.5 mg of 4:1 mixture of (I) and (II), caused the highest attractivity of 26,5± 5.7 per trap per three days. In case of 0.4 mg of (III) was added, the trap catch diminished to 8.0± 2.4 males per trap for the same period. It may be concluded from our experiments that only (I) causes the orientated flight of male *L. capitella* toward the pheromone source, maximum 2.5% adding of (II) to (I) is needed for stable attractivity, but there is lack of optimal ratio of (I) and (II). Small amounts, up to 10% of (III) may result the rise in attractivity, possibly it serves as navigation components of the pheromone blend.

DEVELOPING AN ATTRACTIVE PHEROMONE BLEND FOR THE LEGUME PODBORER, *Maruca vitrata* (F.) (LEPIDOPTERA: PYRALIDAE)

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The legume podborer, *Maruca vitrata* (syn. *M. testulalis*) (F.) (Lepidoptera: Pyralidae) is a pan-tropical pest of a range of subsistence legume crops. Without control measures, flower and seed infestation rates up to 80% and 50% respectively have been reported in cowpea in West Africa [1, 2]. Pheromone-baited traps for *M. vitrata* could make possible monitoring by researchers to develop pest management strategies, and by farmers to time the application of control measures.

Previously, (*E,E*)-10,12-hexadecadienal (EE10,12-16:Ald) was reported as an EAG-active component in extracts from female *M. vitrata* abdominal tips [3], and was highly attractive to male moths in laboratory bioassays. The corresponding alcohol, (*E,E*)-10,12-hexadecadienol (EE10,12-16:OH), was also noted as being present. No field testing of the compounds was carried out.

In the present study sex pheromone was collected by gland extraction or trapping of volatiles from virgin female moths. Analysis by GC-EAG and GC-MS confirmed that (*E,E*)-10,12-hexadecadienal is the most abundant pheromone component with 2-5% of (*E,E*)-10,12-hexadecadienol also present. A monounsaturated hexadecenal isomer at <2% of the major component was also detected. Laboratory wind-tunnel bioassays and field bioassay of blends of (*E,E*)-10,12-hexadecadienal with (*E,E*)-10,12-hexadecadienol and a range of monounsaturated hexadecenal isomers indicated greatest attraction of males was to those including (*E,E*)-10,12-hexadecadienol and (*E*)-10-hexadecenal as minor components.

In subsequent trapping experiments in cowpea fields in Benin, traps baited with a three-component blend of (*E,E*)-10,12-hexadecadienal and the two minor components in 100:5:5 ratio caught significantly more males than traps baited with the major component alone, either two-component blend or virgin female moths. Significant numbers of female *M. vitrata* moths were trapped with synthetic blends but not with virgin females. Further blend and lure optimization experiments did not produce a more attractive blend or eliminate female catches. High isomeric purity was shown not to be critical to attraction in the field, contrary to the findings of [1].

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GEOGRAPHIC VARIATION IN PHEROMONE CHEMISTRY, ANTENNAL ELECTROPHYSIOLOGY AND PHEROMONE-MEDIATED BEHAVIOR OF EASTERN NORTH AMERICAN POPULATIONS OF THE OBLIQUEBANDED LEAFROLLER

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The total and relative amounts of (Z)-11-tetradecenyl acetate (Z11-14:Ac), (E)-11-tetradecenyl acetate (E11-14:Ac), (Z)-11-tetradecen-1-ol (Z11-14:OH) and (Z)-11-tetradecenal (Z11-14:Al), and the response of male antennae to these pheromone gland compounds was compared in laboratory reared *Choristoneura rosaceana* Harris (Leptidoptera: Tortricidae) from British Columbia, Michigan, Ontario, New York and Quebec. A field trapping experiment was conducted in each of these locations to determine the effect Z11-14:Al on the numbers of moths captured. Moths from British Columbia contained the greatest, and those from New York contained the smallest amounts of each of the four pheromone compounds. The relative amount of Z11-14:Ac was greatest in moths from New York and smallest in moths from Ontario. Moths from Ontario, Quebec, British Columbia, Michigan and New York contained decreasing relative amounts of Z11-14:OH and Z11-14:Al. The intercept of the antennal stimulus concentration-response relationship of males from a location was negatively correlated with the average amount of a pheromone compound in females from that location. The behavioral activity of Z11-14:Al was statistically detectable in the results of the trap tests conducted in British Columbia, Ontario and Quebec, but not in those conducted in Michigan or New York. A three compound blend of Z11-14:Ac, E11-14:Ac and Z11-14:OH (97:2:1) with the addition of either 1 or 2% Z11-14:Al is recommended for monitoring *C. rosaceana* in all locations included in the study.

AN ELECTRIC GRID TRAP FOR ATTRACTANT TESTING, MARK-RECAPTURE AND AUTODISSEMINATION APPLICATIONS.

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Electric grid traps have been occasionally used for testing pheromone formulations [1], usually with lethally high voltages and with bulky generator-driven electronics. We have developed a light-weight, 12V battery-powered grid trap which shows great promise for a variety of applications. The trap is up to five times more efficient than conventional canister-funnel type traps, requires minimal servicing attention, and can be used as a non-lethal marking and/or autodissemination device as well as a standard killing trap for use with pheromones and other attractants. Moths are stunned by the electric shock and temporarily lose the ability to fly. They can be captured, or marked and contaminated whilst immobilised in this way. There are no lasting effects and minimal disruption to behavioural patterns if the moth is released immediately after receiving the shock.
Results of trapping experiments with *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) are presented to illustrate the potential uses of this trap design. A mark-recapture experiment using fluorescent dyes was conducted to assess numbers of *H. armigera* in a 21 ha sorghum field, and determine the proportion of the population which could be captured for the purposes of attract-and-kill or autodissemination of pathogens or semiochemicals. Up to 2000 moths per night were marked using 12 such traps, and it was estimated that this constituted up to 8% of the population.

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BIOEFICACY AND PERSISTENCE OF DIFFERENT PHEROMONE BLENDS OF *HELICOVERPA ARMIGERA* (HÜBNER)

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Studies on bioefficacy and persistence of different pheromone blends of *Helicoverpa armigera* (Hübner) were conducted so as to use the pheromone technology in the management of the most dreaded insect pest of several crops in agriculture accounting for the consumption of over 30% of total insecticide all over the world [1]. In the sex pheromone of female *H.armigera* a number of chemical components have been reported [2]. Out of these the major components are Z-11-Hexadecenal and Z-9-Heaxadecenal. The indigenous traps with rubber septa were baited with ratios of 96:4, 97:3, 98:2 and 25:1(Z-11-HDAL and Z-9-HDAL respectively) and used in chickpea field at Pantnagar, India. On the basis of the trap catches the ratio of 97:3 proved most effective followed by 25:1. The trapping effectiveness of these ratios increased up to six days and then slowly decreased. There were very few trap catches after eleven days. GC-analysis of the eluted pheromone components from the septa showed that at the end of one month very small amounts were present and their ratios were changed from the original blends. The reduction in trapping efficiency may be due to decrease in concentration or change in the original blend in the air-born pheromone plume.

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ON-COLUMN HEADSPACE ANALYSIS OF PHEROMONE DISPENSERS

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Measuring the ecochemical communication channel requires precise analysis of effluvia. In our experiments a special collection cell (CC) was used for on-column analysis of dispensers' effluvia. The apparatus used for the experiments enables us to pass the carrier gas to the evaporator following two paths. The first path used for the analysis of effluvia is that built into the gas chromatograph. In the second pathway, used for collection of headspace, the carrier gas proceeds via the CC, which is connected to evaporator through the septa. Gas switcher canalises the carrier gas (He) either to the CC (collection regime) or straight to the evaporator (analysis regime).
Miniket dispensers and filter paper pieces were used as substrates of pheromone compounds. Volatiles, carried by the gas from CC, were adsorbed at the beginning of the capillary column at temperatures of 45 – 80 ºC. We used columns (30 m x 0.32 mm ID) with DB-5 or SP2335, precolumn was of 4 meters.
For the analysis of headspace, two different methods were used. For the first method, the "stationary headspace method", we used a 7.5 ml CC. After the stationary headspace was formed in 0.5 - 2.0 hrs, the collection regime was switched on and after the required collection time analysis followed. At carrier gas velocity of 2 ml/min, total amounts of decanol (10:OH, $A_{10:OH}$) and dodecanol (12:OH, $A_{12:OH}$) increased linearly during the collection time of 0.5 - 1.5 minutes, $A_{10:OH} = 8.6+0.27*t$, R= 0.867, $A_{12:OH} = 8.6+0.103*t$, R= 0.923. Ratio of 10:OH and 12:OH (m_{OH}) was not constant and increased linearly, $m_{OH} = 4.2+0.18t$. At time t=0, m_{OH} was equal to 4.2, which is close to the ratio of first order evaporation constants [1].
Leading the carrier gas through the CC into the heated evaporator and capillary column, long time periods of aging of dispensers may be used to achieve the so-called dynamic headspace. After a rapid cooling of the evaporator and the column, the collection process may be started. Measurements using loaded with 2 mg of 1:1 mixture of dodecenyl acetate (12:Ac) and decyl acetate (10:Ac) Miniket-Si dispensers, showed that even at elevated temperatures (>30ºC) the headspace achieved dynamic equilibrium in some hrs for 12:Ac and in less than one hr for 10:Ac. Emission rates were dependent on temperatures, $m_{12:Ac} = -0.02+3.187*t$, R=0.865, and $m_{10:Ac} = -397 +32.15*t$, R=0.964. As a result, the ratio of substances in effluvia changed from 3.5 at 19 ºC to 7.7 at 38 ºC.
This indicates that stationary headspace, corresponding to Raoul's rule, is achieved duration of different time periods for different compounds, and that the emission from dispensers is limited mainly by diffusion and not by evaporation energy.
Collection of (*Z*,*Z*)-9,11-tetradecadie-1-al, a pheromone component of *Lampronia capitata*, did not demonstrate any increase of geometric isomers content in effluvia in case of Miniket-Si dispensers to compare with the loaded compound.

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HOW DO MALE MOTHS TRADE BETWEEN PHEROMONE-BASED MATE-FINDING AND PREDATOR AVOIDANCE?

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Many insects, including moths, have evolved hearing organs to detect the high-frequency calls produced by echolocating bats. Equipped with ultrasound-sensitive ears and a repertoire of defensive manoeuvres, moths are able to evade attacking bats. Although species of the families Pyralidae and Noctuidae have evolved hearing organs independent of each other, they show similar responses when exposed to attack-sounds produced by bats. In both families, females produce long-range sex pheromone to attract males. Thus, flying males searching for calling females may be under high predation risk by foraging bats. How do they trade between pheromone-based mate-finding and predator avoidance? We have studied the male flight response of two eared moth species, *Plodia interpunctella* (Pyralidae) and *Agrotis segetum* (Noctuidae) when exposed to ultrasound while orientating in a sex pheromone plume in a flight tunnel. Males orientating in sub-optimal odour plumes including incomplete pheromone blends or low concentrations of optimal blends significantly reduced their source contact rate and increased their orientation time to the odour source when compared to unexposed moths. In contrast, males flying towards optimal pheromone blends and doses showed no difference in source contact rate or orientation time to the odour source when compared to unexposed moths. Thus, male moth flying towards an odour signalling a high adaptive value react less to the acoustic cue compared to when flying towards odours signalling a low adaptive value. Our results show that simultaneous exposure of moths to conflicting olfactory and acoustic stimuli with different adaptive values could give important information about integration of multiple sensory modalities as well as behavioural trade-offs by prey under the risk of predation.

INTEGRATED PEST MANAGEMENT OF THE TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* ON HOPS.

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The two-spotted spider mite, *Tetranychus urticae* Koch, is an important pest of hops, *Humulus lupulus*, (Cannabaceae), worldwide. Biological control by inoculative release of the predatory mite *Phytoseiulus persimilis* Athias Henriot, offers good prospects for the long-term management of the pest on dwarf hops, [1] but this predator has seldom produced commercially acceptable control on conventional tall varieties of hops in Europe and USA [1,2]. Hop β-acids, a by-product of hop processing for brewing, have repellent and oviposition-deterring effects on the behaviour of *T. urticae* but are relatively harmless to *P. persimilis* [3,4].

The aim of this work was to investigate the potential of developing a novel control strategy for *T. urticae* comprising biological control with the predatory mite and β-acids. In the field, the combination of β-acids with the predator was more effective than either treatment alone. The highest numbers of spider mites and their eggs were recorded on untreated plots. Application of β-acids slowed the rate of increase of the pest and contributed more to the effect of the combined treatment early in the season when the predator was establishing itself on the crop. By harvest time, the predator was the more influential of the two factors.

T. urticae is polyphagous and an important pest of a wide range of crop and cultivated ornamental plants. Whilst this study demonstrates the efficacy of the combined application of β-acids and predators for spider mite control in hops, the approach may also be exploitable in many other crops and in situations where opportunities for applying synthetic acaricides are limited, for example on ornamentals displayed in enclosed public spaces.

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ATTRACTANTS OF THE SUMMER CHAFER *AMPHIMALLON SOLSTITIALE* (L.) (COLEOPTERA: SCARABAEIDAE).

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The summer chafer, *Amphimallon solstitiale* (L.), is distributed throughout nearly whole Europe and is almost as popular as the well known cockchafer species. The grubs undergo a two- or three-year development in the soil where they feed on roots of several plants, sometimes causing serious damages in lawns.
During the flight period at dusk in late June and July, the females sit motionless on exposed places in the vegetation, while the males perform a remarkably swarming flight during which mate finding occurs. In contrast to the cockchafers, which are known to be attracted to several green leaf volatiles induced by feeding females [1], the summer chafer does not feed during the flight period.
In order to identify the compounds involved in mate location, both head space and whole body extracts were examined using GC-EAD and GC-MS.
Acetoin as well as *rac*- und *meso*-2,3-butanediol, which where present in both male and female extracts, elicited strong responses on male antennae, while they were not recognized by female antennae. In contrast, several green leaf volatiles, which also turned out to be EAD active, were equally well perceived by male and female antennae.
During preliminary field bioassays, the effect of the EAD active compounds on swarming males of *A. solstitiale* was investigated.

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THE AGGREGATION PHEROMONE OF *DINODERUS BIFOVEOLATUS* WOLLASTON (COLEOPTERA: BOSTRICHIDAE).

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The bostrichid beetle *Dinoderus bifoveolatus* WOLLASTON, which is widespread throughout the tropical africa, is an important pest on so called „*cassava chips*“, i.e. dried roots of the manioc plant, *Manihot esculenta* CRANZ (Euphorbiaceae). In addition, the species is quite frequently introduced into other climatic zones with several stored products.
Similar to other bostrichid pests as *Rhyzopertha dominica* (F.) [1] and *Prostephanus truncatus* (HORN) [2], aggregation of *D. bifoveolatus* is induced by a pheromone released by males feeding on the host material [3].
In order to identify the components of this aggregation pheromone, head space extracts of male *D. bifoveolatus* feeding on cassava chips were prepared and examined by GC-MS and GC-EAD. Among the seven male specific compounds, two proved to be highly EAD active. In contrast to the pheromones of *Rhyzopertha dominica* and *Prostephanus truncatus* [1,2], which are represented by low boiling esters of branched, unsaturated fatty acids, the *Dinoderus bifoveolatus* compounds could be identified to be the hydroxy ketone (4*R*,6*S*,7*R*)-4,6-dimethyl-7-hydroxynonan-3-one (**1**) and the shorter homologoue (3*R*,5*S*,6*R*)-3,5-dimethyl-6-hydroxyoctan-2-one (**2**). The structures of **1** and **2** were confirmed by stereoselective synthesis of the corresponding acetates.
The structural similarity between these compounds and (4*S*,6*S*,7*S*)-4,6-dimethyl-7-hydroxynonan-3-one („serricornin“, **3**), the aggregation pheromone of the cigarette beetle *Lasioderma serricorne* F. (Anobiidae) [4], may serve as a further proof for the close relationship between the families Bostrichidae und Anobiidae, which both, together with the Ptinidae und Lyctidae, belong to the superfamilia Teredilia.

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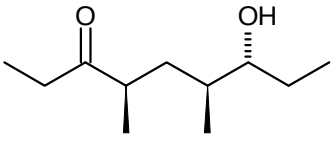

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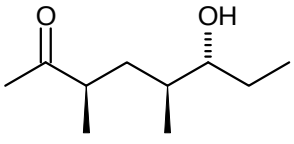

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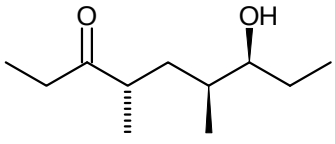

3

SEX PHEROMONE OF THE SWEET POTATO WEEVIL *CYLAS FORMICARIUS*. SYNTHESIS AND BIOLOGICAL ACTIVITY

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The sweet potato ranks seventh among all food crops worldwide with an annual consumption of 115 million metric tons from which up to 98% is produced by developing countries. One of the most important constraints that limit crop production is pre- and post-harvest losses from insects, particularly the sweet potato weevil *Cylas formicarius* (Fabricius). This pest occurs in all parts of the tropics where sweet potatoes are grown and low level infestations can reduce both quality and marketable yield, since the toxic sesquiterpenes produced by the crop tissues in response to insect feeding induces a extremely bitter tasting to the roots.The cryptic feeding habits of the weevil larvae and the nocturnal activity of the adults make difficult for farmers to detect weevil infestations limiting the effectiveness of chemical insecticides.
The sex pheromone of the sweet potato weevil has been identified from virgin females volatiles as (*Z*)-3-dodecenyl (*E*)-2-butenoate by Heath et al. [1] and found to be active in laboratory and field tests. In this communication we present the stereoselective synthesis of the pheromone by two different routes, i.e. direct alkylation of 1-decyne with ethylene oxide and hydrogenation and esterification (route A) and reaction of methylene triphenylphosphorane with ethylene oxide followed by "in situ" metallation and reaction with nonanal in a one-step process to yield (*Z*)-3-dodecenol as a mixture of isomers, followed by esterification (route B). In dual-choice olfactometer tests the highest activity of males has been found between the fourth and sixth hour into the scotophase at a pheromone concentration between 10 ng and 1 μg. In field tests carried out in Cuba in 2000 and 2001 with several stereomeric mixtures of the pheromone, lures containing the synthetic compound in 98:2 Z,E:E,E ratio attracted significantly more insects than those containing higher proportion of the E,E and Z,Z isomers. Our results appear to confirm that (*Z*)-3-dodecenyl (*E*)-2-butenoate is also the pheromone of the sweet potato weevil in Cuba.

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CHEMICAL COMMUNICATION IN CEREAL LEAF BEETLE, *OULEMA MELANOPUS* (COLEOPTERA: CHRYSOMELIDAE).

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The male-produced pheromone of the cereal leaf beetle has been identified as (*E*)-8-hydroxy-6-methyl-6-octen-3-one [1]. Pheromone was detected in volatiles collections of single males, grouped males, and mixed sex groups, but was absent in volatiles collections of oat plants infested with female beetles. Pheromone emission was highest during the photophase and decreased during the scotophase Average pheromone emission for single virgin males was 217 ng/hr during the daylight hours and 37 ng/hr during the nighttime, with a maximum emission of 5.3 µg/16 hrs. Pheromone emission was ~ 800x higher under greenhouse conditions compared to emission under artificial light in incubators. With single virgin males, pheromone emissions started 4 days after emergence from hibernation and lasted for up to 5 weeks. The pheromone was electrophysiologically active on male and female antennae was well as several host derived compounds, such as benzaldehyde, (*Z*)-3-hexenyl acetate, nonanal, and methyl salicylate. The pheromone was attractive to males and females in the field and the behavioral effect of the pheromone was synergized by the plant compound (*Z*)-3-hexenyl acetate.

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CONTACT SEX RECOGNITION PHEROMONE FOR THE ASIAN LONGHORNED BEETLE, *ANOPLOPHORA GLABRIPENNIS*

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A series of long-chain hydrocarbons comprise the cuticular waxes of both sexes of Asian longhorned beetle (*Anoplophora glabripennis*) adults. Although for the most part the gas chromatographic profiles are similar for the two sexes, five monounsaturated compounds were consistently more abundant in samples from females than in those from males. These compounds were identified as (*Z*)-9-tricosene, (*Z*)-9-pentacosene, (*Z*)-7-pentacosene, (*Z*)-9-heptacosene, and (*Z*)-7-heptacosene in the approximate ratio of 1:2:2:8:1, respectively. Antennal contact to a polypropylene micro-centrifuge tube coated with a synthetic mixture of the five compounds stimulated copulatory behavior in males. This is the first time that monounsaturated hydrocarbons, (Z)-9-tricosene, (Z)-9-pentacosene, (Z)-7-pentacosene, (Z)-9-heptacosene, and (Z)-7-heptacosene, have been implicated as contact sex recognition pheromone components in the family Cerambycidae.

IDENTIFICATION OF A PUTATIVE AGGREGATION PHEROMONE FROM MALES *PLATYPUS CYLINDRUS* (COLEOPTERA: PLATYPODIDAE)

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In south of Portugal, the ambrosia beetle *Platypus cylindrus* (Balachovski 1963) relies mainly on cork oak (*Quercus suber*) for reproduction. Males initiate tunnel digging on the host tree, and wind tunnel assays have shown that males and females are attracted to an odour released from male-infested host tree log as well as to that released from crushed males. The aim of this study was to identify a putative aggregation pheromone released by males as well host-related semiochemicals that may be involved in host finding.
Volatiles released from a) a cork oak log (25 cm length, 12 cm diam), b) a cork oak log where 56 males initiated tunnel digging, c) crushed males (n=63), and d) crushed females (n=63) were adsorbed on Porapak Q glass columns (80-100 mesh 0.15 g) and then eluted with *n*-hexane. Samples from a) and b) were collected for 7 days at 30º C using a clean airflow (90 ml/min), and those from c) and d) were collected for 24 hours at 35º C using a nitrogen flow (90 ml/min). Gas chromatography linked to electroantennogram recordings (GC-EAG) was employed to locate GC peaks of each volatile sample eliciting EAGs from males and females. A nonpolar capillary column (30 m × 0.32 mm ID) was used for 12 experiments with beetles from each sex, and a polar capillary column was used for 3 experiments. Tentative chemical identification of compounds giving GC peaks associated with electrophysiological activity was obtained by coupled GC-mass spectrometry. Confirmation was made by peak enhancement in GC on coinjection with authentic samples using the two capillary columns. Finally, biological activity of identified compounds was confirmed by EAG dose-response studies using 12 specimens of each sex. Optical isomerism was not investigated.
During GC-EAG recordings, the largest amplitude EAGs were elicited by three GC peaks detected only in the samples of male-infested log and crushed males. These were identified as hexan-1-ol, 6-methyl-5-hepten-2-one, and 6-methyl-5-hepten-2-ol. In both samples, hexan-1-ol was a major GC peak (>10000 ppm) whereas 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol were smaller peaks (160 and 1600 ppm, respectively). The dose-response studies revealed that males and females detected these compounds similarly with thresholds below 0.1 ng. 6-methyl-5-hepten-2-ol was the most potent stimuli at all doses (0.1, 1, 10 100 and 1000 ng). Hexan-1-ol and 6-methyl-5-hepten-2-one differed only at the two highest doses, with hexan-1-ol inducing larger EAG amplitudes.
Seven other peaks in the chromatograms of cork oak log and male-infested log elicited EAGs in both sexes. However, these host-related volatiles elicited EAGs of much smaller amplitude than those elicited by the above three compounds. Five compounds have been tentatively identified by GC-MS as α-pinene, camphene, sabinene, and β-pinene. The sample of crushed females did not contain any GC peak that elicited EAG.
The results suggest strongly that hexan-1-ol, 6-methyl-5-hepten-2-one, and 6-methyl-5-hepten-2-ol are components of an aggregation pheromone release by males *P. cylindrus*. The host-related active volatiles can be kairomones involved in host-tree finding and/or acting together with the pheromone in beetles' attraction to a single host tree.

CORK OAK MALE BEETLES (*PLATYPUS CYLINDRUS*) RELEASE A PUTATIVE AGGREGATION PHEROMONE THAT ATTRACTS BOTH SEXES

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The ambrosia beetle, *Platypus cylindrus* (Balachovski 1963), relies mainly on cork oak (*Quercus suber*) for reproduction in south Portugal. Their population levels have been related with the decline of the cork oak in this region [1]. The beetle is monogamic, and once on the host tree both sexes share the construction of a complex branched-tunnel where egg laying will occurs. The adults carry fungi spores of the genus *Ambrosia* that grows on the tunnel walls and serve as food for all stages of the insect [2]. The beetles can aggregate in large numbers on a single host tree [1, pers. obs.]. The aims of this study were: a) to determine whether a pioneer sex is responsible for initial colonization of the host tree; b) to test chemically mediated attraction to Q. suber; c) to test the involvement of a pheromone mediating the aggregation on the host tree.

In the laboratory, a cork oak log was given to recently emerged beetles of each sex (n=151 per sex) in two glass containers for 7 days. Only males (38%) initiated tunnel digging in the log, whereas all females died at the end of that period without digging any tunnel.

In a first experiment in wind tunnel, beetles of each sex were subjected to a perforated cooked-clay cylinder (65 cm height; 25 cm diam), placed vertically at the upwind end, and containing one of the following stimuli: a) cork oak log; b) cork oak log infested with 187 males that initiated tunnel-digging; c) cork oak foliage (300 g). In the control experiment, the clay cylinder was empty. In each experiment 44 beetles of each sex were tested for their flight behaviour. Three-way loglinear statistics showed that the stimuli affected similarly flight occurrence and landing of each sex on the clay cylinder. In the presence of the male-infested log, higher frequency of beetles landed on the clay cylinder (67%; $P<0.005$) than in the presence of any other stimuli. Foliage induced higher frequency of landing on the clay cylinder (37%; $P<0.05$) than the log (20%) or control (20%). The male-infested log induced a decrease of flight occurrence (57%; $P<0.05$) when compared with the other stimuli (88%), which did not differ in their effect.

In a second experiment, the stimuli introduced in the clay cylinder were: a) filter paper on which 151 males were crushed; b) filter paper on which 151 females were crushed; c) filter paper (control). The number of beetles tested for each stimulus varied between 48 and 68. Males and females did not differ in landing frequency on the clay cylinder across stimuli. Crushed males induced higher frequency of beetles landing on the clay cylinder (56%; $P<0.005$) than crushed females or control (20%), which did not differ in their effect. Crushed males decreased flight occurrence in males (78%; $P<0.05$) when compared with the other stimuli (94%), but female's flight occurrence was not significantly affected by the stimuli.

Altogether, the results indicate that a host-tree odour attracts pioneer males that initiate tunnel digging on the tree. Further host colonization and reproduction is promoted by a putative aggregation pheromone from males that may act together with host-tree odour in the attraction of conspecifics to a single tree.

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ATTRACTANTS OF RED TURPENTINE BEETLE, *DENDROCTONUS VALENS*, THE SPECIES IN OUTBREAK ON *PINUS TABULAEFORMIS* IN CHINA

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Root and lower stem solitary bark beetles *Dendroctonus valens* (accidentally introduced and established species in eastern China and first recorded in Shanxi in 1998, in Hebei in 1999, respectively) attack living trees in the monoculture of *Pinus tabulaeformis* in the Taihang mountains on the border between Hebei and Shanxi provinces. SPME analysis of volatiles from *P. tabulaeformis*, both quantitative and enantiomeric ratios of identified monoterpenes was enable to suggest host kairomone lures for monitoring and/or further management of the Chinese population of red turpentine beetle. Field trials performed in the beginning of this season are in progress.

A NEW AGREGATION ATTRACTANT FOR THE SUGAR-BEET WEEVIL (*BOTHYNODERES PUNCTIVENTRIS* GERMAR.) (COL., CURCULIONIDAE)

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The beet root weevil (*Bothynoderes punctiventris* Germar) (Coleoptera, Curculionidae) is an important pest of sugar beet throughout the eastern and southeastern parts of Europe. A trap for detection and monitoring would be sought for by farmers in these areas.
Recently we discovered through systematic field screening of compounds known to be attractants for taxonomically related species of the Curculionidae an aggregation pheromone-like attractant for this pest.
In 1995-1997, we tested grandlure I, II, and (III+IV) (alone and in mixtures), components of the aggregation pheromone of *Anthonomus grandis* Boh., racemic grandisal, an attractant of *Pissodes* spp., and grandisoic acid, a component of the aggregation pheromone of the plum curculio, *Conotrachelus nenuphar* Herbst. It was found that the (*E*/*Z*)-3,3-dimethylcyclo-hexylidene)acetaldehyde isomeric mixture (grandlure III+IV) was active in attracting individuals of *B. punctiventris* adults. Both males and females were attracted to the traps.
Efforts to determine whether the *Z* or the *E* isomers are responsible for biological activity remained inconclusive. It was also unclear whether the compounds are produced by the beetles as components of the natural pheromone.
A special challenge in the development of a sugar-beet weevil trap was that in contrast to other, widely used pheromone trap designs, which are suitable for capturing flying insects, in this case the beetle to be captured crawls on the soil surface. We tried to improve the design of soil level pitfall traps (i.e.Barber's trap) which are generally used by insect collectors to make the new trap more effective and easier to use.
Having compared several trap designs we developed a modified pitfall trap codenamed "TAL", which, due to its design which takes into consideration the behavior of the target insect is generally more sensitive than conventional pitfall traps. The trap has a high catching capacity (up to ca 800-1000 beetles); when setting up the trap it is not necessary to dig a hole in the soil, the trap can be placed at the soil surface. The bait is selective, it attracts only adults of the sugar-beet weevil and both male and female specimens, in the natural sex ratio of the population. The traps retain their activity throughout the full flight season, there is no need for renewing the bait within one season.
Such TAL traps have been used sucessfully in application studies for the early detection of sugar-beet weevils at overwintering sites; for the following of movement of weevils into newly planted sugar-beet fields; and for monitoring (qualitative and quantitative) of changes in the local weevil population. Preliminary results show promise also for application of the traps in direct control through mass trapping.

CHEMICAL MIMICRY BY CLEPTOPARASITIC DARKLING BEETLES IN THE OPATRINI SUBTRIBE STIZOPINA (COLEOPTERA: TENEBRIONIDAE)

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Tenebrionid beetles of the subtribe Stizopina (tribe Opatrini) are all nocturnal detritivores which are restricted to the arid regions of southwestern Africa. The subtribe is divided into the *Stizopus* and the *Planostibes* group [1]. Some members of the *Stizopus* group show subsocial behaviour with division of labour [2]. In all known cases of brood care, the species are associated with members of the *Planostibes* group, which lay their eggs as cuckoos in the burrows of their hosts. Each host species or subspecies lives together with an other parasite species [3] and defend their burrows against all intruders, expect their special parasite. Odourless dummies spiced with cuticular hydrocarbon (CHC) extracts of the parasite *Eremostibes opacus* are accepted by its host *Parastizopus armaticeps*, in contrast to the solvent control. Analysing the CHC of 37 Stizopina species, subspecies or populations, we found mainly *n*-alkanes, mono-, di- and trimethyl alkanes with carbon backbones between C23 and C39. Unsatured hydrocarbons are uncommon in Stizopina. To compare the CHC we used the CHC index, a combination of Nei's index and Jaccard's index. The clustering of the species does not reflect the phylogeny in the subtribe, rather it correlates with the host/parastite associations. The cluster of *P. armaticeps* subspecies groups together with the cluster of their parasites *Eremostibes bushmanicus*, *E. opacus*, and *Planostibes namaqua*, just as the other host/parasite pairs *P. transgaripinus* / *Pl. dentipes*, *Ennychiatus caraboides* / *E. barbatus*, and *Adoryacus bidens* / *Pl. rufipes*.
The response of two *P. armaticeps* subspecies to different parasite species corresponds to the results of the clustering. Both subspecies show significantly more often agonistic behaviour against *E. barbatus*, the species with the greatest CHC distance to them, and ignore their own parasite in most cases.

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THE AEDEAGAL GLAND OF PARASTIZOPUS ARMATICEPS (COLEOPTERA: TENEBRIONIDAE) AS SOURCE OF THE 'STERZEL' PHEROMONE

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Parastizopus armaticeps Pér. is a nocturnal detritivorous desert tenebrionid which can only reproduce after heavy rainfall. The beetles court in small mixed-sexed groups on the surface at night. These courting groups are formed when a male, after emerging from the burrow in the early evening, show a specific behaviour, called 'sterzeln' [1]. The beetle perform a 'head stand' and expose the aedeagus. The 'head stand' position is hold for less than 10 s, then interrupted by a period of jerky walking in a circle for about 50 s, terminating in a 'head stand' again. This behavioural sequence can last for up to an hour. Other beetles are attracted to this 'sterzeling' beetles from more than 3 m downwind. We adsorbed the volatiles emitted by 'sterzeling' males with SPME (100µ PDMS), by holding the fibre for 1 - 5 s as close as possible to the everted aedeagus, without contact. We identified *m*-cresol, ethyl-1,4-benzoquinone and 3-ethylphenol by GC/MS. These volatiles are part of the secretion of the aedeagal gland [2]. In addition to the three volatiles, we identified ethylhydroquinone, several methyl-, ethyl-, propyl- and isopropyl esters of fatty acids (C18-C22) and hydrocarbons (C27-C35) from dissected glands, extracted in *n*-pentane. The volatiles may act as a sex attractant pheromone, but also attract competing males. In addition, the brood parasite *Eremostibes opacus* is using the pheromone to locate the courting groups.

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'HANDEDNESS' IN THE 7-SPOT LADYBIRD BEETLE, *COCCINELLA SEPTEMPUNCTATA*: A PROBLEM FOR Y-TUBE OLFACTOMETRY.

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A Y-tube olfactometer with a linear track was used to investigate biases in directional choice of female *Coccinella septempunctata*. Tests were conducted by running experiments with no odour sources on either side of the Y-tube. *C. septempunctata* showed a bias to the right-hand arm of the olfactometer. To check for biases in relation to possible environmental gradients across the room, tests were repeated aligning the olfactometer to all four points of the compass. *C. septempunctata* still maintained a bias to the right-hand arm of the olfactometer irrespective of its orientation.
To test for bias on an individual basis, the same, numbered individuals were tested repeatedly in the olfactometer. A small proportion of individuals (14%) were shown to have a consistent 'left-handed' bias, more (50%) individuals displayed a 'right-handed' bias while 36% were 'ambidextrous'. These results indicate that most individuals of *C. septempunctata* show an inherent bias to the direction they pursue.. Evidence of such 'handedness' has also been found for other insect species [1]. We conclude that it is important to consider 'handedness' bias when designing experiments using Y-tube olfactometers.

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CHEMICAL ECOLOGY AND INTEGRATED CONTROL OF THE ORANGE WHEAT BLOSSOM MIDGE, *SITODIPLOSIS MOSELLANA*.

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Sitodiplosis mosellana is a common pest of wheat in the UK but infestation varies from year to year. Due to difficulties in detection of larvae within the ear the actual degree of damage is hard to predict [1]. This has led to a considerable increase in prophylactic spraying against the pest (currently estimated 250 000 ha). A reliable, semiochemical based monitoring system would reduce the unnecessary use of pesticides against lower levels of midge infestation and allow populations of parasitoids to increase and provide a greater level of natural control. It is proposed to use:
sex pheromone traps to detect presence of male *S. mosellana* in a locality
host plant volatile traps to determine abundance of ovipositing females and likelihood of crop damage
As a prelude to trap development, potentially active semiochemicals were tested in laboratory and field trials. Wheat volatiles from air entrainment samples elicited electrophysiological responses from female *S. mosellana* and attracted them in an olfactometer bioassay. The sex pheromone, 2,7-nonanediyl dibutyrate [2], was synthesised and found to be attractive to newly emerged males of a UK strain of *S. mosellana* in olfactometer bioassay. Initial field trapping experiments are also described.

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BENZOQUINONES EMITTED BY DIPLOPODS ARE EXPLOITED AS KAIROMONES BY PARASITOID SARCOPHAGID FLIES

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The defensive secretion of pachybolid and spirostreptid millipeds from the sub-humid Guinea savannah of the Ivory Coast mainly consists of 2-methyl-1,4-benzoquinone and 2-methoxy-3-methyl-1,4-benzoquinone, accompanied by numerous other benzoquinones and corresponding hydroquinones. The secretion from the segmental glands is not only discharged in the case of defence, for instance against vertebrates or army ants, but also during sexual interactions. Reluctant females of *Pelmatojulus tigrinus*, which were approached by a courting male, emit large amounts of the secretion while escaping, as do males erroneously annoyed by sexual attempts of other males. If a rival male bothers a copulating pair, the intruder is also offended by a charge of the secretion. During all these encounters, tender membranes and body orifices especially at the genitalia and gonopods are exposed by the diplopods, which otherwise are protected by a heavily sclerotized exoskeleton. Coincidently with the discharge of chemicals during defence or sexual interactions, larviparous flies of the genus *Sarcophaga (Liosarcophaga)* are attracted within a short time and deposit their offspring on the exposed membranes, where the parasitoid larvae enter the host. The secretion also emanates from injured or freshly killed millipeds, e.g. trampled by a mammal, and attracts sarcophagid flies as scavengers as well. Defensive secretion milked from the millipeds, as well as both major synthetic benzoquinones spiced to filter papers lured *Liosarcophaga* spp. in the field. The quite simple chemical composition of the secretion is not species specific for numerous co-occuring diplopods, some of which live in high densities in West African savannahs. Additionally, numerous syntopic sarcophagids and other fly species, which are attracted to benzoquinones, coexist in the region. Thus, diplopods as a common resource are just exploited by a specialized facultative parasitoid by using common defensive compounds as kairomones and circumventing the mechanical barriers of a well armoured arthropod.

o-NITROBENZALDEHYDE SPRAYED AS A SNEEZING-POWDER BY AN ASSASSIN BUG (ECTRICHODIA SPEC.)

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True bugs are well known as versatile chemists, however, the assassin bugs (Heteroptera, Reduviidae) have been nearly neglected so far. In the West African Guinea savannah, a large aposematically coloured species of the genus *Ectrichodia* is an exclusive predator of diplopods. The bug's toxic saliva immediately paralyses even large millipedes (e.g. *Pelmatojulus tigrinus*). The Brindley´s gland contains short chain fatty acid derivatives. The most conspicuous defence system of *Ectrichodia* adults, however, is the metathoracic gland secretion with an unique chemical composition and its allergenic properties, an extraordinary gland morphology and the mode of atomizing a powder.
GC/MS-analyses of the emitted secretion or reservoir contents revealed only one major peak (> 95 % total peak area). *o*-Nitrobenzaldehyde was identified from the secretion by its mass spectrum and co-chromatography of synthetic isomers. Minor components were other N-containing aromatics. No organic solvents could be detected. Insect collectors hit by this secretion during handling report heat and a stinging pain on their skin, and suffer from irritation of their eyes and respiratory tract. Some individuals reported general allergic responses with intense sneezing attacks. *o*-nitrobenzaldehyde was formerly a constituent of 'sneezing-powders' offered by manufacturers of gag gifts, but its use has now been strictly prohibited by law due to its hazardous properties. Thus, the bugs may use this irritant in defence against insect feeding vertebrates.
When disturbed by handling, *Ectrichodia* adults eject a white glittering powder flying off over a distance of up to 50 cm from the metathoracal gland, sometimes with an audible puff. Cover slides hit by the jet at a distance of a few centimetres are sprinkled with small crystals. Legs and other body parts of the bug show a white crust of crystalline powder. High speed video sequences showed a thin continuous jet ejected from the glandular orifice of about 27 msec duration, waving from back to forth and back again and covering an angle of about 90°. Three subsequent shots may be fired during 200 msec. It is not clear yet, whether the jet consists of a liquid crystallizing only at farer distance. A possible explosive reaction under heat and gas production, which sprays a liquid or powder like a fire extinguisher, needs to be examined.
The metathoracic gland seems to be adapted to withstand high pressures. The reservoir is hard to grasp by forceps during dissection because of its elastic consistence like a rubber ball. The very thick cuticle is conspicuously wrinkled in the interior of the bulb. The glandular cells are situated in ramified tubules and their duct joins the duct from the reservoir close to the tiny opening dorsally to the hind coxa. Both ducts run in parallel in a cuticular channel. A chitinous rod moves a cuticular flap at the orifice. Reservoirs dissected in buffer solution contain a white crystalline mass of secretion.
Ectrichodia larvae own dorsal abdominal glands, the last one comprising whitish glandular cell clusters and a thin-walled reservoir. During molestation of the bug, a clear liquid is oozing from the slit like orifice, covers the surroundings and evaporates only after minutes to leave a faint white crust. The secretion also contains *o*-nitrobenzaldehyde, however, accompanied by 1-pentadecene and several monoterpenes probably serving as solvents. The enemies of the small gregarious larvae may be arthropods, however, the irritant or repellent action of *o*-nitrobenzaldehyde against arthropd predators needs to be tested.

CHEMICAL AND OTHER SIGNALS MEDIATING AGGREGATION OF 1ST AND 2ND INSTAR STINK BUGS (HETEROPTERA: PENTATOMIDAE).

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First and 2nd instar nymphs of pentatomid bugs form aggregations. We investigated possible signals that might mediate the formation of these aggregations, for nymphs of six different pentatomid bug species (*Nezara viridula, Acrosternum hilare, Chlorochroa ligata, Chlorochroa sayi, Thyanta pallidovirens*, and *Euschistus conspersus*). The chemical profiles of nymphs of each species were determined by solvent extraction of groups of nymphs with pentane, followed by GC-MS analysis. The profile of volatile chemicals produced by groups of live nymphs also was checked by solid phase microextraction (SPME) and GC-MS analysis. Immature bugs of the different species had some compounds in common, and some compounds that were more species-specific. The solvent extracts were tested over a range of concentrations for their ability to induce or maintain aggregations, by treating filter paper discs with crude extracts. The crude extracts did not induce aggregation behavior, for the 1st and 2 nd instars of the six different species. However when live 1st instars of any two species were put together in a small Petri dish, they formed heterospecific aggregations similar to their natural conspecific aggregations. Further bioassays were conducted with polysulfone beads (1 mm diam) that were glued together in groups approximating bug egg masses. First instar bugs tended to aggregate on the beads, suggesting that tactile cues may be important in maintaining the aggregations. Bioassays are continuing with beads treated with individual components of the bug extracts. The relative roles of tactile, chemical, and other possible signals will be discussed.

CUES MEDIATING HOST LOCATION IN *TRISSOLCUS BASALIS*: THE ROLE OF VISUAL AND SEMIOCHEMICAL FACTORS FROM THE EGG HOST UNIT.

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When seeking their hosts for reproduction, insect parasitoids follow a hierarchical sequence of phases which eventually leads to host acceptance and oviposition [1]. Chemical and/or physical cues from the host, the substrate and the associated material and/or organisms play a fundamental role in mediating the different steps of host selection [1, 2, 3, 4], and the ensemble of such cues for a given host-parasitoid association has been defined as a "host unit" [5]. When dealing with *Trissolcus* spp. (Hymenoptera: Scelionidae) which attack pentatomid bugs (Heteroptera: Pentatomidae), an "egg host unit" may be represented by the characters of the host egg and the egg's immediate surroundings. Details on such egg host unit are best known for the association *Nezara viridula* (L.) - *Trissolcus basalis* (Wollaston). The secretion present on *N. viridula* eggs contains the host recognition kairomone used by its parasitoid *T. basalis* [6]. In addition, parasitoid females are attracted by volatile chemicals from *N. viridula* eggs, gravid females and males [7, 8, 9]. This parasitoid also shows an arrestment response to patches contaminated by contact chemicals from adults of *N. viridula* [9].
However, very little has been done on the cues used during the final steps, when the parasitoid is walking on the plant substrate in the vicinity of the host eggs. Recent data indicate that the parasitoid respond to volatile cues induced by host oviposition on fava bean leaves [10]. Here we report laboratory observations on the influences of volatile cues from *N. viridula* host eggs, as such or associated with visual cues, on *T. basalis*. Bioassays of host egg clusters or glass models of the clusters were conducted in a filter paper open arena and in a closed arena with air flow, and were recorded with the aid of a video tracking and motion analysis system. *T. basalis* females show an orientation response to host egg clusters, to glass dummies treated with chemical egg extracts and to egg extracts directly applied on the substrate. In contrast, *T. basalis* did not respond to untreated cluster dummies differently coloured. Results are discussed comparing data from open and closed arenas, and also considering those obtained from bioassays conducted in a Y-tube olfactometer, which show a response by *T. basalis* females to volatiles induced by host egg - plant interaction [10].

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SEX PHEROMONE COMPONENTS OF THE COCOA MIRIDS, DISTANTIELLA THEOBROMA AND SAHLBERGELLA SINGULARIS (HETEROPTERA: MIRIDAE)

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Mirids are the most important insect pests of cocoa in West Africa, typically causing losses of more than 25% of the crop. Currently conventional insecticides provide the only effective methods of control, but the recommended spray programmes are rarely used by farmers because of expense and difficulty of application, as well as being environmentally and ecologically damaging.

There are two main mirid species in Ghana, *Distantiella theobroma* Dist. and *Sahlbergella singularis* Hagl. King [1] showed that males of *D. theobroma* are attracted to traps baited with conspecific virgin females. He could not demonstrate this for *S. singularis*, but, as part of the work described here, *S. singularis* males were caught in traps baited with conspecific virgin females aged between 9 and 43 days old.

Volatiles were collected from virgin females of both species. Amounts of pheromone components were extremely low compared with amounts produced by other mirid species [e.g. 2] – typically less than 5 ng/insect/day. Analyses using gas chromatography (GC) directly linked to electroantennographic (EAG) recording from the male antennae were hampered by the difficulty of transporting insects and the short life of EAG preparations. GC-EAG analyses with *D. theobroma* showed a single EAG-active component, and similar analyses with *S. singularis* showed two active components, one being probably the same as that observed with *D. theobroma*.

The common component was identified as a novel diester by consideration of EI and mass spectra, GC retention data and high resolution MS data and synthesis of over 40 potential candidate compounds. GC and MS data for the second active component fitted with those for the corresponding monoester. GC analyses on a cyclodextrin GC column showed the natural component to have the *R* configuration, and the *R* enantiomers of diester and monoester elicited larger EAG responses from male *S. singularis* than the corresponding *S* enantiomers. Re-examination of volatile collections from female mirids showed that both the diester and monoester were produced by *D. theobroma* and *S. singularis* in similar 2:1 ratios.

Blends of the *R* enantiomers of the two components were attractive to male *S. singularis* in field trapping trials and the *S* enantiomers were completely unnattractive. *D. theobroma* has become much less prevalent in Ghana in recent years, and only very low numbers of this species were trapped. Work is in progress to optimise the blends for the two species and develop pheromone traps for monitoring these pests and possibly control.

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OLEANDER SCALE SEX PHEROMONE CHEMICAL INVESTIGATIONS OF BOTH SEXUAL AND PARTHENOGENETIC STRAINS.

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The Oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) is a cosmopolitan phytophagous of several plants species. In Sicily have an important role as pest of lemon tree (orchards). In nature this species presents different strains, characterized by sexual or parathenogenic reproduction. The sex pheromone of the Oleander scale was recently chemically identified. In a previous study about its pattern of emission, using solid phase microextraction (SPME) technique, we determined the out line production of sex pheromone released by females of sexual strain.

The aim of this study is to verify the pheromone production of the parthenogenetic population. A chemistry investigation was conducted to follow the sex-pheromone time production, using headspace solid phase microextraction (SPME) subsequently analyzed by gas chromatography/mass spectrometry (GC/MS).

The headspace SPME of the volatile emission from two lemons infested by about 100 oleander scales virgin females monitored for several days allowed to individuate no pheromone emission, on this strain.

On the basis of these results we carried out a set of experiments about behavioral and bio-molecular components, to better characterize the differences between the sexual and a sexual populations and determine if these are two differences species rather than two different strains within a single species.

A set of breeding experiments between the parthenogenetic females and males of sexual population was carried out: only female progeny was achieved (parthenogenetic). A 1000bp fragment of the mitochondrial genome (including portions of the CO I, COII and tRNA-Leu) has been sequenced from the sexual and parthenogenetic populations. The two haplotypes are highly divergent with an uncorrected pairwise difference of 5.7 between them.

PHEROMONALLY MEDIATED REPRODUCTIVE ISOLATION BETWEEN TWO SYMPATRIC SIBLING SPECIES OF SEA-SNAKES

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Two sibling species of amphibious sea-snakes (*Laticauda colubrina* and *L. frontalis*) occur on the island of Efate, in the Pacific Ocean republic of Vanuatu. The two taxa are almost identical morphologically, except that *colubrina* grows much larger than *frontalis*. No natural hybrids have been reported. Our fieldwork shows that the two taxa are often syntopic, and that both breed in November-December. Behavioral studies in outdoor arenas show that the separation between these two taxa is maintained by species-specific cues that control male courtship. Males of both species courted conspecific females but not heterospecific females. The proximate mechanism driving this separation involves chemical cues. Adult females of both taxa possess distinctive lipids in the skin. Males directed courtship behavior (chin-pressing) to hexane-extracted samples of lipids from conspecific but not heterospecific females. Males of the dwarf species (*frontalis*) were move selective courters than were those of the larger taxon (*colubrina*), perhaps because a preference for courting larger females means the *colubrina* males would be unlikely to court *frontalis*-sized females even in the absence of pheromonal barriers.

IDENTIFICATION OF A PHEROMONE RESPONSIBLE FOR MEDIATING REPRODUCTIVE TRAILING BEHAVIOR IN GARTER SNAKES

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Male garter snakes locate females during the breeding season utilizing conspecific trailing behavior. It has been hypothesized that the female-derived chemical cue responsible for mediating male reproductive trailing behavior is the sexual attractiveness pheromone, a previously characterized sex pheromone composed of a homologous series of saturated and unsaturated methyl ketones sequestered in the skin lipids of females. To examine this hypothesis, we tested the response of male red-sided garter snakes, *Thamnophis sirtalis parietalis*, to hexane-extracted female skin lipids collected during the breeding season. When tested on a Y-maze, males were found to detect and follow female extract trails, demonstrating that the pheromone is skin lipid-derived. Subsequent fractionation of the skin lipids revealed that males preferred to follow the fraction containing the sexual attractiveness pheromone (fraction 5 – verified utilizing gas chromatography/ mass spectrometry) over a combination of all other fractions (Y-maze; simultaneous choice tests). These results provide convincing evidence that the sexual attractiveness pheromone is indeed the chemical cue utilized by male garter snakes to mediate reproductive trailing behavior.

FRACTIONATION OF KAIROMONE(S) IN THE *DAPHNIA – SCENEDESMUS* SYSTEM BY MEANS OF HPLC

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True chemical communication in aquatic systems has recently been reported. Uni-cellular *Scenedesmus obliquus* algae started to form eight-celled colonies in the presence of *Daphnia magna* or water that had previously contained *D. magna* [1]. The larger size of the colony prevented predation by *D. magna*. This colony formation was the basis of the bioassay, where fractions were added to fixed amounts of medium and algae. After 48 hours, algal size distributions and densities were determined using an electronic particle counter. The Mean Particle Volume was 2 to 3 times higher in the presence of the kairomone(s) [2].
Previous work showed that the kairomone(s) could be extracted from a C18 SPE-cartridge with 85% aqueous methanol [3]. Comparing HPLC profiles of extracts with or without kairomone(s) showed several differences, especially between 17 and 24 minutes. These extracts were fractionated using HPLC. After testing in the bioassay, one active fraction was found between 18.5 and 23.5 minutes. This fraction was fractionated again and yielded two active fractions: from 20 to 21 minutes and 23 to 24 minutes. These fractions will be further investigated using LC-MS and possibly LC-NMR.

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CONVERSION OF SULPHUR COMPOUNDS TO SULPHUR IN URINE OF CHEETAH TO AVOID DETECTION BY STRONGER PREDATORS?

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The cheetah, *Acinonyx jubatus*, sometimes referred to as 'the greyhound of the cats', is probably the most elegant member of the cat family. Formerly widespread in Southern Africa, it is now threatened with extinction. It occurs in open savannah and light woodland, but also occasionally in hilly country. The cheetah is a predator that can reach speeds of more than 110 km/h in short bursts. With a mass of between only 40 to 60 kg, it is not very powerful and cannot defend itself very effectively against carnivores such as lion and hyaena. This might be the reason why, in order not to advertise its presence, the urine of this animal is practically odourless. In turn, this might explain why no research has so far been devoted to the urine of this animal. In contrast extensive work has been done on the chemical characterization of the urine of many other carnivores, notably that of the lion.
Extraction of the urine with dichloromethane gave too little material for GC-MS. Sample enrichment by solid phase microextraction (SPME) of the headspace of the urine or SPME of the liquid itself gave very weak gas chromatograms. Better results were obtained when an SPME-type of headspace sampling, using a larger mass of polydimethylsiloxane, was employed, although many of the constituents of the urine could still barely be detected. The compounds identified include a large number of ketones, aldehydes, cyclic and acyclic ethers, carboxylic acids, amides, two sulphur compounds in barely detectable quantities, and elemental sulphur. Perhaps the most remarkable result of this research is that the urine of the cheetah, although it is a carnivore, does not contain the many sulphur-containing compounds present in relatively high concentrations in the urine of other carnivores. However, it does contain elemental sulphur. Perhaps some mechanism exists by which the cheetah converts strongly odorous sulphur-containing compounds into the less strongly smelling sulphur.

METHYL-BRANCHED ALKANES OF THE ADULT FLEA BEETLES, *APHTHONA LACERTOSA* AND *APHTHONA NIGRISUTIS*.

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The adult flea beetles, *Aphthona lacertosa* and *Aphthona nigriscutis*, used as biocontrol agents for leafy spurge, had a complex mixture of hydrocarbons on their cuticular surface consisting of alkanes, methylalkanes, alkenes and alkadienes as determined by gas chromatography-mass spectrometry. A trace amount of wax esters were present. In both species, the hydrocarbons were the major cuticular lipid class and the gas chromatographic profiles of the total hydrocarbons were similar. However, the profiles for the saturated hydrocarbon fraction were distinct for each species. Alkanes (*n*-alkanes and methyl-branched alkanes), alkenes and alkadienes comprised 26%, 44% and 30%, respectively, for *A. lacertosa*, and 48%, 26% and 26%, respectively, for *A. nigriscutis*, of the total hydrocarbons. The major methyl-branched hydrocarbons were 2-methylalkanes: 2-methyloctacosane and 2-methyltriacontane. The major monoene was hentriacontene and the major diene was tritriacontadiene. The species were unique in that a number of di- and tri-methyl-branched alkanes were present in minor quantities in which the first methyl branch was on carbon 2 or 3. Examples of structures were 2,10-and 2,12-dimethylalkanes, 2,6- and 2,4-, and 3,7-dimethylalkanes. 2,10,12-Trimethylalkanes with one methylene between adjacent methyl branch points were identified. The adjacent methyl branch points appear to cause an additional fragmentation in their mass spectra. Methylalkanes with an odd number of carbons in the backbone of the molecule were identified as 2,23-dimethylnonacosane and 2,25-dimethylhentriacontane; their mass spectra also corresponded to mass spectra for a 2,6 branching sequence. However, a 2,6 branching sequence is not biosynthetically feasible because such a structure has a straight-chain tail with an odd number of carbon atoms beyond the last methyl branch point. The 2,23 and 2,25 branching sequences could be synthesized starting with a primer derived from the amino acid leucine.

VARIATION OF CUTICULAR HYDROCARBONS AND GENETIC RELATEDNESS OF PARISIAN COLONIES OF THE TERMITE *RETICULITERMES SANTONENSIS*

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In a previous study [1], we used a technique of molecular genotyping (microsatellite markers) that gave some first informations of how colonies and populations of the subterranean termite *R. santonensis* were organized in Paris. In the present analysis, twenty workers from five different sampling points were genotyped at five microsatellite loci originally developed for *R. flavipes* [2]. We could determine relatedness among individuals, and attribute the sampling points as same or different colonies. Such assignments are also possible using cuticular hydrocarbons. Thus we determined the individual chemical signature of twenty other workers from the same colonies used in genetic analyses. All the five investigated colonies contained the same cuticular hydrocarbons that were species characteristic [3], but showed quantitative variation in percentage composition between individuals. From these preliminary data, we tried to correlate the genetic distances of colonies and the cuticular hydrocarbon patterns.

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VOLATILE COMPOUNDS FROM TWO LABORATORY NESTS OF *ATTA SEXDENS RUBROPILOSA* AND THEIR SEASONAL CONVERSION IN UNMATED MALES

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Colony recognition by ants is related to some volatile compounds presents in their metabolism. This can also be true for determining the period for nuptial fly. This communication presents the results obtained in two experiments that can contribute to elucidation this hypothesis.
Experiments with two laboratory nests were carried out using dynamic headspace extraction with active charcoal, ODS, Floresil®, Porapak® and Tenax®. The volatile compounds from unmated males and females and unmated males seasonal variation were obtained using SPME Fiber (100 μm, polydimethylsiloxane, Supelco).
The compounds identified from the two nests were α-pinene, limonene, undecane, dodecane, isobuthyl benzoate, copaene, cadinene and tetradecane. Using Tenax® as support for headspace extraction, all these compounds were identified using GC/MS, while when was used charcoal and ODS only few compounds were identified.
The main volatile compounds found in the head, gaster and thorax of unmated males and females were 4-methyl-3-heptanone and 4-methyl-3-heptanol.
The analysis of the seasonal variation of these two compounds, in unmated males, showed that 4-methyl-3-heptanol (**1**) is practically the only compound present in the ants on the 1st day (**1** > 99%) and it is gradually transformed in 4-methyl-3-heptanone (**2**). On the 4th day, the relationship between these two compounds is 96:4 (**1**:**2**) and it progresses to 70:30 and 35:65, respectively for the 15th and 30th days.
The results indicated that: 1) there are no difference among the volatile compounds found in the two laboratory nests analyzed using several support for dynamic headspace extraction and little variation in their concentration was found; 2) Tenax® was the best tool to obtain the volatile compounds of the ant nests and 3) the seasonal conversion of **1** to **2** indicates that these volatile compounds probably play an important role indicating when it is the time for unmated males ants fly out of the nest in order to couple with females from other nests.

OVARIAN ACTIVITY AND AGE DEPENDENT QUEEN EFFECTS ON COLONY LABEL IN *CAMPONOTUS FLORIDANUS* (HYMENOPTERA: FORMICIDAE)

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Generally in social insects it is very important that nestmates do not allow the access of alien individuals to their colony to prevent exploitation of colony resources. In ants nestmate recognition is mediated by a chemical label or "colony odor" which could be derived from any or all of the following: environmental odors, the individual´s own genetically-determined recognition pheromones, a mixture of transferable discriminators produced by each nestmate and absorbed by all and the discriminators of the queen applied to all nestmates. However, the relative importance of these factors is not yet clear. Carlin and Hölldobler's (1983, 1986, 1987) investigations favor a strong contribution of the queen whereas Lahav et al. (1998) suggest that the queen is not important for the formation of colony odor. In our study we try to solve this apparently inconsistent pattern. We hypothesize that the influence of the queen pheromone is limited to a temporal window in which the colony is still relatively small (200-300 workers) but the queen already reached a distinct pheromonal activity which correlates with ovarian activation.
We analyzed the cuticular hydrocarbon profiles (SPME/GC) from the queen, one nestmate worker and one alien nestmate worker (adopted to the colony as pupa) of *C. floridanus* in four experimental groups each consisting of 20 colonies: founding colonies with less than 10 workers, colonies with 40 to 60 workers, medium colonies (200 to 300 workers) and large colonies over 1000 workers. The number of cuticular hydrocarbons (CHCs) in queens increases with her age and her reproductive status. We found qualitative differences in CHCs between a fully functional queen and her workers. There are up to 45% special queen compounds in large colonies but the compounds are not yet completely identified. Because of that we divided the CHCs from queens and workers in two groups: I) special queen compounds, II) common worker-queen compounds and compared the transformed proportions of the total peak area of group I with group II between workers and their queen. We expect a transfer of CHCs from queen to worker which is correlated with the CHC production of the queen.
There are no significant correlations between the proportion of CHCs from group I between queen and worker in founding colonies (Pearson, n.s.) and colonies with 40 to 60 workers (Pearson, n.s.). The queen in these colonies did not yet show a reproductive activity and the CHCs from group I were almost absent. However, the correlation in colonies with 200-300 workers was significant which demonstrates a transfer of compounds that relates to the relative production of CHCs by the queen (Pearson, p=0,015). Large colonies are analyzed at the moment. We also found an effect on individual CHC profiles. There are quantitative differences between alien workers from investigated colonies and their relatives from the original colony. But these mixed CHC profiles of alien nestmates are caused by the queen and/or other nestmates. The transfer of CHCs may either have a function in the context of the formation of colony odor or it may communicate the presence of the queen to her workers.

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HARVESTER ANT MIDDEN PEBBLES ARE LABELLED WITH COLONY-SPECIFIC CUTICULAR HYDROCARBONS.

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The nest-mounds of the harvester ant, *Pogonomyrmex barbatus*, are covered by a layer of small pebbles, that are collected from the surrounding area by workers, along with piles of midden composed of seed husks and corpses. A specific task of workers, midden workers, tend to the midden and can often be observed manipulating these pebbles with their mouth parts and moving them to new locations on the mound. The attention paid to the midden pebbles suggests that they play a role in colony function. The aims of this study were: 1) to determine if midden pebbles are labeled with the colony odor, known to be composed of cuticular hydrocarbons, 2) to determine if *P. barbatus* workers can discriminate foreign midden (collected from both neighboring *P. barbatus* colonies and from colonies of a competing ant species, *Aphaenogaster cockerelli*) from their own, and 3) to demonstrate that *P. barbatus* workers can discriminate cuticular hydrocarbons isolated from foreign midden from their own. Chemical analysis showed that midden pebbles of both species are coated with low levels of colony specific hydrocarbons. Behavioral data, collected from bioassays that measured aggression, demonstrated that *P. barbatus* workers could discriminate their own midden from foreign midden and that this behavior was displayed in response to hydrocarbons isolated from midden pebbles that differed from their colony odor.

(*S*)-(+)-LINALOOL, A MATE ATTRACTANT PHEROMONE COMPONENT IN THE BEE *COLLETES CUNICULARIUS*

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Enantiomerically pure (*S*)-(+)-linalool was the main constituent in the extracts of the cephalic secretions of virgin females, mated females, freshly emerged males and patrolling males of the solitary bee *Colletes cunicularius (Hymenoptera, Colletidae)*. After copulation, the content of (*S*)-(+)-linalool in the secretions was strongly reduced. Electrophysiological experiments revealed that the two enantiomers of linalool were equally well received by the antennae of the males. Field tests using pure enantiomers and the racemate of linalool showed that the number of male bees attracted was highest for (*S*)-(+)-linalool. The search flight activity in the mating flight area increased dramatically when (*S*)-(+)-linalool was presented to patrolling males instead of (*R*)-(-)-linalool. This indicated a mate attractant pheromone function of (*S*)-(+)-linalool.

MARKING PHEROMONES OF BUMBLEBEES: COMPOSITION OF THE LABIAL GLAND SECRETION OF MALES OF *BOMBUS MAGNUS*.

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Bombus magnus is a bumblebee species belonging to the subgenus *Bombus sensu stricto* and closely related to *B. cryptarum* and *B. patagiatus*. It is abundant in Atlantic heaths from Portugal to Norway [1,2]. Characters given by Rasmont to separate *B. magnus*, *B. cryptarum*, *B. terrestris* and *B. lucorum* show a great variability in coloration and a great homogeneity of genitalia which make them difficult to identify [2]. Because of this variability Williams [3] does not recognise *B. magnus* as a good species. As application of the Paterson species recognition concept [4], the composition of the labial gland secretion, used by males for scent-marking their patrolling nuptial behaviour, seems to be the most reliable trait for recognising the validity of the bumblebees species [5].
Main component of the labial gland secretion of *B. magnus* is ethyl dodecanoate (23 %) as it is the case also for *B. patagiatus* [5] and *B. cryptarum* [6]. This is also a major or minor characteristic component in all known sexual pheromones of other species of the subgenus *Bombus* [5-7]. Unsaturated C_{18} alcohols and ethyl esters of the corresponding acids are found in *B. magnus* and *B. lucorum* [7], but they are not reported in *B. cryptarum* [6]. Beside the main component, many other lower-abundant components are in common for *B. magnus* and *B. cryptarum*.. However, their proportions are different. Another characteristic of *B. magnus* sexual pheromone is that it contains small amount (0.4 %) of geranylcitronellol and other terpene derivatives. The same is true for *B. lucorum* (0.3 % of isoprenoids). However, *B. terrestris* is the only other species of the subgenus *Bombus* which contains large amounts of isoprenoids (2,3-dihydrofarnesal 5 %, 2,3-dihydrofarnesol 18 %, 2,3-dihydrofarnesyl acetate 2 %, geranylcitronellal 4 %, geranylcitronellol 8 %, and 2,3-dihydrofarnesyl dodecanoate 6 %).

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SEMIOCHEMICALS FROM HONEY BEE ROYAL JELLY INVOLVED IN THE COMMUNICATION WITH THE VARROA MITE

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The varroa mite, *Varroa destructor* (formerly *V. jacobsoni*), is a parasitic mite of the honey bee *Apis melifera*, and is still a serious threat to the honey bee keeping industry. Controlling this pest with acaricides has been successful, but reinfestation after the treatment is not uncommon and treatments must thus be repeated once or twice a year. Therefore much research effort is done on obtaining more information on the communication between the honeybee, *A. melifera*, and the varroa mite.
Mites reproduce on both worker and drone brood, but rarely enter queen cells [1, 2]. In addition to this, we recently discovered that royal jelly from the honey bee queens was found to be repellent towards the mites [2]. This study, therefore, focuses on the identification of the compounds present in the royal jelly which cause the repellent activity. Royal jelly was fractionated in several steps on silica gel in order to remove non-active compounds. Identification of the compounds in the active fractions was achieved by GC/MS after methylation and silylation of the compounds. The active fractions consisted mainly of several fatty acids, fatty di-acids and hydroxy fatty acids.
Identification, synthesis of and bioassays on these compounds are to be discussed.

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FRONTALIN BIOSYNTHESIS IN THE JEFFREY PINE BEETLE, *DENDROCTONUS JEFFREYI* HOPKINS (COLEOPTERA: SCOLYTIDAE).

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Frontalin, discovered over 30 years ago through the efforts of pioneering chemical ecologists such as Silverstein, Pitman, Wood and Vité, is an aggregation pheromone component of many *Dendroctonus* species bark beetles. Although frontalin is critical to the aggregation behavior of these economically important pests of pines, its biosynthesis has not been well studied. We are studying this biosynthesis in the Jeffrey pine beetle, *Dendroctonus jeffreyi*, a major pest of pine in the Sierra Nevada Mountains of western North America. Topical application of juvenile hormone III (JHIII) induces the production of frontalin in adult male *D. jeffreyi*, and we have evidence that JHIII is the developmental cue that triggers frontalin production in males emerging from their brood tree. Radiotracer experiments in JHIII-treated male *D. jeffreyi* were conducted to examine the following hypothetical *de novo* pathways of frontalin biosynthesis: (**1**) a fatty acid-like elongation of leucine followed by cyclization to frontalin, (**2**) an isoprenoid pathway synthesis followed by the same cyclization, or (**3**) a hybrid of the two pathways. [^{14}C]Frontalin was extracted from groups of male *D. jeffreyi* that had been injected with the radiolabeled isoprenoid intermediate [^{14}C]mevalonolactone or [^{14}C]isopentenol, providing evidence for (**2**). A key regulatory enzyme in isoprenoid synthesis is HMG-CoA reductase (HMG-R). Following JHIII treatment, frontalin mass and HMG-R transcript increase coordinately in a time- and dose-dependent manner. These results further support an isoprenoid synthesis (**2**) of frontalin. In a stable-isotope labeling study, [^{13}C]acetate was injected into adult male *D. jeffreyi*, and isotope ratio monitoring-GC-MS analysis showed a high incorporation of ^{13}C into frontalin. Taken together, these frontalin production, gene expression, and isotope labeling results provide strong evidence for the *de novo* isoprenoid biosynthesis of frontalin in male *D. jeffreyi*.

PHEROMONE BIOSYNTHETIC PATHWAY FOR DISPARLURE IN THE GYPSY MOTH, *LYMANTRIA DISPAR*

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The pheromone biosynthetic pathway for production of disparlure, 2-methyl-7R,8S-epoxy-octadecane, was investigated in the gypsy moth. Disparlure was first identified in 1970 in part by chemical epoxidation of the alkene[1]. The alkene is not required for attraction but may inhibit male behavior [2]. A previous report has indicated that radiolabeled alkene can be converted to disparlure although it was not determined in which tissue the conversion was taking place [3]. In this report we utilized deuterium labeled precursors and intermediates to follow specific reactions in the pathway. It was determined that the alkene precursor, 2-methyl-Z7-octadecene, is most likely made in oenocytes cells. This pathway uses valine to contribute the methyl carbons for chain initiation, followed by chain elongation to 19-carbons. The double bond is introduced with an unusual Δ12 desaturase followed by decarboxylation to the hydrocarbon. The alkene is then transported to the pheromone gland through the hemolymph. This is similar to that already reported for two arctiid moths [4]. We have already identified the alkene and 2-methyl-octadecane in the hemolymph of female gypsy moths [5]. At the pheromone gland the alkene is unloaded and transformed into the epoxide. This last step is regulated by PBAN. Each step in this pathway was followed using deuterium labeled compounds that could be identified using GC/MS. This provides unequivocal determination of specific reactions in the pathway.

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PHEROMONE PROCESSING IN THE GYPSY MOTH, LYMANTRIA DISPAR.

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Pheromone olfaction begins with the pheromone-binding protein binding the pheromone (PBP) at the sensory hair cuticle.. The PBP desorbs the pheromone from the cuticle [1] and transports the pheromone to the dendritic membrane, where the pheromone most likely stimulates a transmembrane receptor [2]. The sequence of events ends with degradation and/or active removal of the pheromone from the sensory hair lymph [3]. Pheromone is also actively degraded in the scales that cover many body parts, such as the wings [4]. this prevents excessive accumulation of pheromone on the surface of the moth. Previous studies have revealed that the gypsy moth has an antennal epoxide hydrolase, which hydrolyses both enantiomers of *cis* 7,8-epoxy-2-methyloctadecane (disparlure) to the (7R, 8R) *threo* diol [5]. The inhibition of this enzyme was also studied [6]. We have further investigated processing of the disparlure enantiomers and of (7Z) 2-methyloctadec-7-ene. Our findings suggest that the epoxides are processed to the diol and to compounds more polar than the diol. A UDP-glucosyl transferase as well as an NADPH-requiring enzyme are involved in these processes. The alkene is oxidized to a mixture of less saturated alcohols, which are further processed to more polar compounds. At least one membrane-bound monooxygenase is involved and one soluble enzyme. One step is sensitive to CO inhibition.

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cDNA CLONING OF BIOTRANSFORMATION ENZYMES BELONGING TO CYTOCHROME P450 FAMILY IN THE ANTENNAE OF A NOCTUID MOTH

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The involvement of cytochrome P450 enzymes in olfaction has been demonstrated in vertebrates long time ago. In insects, these enzymes are well known for their role in insecticide resistance, but the implication of P450 in pheromone degradation was just recently demonstrated. Using a PCR strategy, we have isolated two cDNAs from the antennae of the cabbage armyworm *Mamestra brassicae*, *CYP4L4* and *CYP4S4*, which encode microsomal P450s. *CYP4S4* expression is restricted to the antennae, whereas *CYP4L4* is also found in the proboscis and legs. Moreover, the two genes are strongly expressed in one type of sensory unit of the antennae, *i.e.* the sensilla trichodea, which are tuned to odorant detection. The putative function of the corresponding enzymes is discussed in regard to their respective expression patterns.

MOLECULAR EVOLUTION OF ODORANT-BINDING PROTEIN GENES IN MOTHS

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In moths, olfaction is vital to find suitable food sources, host-plants and mating. Olfaction is first mediated by sensillar hairs that capture and detect odorants through specialized sensory neurons. A classical feature of these olfactory hairs is a sensory neuron surrounded by an aqueous environment, the sensillar lymph. Therefore, when entering a sensillum, hydrophobic odorants have to cross this lymph before to reach the olfactory receptor neurons. The odorant binding proteins (OBPs) are proteins from the lymph that are thought to permit the solubilization of odorants [1]. In noctuids, OBPs may be divided into pheromone binding proteins PBP Grp-1 and Grp-2 and general odorant binding proteins GOBP1 and GOBP2 [2-3]. The PBPs are small water-soluble proteins (14-15kDa) characterized by six highly conserved cysteines and an amphiphilic structure. A similar structure is found in GOBPs that retain up to seven conserved cysteines but the different OBPs show a specific sensillar distribution. The PBPs are highly expressed in the male antennae whereas GOBPs are found in the antennae of both sexes with higher levels detected in females. On the male antennae, PBPs are found in pheromone-sensitive sensilla whereas GOBPs are located in sensilla responding to general odorants such as plant volatiles [4-5]. Unlike PBPs, GOBPs show highly conserved amino acid sequences between species (78 to 92% identity within GOBP subfamilies) suggesting that GOBP may bind common ligands while the more variable PBP may be tuned to the recognition of specific pheromone blend.
Earlier studies reporting two PBP (Aips-1 and Aips-2) genes in the noctuid species *Agrotis ipsilon* have demonstrated that Grp-1 and Grp-2 genes have a similar structure exon-intron-exon-intron-exon but differ in the intron lengths. The Aips-1 and Aips-2 genes show about 45% of identity and are thought to have evolved from gene duplication [6]. In the sphingid *Manduca sexta*, GOBP and PBP genes have been shown to be juxtaposed and both exhibit the 3 exons / 2 introns structure [7]. Thus, the genes encoding general odorant and pheromone binding proteins are found in a cluster of structurally related genes.
To explore the functions of different OBPs, specific experiments using ligand-binding assays, structure crystallography and immunocytochemistry are required. However, analysis of the diversity of gene structures with respect to the changes in pheromone composition and to the species evolution should also be informative about the role of OBP in insect olfaction.
If GOBPs are tuned to bind common general odorants (i.e. plant volatiles) little evolution should be expected in the genes encoding GOBP in contrast to the genes encoding pheromone-binding proteins. This has led us to the characterization of the GOBP gene in *Agrotis ipsilon*. In this species, tissue distribution of RNA by RT-PCR and Northern Blot analyses reveals that expression of GOBP is preferentially associated to the female antennae.

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SEX PHEROMONE EVOLUTION VIA ANCESTRAL GENES

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A great diversity of pheromone structures are used in the Lepidoptera for long-distant mating signals, although the signal/response channel appears to be narrow for each species. The conundrum is how signal divergence has occurred in the face of strong selection pressures against small changes in the signal. Here we present data that support a major shift in the pheromone blend of an *Ostrinia* species by activation of a nonfunctional desaturase gene present in the pheromone gland. We also discovered that rare males exist that respond to the new pheromone blend, which would allow for asymmetric tracking of male response to the new blend and, thus, evolution of an *Ostrinia* species with structurally different sex pheromone components.

CHANGES IN PHEROMONE AND FATTY ACYL PHEROMONE ANALOG TITERS IN THE Z-STRAIN OF THE EUROPEAN CORN BORER.

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The European corn borer, *Ostrinia nubilalis*, produces a blend of (*Z*)- and (*E*)-11-tetradecenyl acetates as its sex pheromone. The two pheromone strains of this species ("Z" and "E" strains), produce blends containing, respectively, approximately 97:3 and 3:97 of these two isomers. In spite of extensive study on the genetics of pheromone biosynthesis in the two strains, relatively little work has been carried out on the mechanisms of pheromone biosynthesis in this insect. A study by Wolf and Roelofs [1] on the fatty acids in the pheromone gland of both these strains showed, surprisingly, that the ratios of the fatty acyl analogs (FAPAs) of the pheromone components were different from those of the actual pheromone components themselves, and indeed similar in both strains (i.e., roughly 30:70 Z:E in both strains). A later study by Zhu et al, [2] showed that the fatty acid reductase was principally responsible for determining the final pheromone component ratio in both strains. Both of these studies analysed the FAPAs as a "snapshot" (i.e., at a single age and photoperiod). Recent work [3] has shown that FAPAs in moth pheromone biosynthesis are dynamic, changing both with age and photoperiod. We have begun a more detailed examination of pheromone biosynthesis in *O. nubilalis* focusing on its dynamics, particularly how it is regulated and how fatty acids are partitioned between lipids and, ultimately, pheromone. In this poster, we report on the dynamics of pheromone and lipid precursor titers in the Z-strain of *O. nubilalis*, with respect to both age and photoperiod.

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ACTIVITY OF A POTENT ESTERASE INHIBITOR ON DEVELOPMENT AND BEHAVIOUR IN *SPODOPTERA LITTORALIS* AND *SESAMIA NONAGRIOIDES*

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The Egyptian armyworm *Spodoptera littoralis* (Boisd.) is one of the most important polyphagous pests, widely distributed in the Mediterranean region and North and East Africa. The corn stalk borer *Sesamia nonagrioides* (Lef.) is one of the most serious pests of corn, particularly in the Mediterranean basin and North Africa countries.
Extensive research has been conducted on trifluoromethyl ketones (TFMKs) as selective "in vitro" and "in vivo" inhibitors of esterases in Lepidoptera, particularly JHE and antennal esterases. In "in vivo" experiments TFMKs have been found to disrupt the orientation flight of *S. littoralis* and *S. nonagrioides* males to pheromone sources (virgin females or synthetic pheromone) [1], whereas in the field the chemicals displayed inhibition of the pheromone action on conspecific males when mixed with the synthetic pheromone [2]. In our current project directed to the development of inhibitors of the pheromone catabolism in insects, we have conducted a series of experiments to examine the effect of 3-octythio-1,1,1-trifluoro-2-propanone (OTFP) on oviposition, growth, development and behaviour of the Egyptian armyworm and the corn stalk borer. The chemical behaved as an oviposition deterrent, and when added to the diet of the 2nd instar larvae of both insects reduced diet consumption and growth, pupation and adult emergence. Treatment of the compound on late instars did not produce significant differences in the amount of diet ingested. Our results suggest that the effect of OTFP is long-lasting and that the inhibitor is not fully detoxified by the detoxification enzymes of the digestive tract of the insects. In behavioural assays, adult males, which had been treated with the chemical at the larval stage, were less attracted to the pheromone source than regular untreated males. When *S. littoralis* untreated females were used as the attractant source, treated males also displayed a significantly lower number of contacts with the cage-containing females than untreated or solvent-treated males. Using previously treated females, only 27% of treated males successfully completed the flight in comparison to animals responding to control or hexane-treated females. By contrast, when *S. nonagrioides* females, which had been subjected or not to the treatment, were used as the attractant source, males were similarly attracted to them regardless they had been treated or not at the larval stage. Analyses of gland extracts of *S. littoralis* treated females showed no difference with regard to control animals in the qualitative or quantitative composition of the pheromone. These results, in combination with other previously reported by us [2], provide new and relevant information about the possible utility of these chemicals in future new biorational approaches to pest control.

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AGE-RELATED CHANGES IN PHEROMONE GLAND COMPETENCY AND PHEROMONOTROPIC ACTIVITY OF BRAIN EXTRACTS IN VIRGIN AND MATED FEMALES OF TWO *CHORISTONEURA* SPECIES.

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Nine-day-old decapitated females injected with different doses of *Hez*- PBAN produced significantly less pheromone than 1-day-old individuals, suggesting that the age-related decline in the pheromone titre of *Choristoneura fumiferana* and *C. rosaceana* virgin females was primarily the result of a reduced ability of the glands to produce pheromone. In *C. fumiferana*, a lower pheromonotropic activity of the Br-SEG may also contribute to the pheromone decline with age but not in *C. rosaceana*, as the pheromonotropic activity of their Br-SEG remained constant throughout the females' life. In both *Choristoneura* species, mating also suppressed pheromone production (pheromonostasis) after 24 h. The Br-SEG of mated females contained PBAN but there was no indication that its concentration changed with time post-mating since Br-SEG homogenates obtained from different-aged mated females showed the same level of pheromonotropic activity in both *Choristoneura* species. However, as observed in virgins, pheromone glands of older mated females were less sensitive to PBAN than those of younger ones. This suggests that the probability of *Choristoneura* females to attract a second mate may decrease with an increase in the refractory period following the first mating.

SPECIFICITY OF RECEPTOR NEURON RESPONSES TO SEX PHEROMONE COMPONENTS AND A BEHAVIORAL ANTAGONIST IN *OSTRINIA FURNACALIS*.

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We recorded receptor neuron responses to female sex pheromone components and behavioral antagonists on the antenna of male Asian corn borer moth, *Ostrinia furnacalis* (Lepidoptera: Crambidae) by using a cut-sensillum technique. Each sensillum was found to include 2-3 neurons.
Five physiological types of responding neurons were observed in short sensilla trichodea. Type 1 neurons responded to (*E*)- and (*Z*)-12-tetradecenyl acetates (E12- and Z12-14:OAc), which are the two pheromone components of *O. furnacalis*. Type 2 responded to Z12-14:OAc alone. Types 3 and 4 responded to E12- and/or Z12-14:OAc, but with small amplitude action potential. Type 5 responded to Z9-14:OAc, which is a pheromone component in sympatric congeners, *O. zaguliaevi* and *O. zealis*, and is preliminarily observed as a behavioral antagonist to *O. furnacalis*.
Cross-adaptation and dose-response studies confirmed that single type 1 neurons were equally responsive to E12- and Z12-14:OAc. Dose-dependent responses in type 3 to E12- and Z12-14:OAc and those in the type 5 to Z9-14:OAc were also observed.
O. furnacalis has a unique coding system for pheromone information built in types 1 and 3 responding to two pheromone components and types 2 and 4 responding to one pheromone component. On the other hand, *O. nubilalis*, which is closely related to *O. furnacalis*, has two types of neurons responding to each pheromone component irrespective of pheromone races.

ANTENNAL SENSILLA OF THE PALM WEEVIL *RHYNCHOPHORUS PALMARUM* (COLEOPTERA: CURCULIONIDAE)

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Antennae of male and female of the palm weevil, *Rhynchophorus palmarum*, were studied by scanning and transmission electron microscopy, to determine types and locations of the sensory structures. The whole antennal structures, except some mechanoreceptors are concentrated on the antennal club, which is restricted in this insect, on a single segment, characteristic of the Rhynchophorinae. This restriction resulted on a high density, and on a homogeneous distribution of the hair like structures on a plane surfaces. We observed five types of hair like structures reaching on four different levels from the cuticular base. Type I is mechanoreceptor sensilla, the longest (250 µm) and the less abundant on the antenna (20 / antenna). Type II is grooved, ridged, thick-walled sensilla that project at nearly right angles from the cuticle. They are 50 µm long and are present on a high density (17,000 / antenna). They contain four dendrites and a terminal pore.. Their contact chemoreception function could be confirmed by tip recording of firing responses using sugar solution as stimulus. Type III are bifid, grooved hairs, the most abundant on the antennae (20,250 / antenna) and their length ranges from 28 to 30 µm. They do not present either wall pores or dendrites in the lumen; they could have a protective function. The olfactory sensilla, trichoidea and basiconica, are located at the lowest level. Type IV is sensilla trichoidea, which length ranges from 20 to 25 µm. They are thick-walled, showing wall pores with low density (20 pores / μm^2), unbranched dendrites and house one to three cells in the lumen. Type V is sensilla basiconica, which are present in three subtypes. The main difference is their lengths that are respectively 20, 14 and 10 µm. Their number on the antenna is estimated to 10,000, but we were not able to distinguish them easily through the dense group of other hairs. The sensilla basiconica are thin-walled with branched dendrites and higher density of wall pores (25 pores / μm^2)

DISTRIBUTION AND RESPONSE OF THE TARSAL CONTACT CHEMO-SENSILLA IN THE BUTTERFLY, *ATROPHANEURA ALCINOUS*

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The oviposition of butterflies is induced by the recognition of the host plant components with their tarsi in the forelegs. We examined distribution and electrophysiological response of the tarsal contact chemosensilla in the Aristlochiaceae-feeding butterfly, *Atrophaneura alcinous.*
The tarsal contact chemosensilla in the foreleg were classified into two groups based on their length: long type and short type using SEM. The long type sensilla were distributed widely much more in females than those in males, whereas the short type sensilla were done at the edge of tarsi in a similar manner between sexes. Each sensillum in both types forms a small pore at the tip of sensilla.
We recorded taste responses from both the long and the short sensilla to the methanolic extracts of *Aristlochia debilis* (host) and of *Citrus* spp. (non-host), some pure compounds by the tip-recording technique. The long type sensilla evoked several types of impulses by the extracts of *A. debilis* and a type of impulse by the extracts of *Citrus* spp. The predominant impulse evoked by the extracts of *A. debilis* was distinguishable from the impulse by the extracts of *Citrus* spp., according to binary mixtures experiment. The short type sensilla were failed to observe any response to the extracts of *A. debilis* and *Citrus* spp., but they responded to sucrose and NaCl depending on the concentration. Thus, the physiological property of each type of sensilla was associated with the morphology.
The results of these experiments suggest that the butterfly can discriminate the taste of host plant components from other chemicals with the long type sensilla during oviposition and may recognize the taste of diets containing sucrose and NaCl with the short type sensilla.

THE VARIATION OF THE NUMBER AND LOCATION OF THE FORELEG 5TH TARSI TRICOID SENSILLA OF JAPANESE PAPILIO BUTTERFLIES

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The number and location of the foreleg 5th tarsi tricoid sensilla of Japanese *Papilio* butterfly (9 species) were examined. Every species, female butterflies had more sensory hairs than male butterflies. This difference was more conspicuous in the species of Subgenus *Menelaides*. In *P.(M.) protenor*, *P. (M.) helenus* and *P. (A.) bianor* which occur both Main area of JAPAN and Nansei Islands and separated into some subspecies by their color patterns of adult wing and larvae, the number and location of sensilla also showed geographical variations that are almost corresponding to the difference of subspecies. In female butterflies, *P. (M.) memnon* who lays eggs only on *Citrus* spp. have most number of sensilla, in contrast, subgenus *Achillides* species, *P. (M.) macilentus* who lay eggs on *Citrus*, *Zanthoxylum*, *Phellodendron* and *Orixa* have not so many amount of sensilla.

CLASSIFICATION OF PLANT ODOUR RECEPTOR NEURONES ACCORDING TO RESPONSE SPECIFICITY OBTAINED BY ELECTROPHYSIOLOGY LINKED TO GAS CHROMATOGRAPHY AND MASS-SPECTROMETRY.

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An important question in studies of olfactory coding is for which chemicals the receptor neurones are evolved. In our laboratory we have employed electrophysiology linked to gas chromatography and mass spectrometry in order to identify odorants in the complex volatile mixtures released by plants that are detected by single olfactory receptor neurones (ORNs) in heliothine moths. All recordings have shown a marked best response by single ORNs to one component and secondary responses to a few structurally related chemicals. Thus, the neurones could be classified in distinct types according to the most effective odorant. So far 17 types of plant ORNs have been identified, showing no overlap of the response spectra (Røstelien et al., 2000a,b, Stranden et al., 2002). For 5 types the active components have been identified by GC-MS and retested on the relevant ORN types, confirming the identification. The primary 5 components are (-)-germacrene D, E-beta-ocimene, E,E-alpha-farnesene, homo-farnesene and (+)-linalool. Interestingly, the ORNs responding to (-)-germacrene D constitutes a major neurone type in the investigated heliothine species (80% of the recordings in Heliothis virescens) and show the same enantioselectivity and specificity. The other ORN types also showed the same specificity in these related species (H.virescens, Helicoverpa armigera, and Helicoverpa assulta). Preliminary stainings of the ORNs show projections in one or two glomeruli. It is expected that more neurone types are present in these species, since the primary olfactory centre, the antennal lobes, contain 62-66 ordinary glomeruli involved in processing plant odour information (Berg et al., 2002). The identification of relevant stimulants for the ORNs have made it possible also to study the behavioural significance of odorants in the interaction with the host plant; whether they are involved in feeding, egg laying or avoidance reactions and whether odorants of the different context can be learned.

TASTE IN HELIOTHINE MOTHS: I. ANTENNAL TASTE RECEPTORS – PHYSIOLOGICAL CHARACTERIZATION AND PROJECTION PATTERNS.

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The heliothine moths can learn odours by pairing odour stimulation (conditioned stimulus) with stimulation of the taste sensilla with sucrose (unconditioned stimulus). With the aim to reveal the neuronal connection between the two sensory pathways, we have studied the taste sensilla (s. chaetica) on the antennae and the projection of the associated receptor neurones in the primary taste centre, the suboesophageal ganglion (SOG), of the CNS. SEM imaging showed that 4 s. chaetica are present on all flagellar segments, except for the most distal having a higher sensilla density. S. chaetica is characterised by one pore at the tip of the hair, which is about 100 micrometer long with a diameter of 8-10 micrometer. Tip recordings were performed using glass microcapillaries filled with 0,1 M KCL and various chemicals to be tested, e.g. sucrose. The recordings indicated the presence of 4 taste receptor neurones, which is in accordance with previous morphological studies. Movement of the sensilla elicited responses of a mechanosensory neurone, displaying large spike amplitudes. In the present study we stained the receptor neurones with tetramethylrhodamine dextran by cutting the sensilla near the base while the antennae was submerged in the dye. Imaging in confocal laser scanning microscope showed that the axons bypassed the antennal lobe laterally and proceeded toward the SOG where they terminated in a fingerlike pattern. Axons from the same sensillum ran tightly together and projected in the same area. A Micro 3D software for analyses of neuroanatomical spatial distribution (Oslo Research Park) was employed to compare the projection pattern of selected receptor neurones. It is expected that neurones located in the SOG will be connected to neurones of the olfactory pathway.
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TASTE IN HELIOTHINE MOTHS: II. CONTACT CHEMOSENSILLA ON THE PROBOSCIS – PROJECTION PATTERNS OF THE RECEPTOR NEURONS.

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Stimulation of the taste sensilla with sucrose elicits extension of the proboscis in nectar feeding insects. This proboscis extension reflex (PER) is used to study appetite learning, where sucrose stimulation represents the unconditioned stimulus and odours the conditioned stimulus (Menzel 19). In this study of Heliothis virescens, we are presenting the morphology of the taste sensilla (s. styloconicae) on the proboscis and the projections in the CNS of the associated receptor neurones. This study is part of a project studying the neuronal connection between the taste and olfactory pathways in heliothine moths. The morphology of the sensilla which are present on the distal part of the proboscis, were studied by light and electron microscopy (SEM, TEM). The sensillae are 100 micrometer long and 20 micrometer in diameter. In cross-section they have a hexagonal, star-like shape and the lumen contain 3 or 4 dendrites enveloped by a dendritic sheath made by the one of the supporting cells. Large epidermal cells extends to the tip of the sensillum, separating the inner dendritic space from an outer lumen. The tip of the sensillum is protruded with a single pore for penetration of taste chemicals. The receptor neurones were stained with tetramethylrodamindextran and the projections were viewed in a confocal laser scanning microscope. By applying software for analyses of neuroanatomic spatial distribution (Micro3D), Oslo Research Park) a further 3D visualisation of selected receptor neurones and brain structures was made. The stained axons of the receptor neurones were traced to the suboesophageal ganglion (SOG). Two pairs of nerves enter the SOG, and the stained axons were found in the dorsal nerve pair, with extensive arborizations in the ipsilateral neuropil.
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INTERACTION OF COMPLEX ECDYSTEROIDS, ECDYSIS-DISTURBING SAPONINS AND SOME LIGNANS WITH THE ECDYSTEROID RECEPTOR

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Ecdysteroid agonist and antagonist activities can be detected and quantified with the *Drosophila melanogaster* B_{II} cell bioassay. We are comparing the biological activities of a series of natural and chemically modified ecdysteroids and also ecdysis disturbing spirostanol saponins in this bioassay, in which the potency of the effect reflects the affinity of binding to the ligand-binding site of the *Drosophila melanogaster* ecdysteroid receptor [1]. The natural ecdysteroids were isolated from higher plants or from fungi. The structural analogues and conjugates were prepared by chemical transformations, and the complex dimeric ecdysteroids by specific phototransformation of 20-hydroxyecdysone, ponasterone A or ajugasterone C. The activity data were used to investigate the structure - activity relationships and to design further targeted structure modifications. The obtained activities [2,3] generally confirmed the activities from previous *in vivo* tests. They also contributed to the expression of the structure-activity relationship, for the first time in a complex form of a pharmacophore hypothesis [4]. In order to explore this phenomenon further, we prepared and tested several new specific analogues and also unique ecdysteroid dimers obtained from phototransformations. The results corrected some of the former general presumptions. We are comparing here the activities of the relatively large ecdysteroid dimeric and glycosidic molecules with their structurally related but considerably smaller monomeric analogues and aglycones. The ecdysis disturbing spirostanol saponins were found inactive at the receptor and thus indirectly confirmed the previously elucidated mode of action [5] concerning a regulatory effect at the early steps of the biosynthesis of ecdysone. A series of other non-steroid plant substances, some of which are known to affect insect development and reproduction, were also assessed in this bioassay [6]. We concentrate here on certain selected lignans [7] and structurally related phenylpropanoids.

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A CLASSIFICATION OF KAIROMONES

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Although the usefulness of the term kairomone was discussed controversially after its introduction, it is now widely accepted by the scientific community. According to the last update of its definition, the term kairomone is commonly used to describe a chemical that is pertinent to the biology of an organism (organism 1) and that when it contacts an individual of another species (Organism 2) evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 2 but not to organism 1 [1]. A look at the chemoecological literature reveals that chemicals classified by the mere term kairomone may have completely different biological functions for the receiving organism. Chemicals meeting the definition of a kairomone are used for the location of food sources and sexual mates, or may be used by potential prey or host organisms to decrease the negative impact of natural enemies. Thus, by describing those diverse mediators merely as kairomones, no information on the actual function of the chemical is given. When considering the terminology of pheromones, another diverse group of infochemicals mediating intraspecific interactions, further subdivision is common practice and useful to describe the multitude of different functions and thus, to prevent terminological confusion.
We propose a classification of kairomones [2] according to the function for the benefiting organism introducing the terms *foraging kairomone* (used in the context of food location), *enemy-avoidance kairomone* (used to reduce the negative impact of natural enemies), *sexual kairomone* (used for sexual purposes), and *aggregation kairomone* (attracting/arresting both sexes of an organism). Additionally, discrimination of two groups of kairomones according to the effect on the benefiting organism is proposed leading to the terms *primer kairomone* (inducing physiological responses) and *releaser kairomone* (inducing behavioural responses). The intention of the proposed classification is to allow a more precise description of kairomones and thus, to aid the discussion of these compounds and to improve the readability of kairomone-related papers.

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THE ROLE OF NATURAL ENEMIES ON THE EVOLUTION OF HOST PLANT SHIFT IN *CHRYSOMELA LAPPONICA* (COLEOPTERA: CHRYSOMELIDAE)

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Palearctic leaf beetles of the genus *Chrysomela* usually feed upon Salicaceae which have been suggested as ancestral host plants of this taxon. The leaf beetle species *Chrysomela lapponica* forms distinct geographically separated populations in Central and Northern Europe. Individuals of a population in the Czech Republic feed monophagously on the birch *Betula pubescens* (Betulaceae) and show morphological and chemical features different from individuals of a population in North Lapland (Finland) feeding on willow species, mainly *Salix borealis*. The specialization of *C. lapponica* on either birches or willows is associated with a change in the composition of the defensive larval secretion. In this study, we were investigating the significance of natural enemies as possible selective forces for the host plant shift of *C. lapponica* from willow to birch. While both types of secretion were shown to be repellent towards generalist predators with chewing mouthparts, secretions were inactive against predaceous bugs. The hoverfly *Parasysrphus nigritarsis*, a specialist predator of eggs and larvae of Chrysomelinae leaf beetles, was significantly less attracted by chemical signals (faeces and secretions) of birch-feeding larvae than by signals of larvae from the willow feeding population. Additionally, high infestations of the eggs of *C. lapponica* and related Chrysomelinae by *P. nigritarsis* were found on willows in both habitats while no infested eggs were found on birches. Moreover, lower abundances of larval and pupal parasitoids were detected on birches than on willows in the field. We hypothesize that the host plant shift of *C. lapponica* from willow to birch has been favoured by the advantage of escaping to a plant with lower risk of predation and parasitization.

HOW TO ENHANCE EFFECTIVENESS OF CARNIVOROUS NATURAL ENEMIES IN TRITROPHIC SYSTEMS.

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To realize sustainable agriculture, the effective use of carnivorous natural enemies of herbivorous insects is of great importance. Plants are known to emit several volatiles that attract carnivorous natural enemies in response to herbivory [1,2]. Therefore, one way to enhance the effectiveness of the carnivores is to modify infested plant-carnivore interactions mediated by herbivore-induced plant volatiles.
Jasmonic acid (JA) activates the expression of genes encoding antifungal proteins such as beta-1,3-glucanase and chitinase, and that of genes encoding protease inhibitors (PIs) against herbivores [3]. Furthermore, it has also been reported that herbivore-specific production of volatiles is controlled by the phytooxylipin pathway leading to JA, based on the finding that the exogenous application of JA induced the production of volatiles [4-6].
In this paper, we will discuss ways to enhance the effectiveness of parasitic wasps by focusing on the JA and phytooxylipin pathways. The following tritrophic systems will be discussed.

(1) Lima bean plants, two-spotted spider mites (*Tetranychus urticae*) and predatory mites (*Phytoseiulus persimilis*)
(2) Corn plants, common armyworms (*Mythimna separata*) and parasitic wasps (*Cotesia kariyai*)
(3) *Arabidopsis thaliana*, cabbage butterfly larvae (*Pieris rapae*) and parasitic wasps (*Cotesia glomerata*).

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GOURMETS, GOURMANDS AND CHEMICALS: TESTING THE CONCEPT OF DIETARY SPECIALISATION AND INFOCHEMICAL USE IN CARNIVORES.

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For the location of hosts and prey, insect carnivores like parasitoids or predators often use infochemical cues that may originate from the host/prey but also from the hosts/preys food plant or feeding substrate. These cues can be either specific for certain host/prey complexes (i.e. host/prey plus food plant or feeding substrate) or generally present in various complexes. The reaction of the carnivores to these cues is either innate or learned. According to the concept of dietary specialisation and infochemical use in natural enemies [1] these traits (origin, specificity, innateness) should depend on the dietary specialisation of the carnivore and its host/prey species. With 300 citations since 1994 (ISI-Web of Science) this concept has been often cited in studies on chemical foraging cues of especially parasitoids to put results in perspective. Only few studies, however, have been explicitly designed to test predictions of the concept. Thus, ten years after being published and despite of its broad acceptance, the general validity of the concept is still unclear.
Using data from about 140 research papers on 95 species of parasitoids and predators, the present literature study comparatively examines the following three predictions of the concept: (1) Extreme generalists do not use infochemicals for foraging; (2) Specialists innately use specific cues and generalists innately use general cues; (3) Learning of infochemicals is expected in generalist carnivores and not in specialists.
In contrast to the first two predictions, the use of infochemicals for foraging has been reported in over 25 generalist species, including spiders, carabids, and lacewings, and the innate use of specific chemical cues was found in many species regardless of dietary specialisation. In accordance with the concept, learning of chemical foraging cues was predominantly found in generalist natural enemies and less frequently for specialist carnivores feeding on specialist herbivores. Thus, current data only support the last prediction. Other, more sophisticated predictions of the concept are difficult to test at present due to a lack of experimental data. To fill this gap, experiments in which the experience and the specificity of cues are established are required.

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BIOCHEMICAL AND MOLECULAR MECHANISMS OF TERPENOID VOLATILE FORMATION IN COTTON PLANTS IN RESPONSE TO INSECT HERBIVORY.

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Plants that are under herbivore attack often release a large variety of volatile organic compounds. These substances can serve as cues for predators and parasitic wasps leading them into the vicinity of their herbivorous prey or host [1,2]. Volatiles released from attacked cotton plants (*Gossypium hirsutum*) can therefore benefit both the plant in attracting natural enemies of herbivores that feed on its foliage, and the parasitoid by indicating the presence of potential hosts on the plant [3]. In cotton, the blend of volatiles released specifically in response to herbivory includes mostly acyclic terpenoids, like (*E*)-β-ocimene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene [4] that are synthesized *de novo* [5]. Terpene synthases catalyze the committed steps in biosynthesis of the different terpenoid carbon skeletons. We found that levels of mono- and sesquiterpene synthase activity are upregulated in response to herbivory. Terpene synthase genes involved in the formation of herbivore-induced volatiles and other defense related genes are now being identified by random sequencing of a cDNA library, constructed from herbivore-induced cotton leaf tissue, and by screening the library with heterologous probes designed to conserved regions of terpene synthases. The isolated genes will be used to understand the physiology and regulation of volatile emission, and to manipulate terpene release to test its ecological significance for the plant, its herbivores and the parasitoid and predator community.

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CHANGE IN ACCEPTABILITY OF BARLEY PLANTS TO APHIDS AFTER EXPOSURE TO ALLELOCHEMICALS FROM COUCH-GRASS (*ELYTRIGIA REPENS*)

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The response of the bird-cherry-oat aphid, *Rhopalosiphum padi*, to barley plants was investigated following exposure of the plants to root allelochemicals from the aggressive weed couch-grass, *Elytrigia* (*Agropyron*) *repens.* Plants were treated either with root exudates from living couch-grass plants, or with the previously identified [1,2] couch-grass root compounds 5-hydroxyindole-3-acetic acid, DL-5-hydroxytryptophan, L-5-hydroxytryptophan hydrate and 6-hydroxy-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (carboline), either separately or in mixtures. In choice and no-choice settling tests, aphid acceptance of barley plants was significantly reduced following treatment with root exudates, and the carboline when tested alone or in combination with the other compounds. In contrast, the other compounds without the carboline were less active in reducing aphid acceptance. In a probing bioassay, individual substances were either neutral or stimulatory to aphids, indicating that the reduced settling was probably not due to direct effects on the aphids, but rather due to effects on the plant. This was confirmed in olfactometer assays, in which aphids were repelled by odours from barley plants following treatment with a mixture containing all four chemicals. The responses of aphids to barley plants exposed to *E. repens* allelochemicals are similar to those reported after exposure of barley plants to volatiles of other barley plants [3].

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ANTENNAL RESPONSES OF BANDED SUNFLOWER MOTH (*COCHYLIS HOSPES*) TO COMPONENTS OF SUNFLOWER HEAD EXTRACTS.

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Female banded sunflower moths have been shown to be attracted to and preferentially oviposit on the bracts of sunflower heads. Extracts were made by dipping heads into hexane, and analysis by GC/MS indicated that the main components were terpenoids and long chain leaf waxes. Fractionation of extracts by silica gel flash column chromatography resulted in non-polar fractions containing terpenes and polar fractions containing terpenols. Gas chromatography coupled to electroantennogram detection (GC-EAD) showed that female banded sunflower moth antennae responded to several terpenoids. GC-EAD results and identification of active compounds are reported.

MOLECULAR ANALYSIS OF APHID-INDUCED RESPONSES IN BARLEY

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We are investigating plant defence mechanisms against piercing and sucking insects, using the bird cherry-oat aphid (*Rhopalosiphum padi*) and barley (*Hordeum vulgare*). The primary host for this aphid during winter is bird cherry while cereals and wild grasses serve as secondary hosts during summer. Barley lines are available that differ in their resistance to *R. padi*. Previous studies have shown that pathogenesis-related proteins are induced upon aphid infestation in barley and that this induction is stronger in resistant than in susceptible barley lines [1]. In addition, there are reports that secondary metabolites (among them gramine in barley) are induced in cereals upon aphid feeding. These proteins and metabolites are presumed to act in defence, but their precise effect on aphids has yet to be shown.
To obtain a broader picture of the reactions that are induced in barley upon aphid attack we have constructed subtracted cDNA libraries from infestated and non-infestated barley plants of the susceptible variety Lina. Subtraction was performed using subtractive hybridization PCR. Subtraction was performed in both directions in order to be able to isolate up- as well as down-regulated cDNA clones. Here we report our results from screening randomly picked clones from both subtracted cDNA libraries for their expression pattern upon aphid attack.

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PERFORMANCE DIFFERENCES IN *OXYOPS VITIOSA*; FED CHEMOTYPES OF *MELALEUCA QUINQUENERVIA*

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An environmental weed, *Melaleuca quinquenervia* constitutes one of the biggest threats to the biodiversity of the south Florida everglade ecosystem. This species is a member of the Myrtaceae family and originates from Australia. Members of the family including the two largest genera *Melaleuca* and *Eucalyptus*, constitute important sources of volatile essential oils of considerable medicinal value. Terpenoid variants or chemotypes occur within the *M. quinquenervia* species that have distinct terpenoid profiles. For example, the essential oils caryophyllene and nerolidol dominate chemotype I and α-pinene, 1,8-cineole and viridiflorol dominate chemotype II. These same terpenoids also function as important repellent and toxic constituents that protect plants from non-adapted generalist herbivores. However, the genus-level specialist weevil, *Oxyops vitiosa*, has been imported from Australia for biological control of *M. quinquenervia*. The adults more readily accept the leaves of the chemotype dominated by nerolidol compared with those dominated by viridiflorol. Larval survival, growth, development and adult fecundity are also greater when fed the nerolidol chemotype. Distinct biotypes of weevils may exist that are each compatible with a particular chemotype. These results suggest that additional collections of weevils from Australia may uncover new biotypes with greater tolerance of the viridiflorol chemotype which would compliment the control exerted by our single biotype.

RESPONSE OF MALE MELOLONTHA COCKCHAFERS TOWARDS DAMAGE-INDUCED PLANT VOLATILES: COMPARISON OF TIME-DEPENDENT BOUQUETS AND ROLE OF INDIVIDUAL COMPOUNDS

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Melolontha cockchafer males are known to be attracted by damage-induced green leaf volatiles (GLV) from leaves of host and even non-host plants. GLV lead males to sites where females are feeding [1,2]. The sex pheromones 1,4-benzoquinone in the forest cockchafer (*Melolontha hippocastani* Fabr.) and toluquinone in the European cockchafer (*M. melolontha* L.) synergistically enhance this response [3,4]. Since the scent of freshly damaged leaf material should most reliably indicate sites with currently feeding females, we investigated the kinetics of volatile emission in damaged host leaves and the response of male cockchafers towards time dependent bouquets asking the following questions:
(a) Do host plant leaves emit different volatile bouquets depending on the time lag since damage has been inflicted? Six-carbon aldehydes were most abundant in the bouquet of freshly damaged leaves (sampling 10 min after damage), whereas (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate predominated in bouquets after 1.5 h [5].
(b) Do *Melolontha* males differentiate between fresh and old damage bouquets? Male *M. hippocastani* were equally attracted towards volatiles from freshly damaged leaves and from leaves with old damage. Significantly more males were attracted towards a synthetic GLV mixture mimicking old leaf damage compared to a mixture mimicking fresh leaf damage [5].
(c) Which individual compounds are responsible for the attractiveness of damaged leaves? Green leaf alcohols but not the respective aldehydes and acetates were attractive. (*Z*)-3-hexen-1-ol was the only attractive compound for *M. hippocastani* males [5], whereas 1-hexanol, (*E*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol attracted *M. melolontha* males [2]. Hence, the hydroxyl group of the tested compounds was essential for attractiveness towards *Melolontha* males. Moreover, *M. hippocastani* males differentiated between leaf alcohols regarding presence, position, and/or configuration of a double bond. However, all tested GLV including aldehydes and acetates elicited antennal responses in electrophysiological experiments [1,2,5].

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DIFFERENTIAL RESPONSE OF POLLEN BEETLE *MELIGETHES AENEUS* (♀) TO DIFFERENT VARIETIES OF OILSEED RAPE.

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Olfactometer and field experiments have shown that different varieties and cultivars of rape show different attractiveness to pollen beetles *Meligethes aeneus* during different stages of flowering. When the plants are in the green bud stage insects are more attracted to turnip rape *Brassica rapa* than oilseed rape *Brassica napus* cv. Canyon and cv. Starlight. When the plants are in full flower this difference in attractiveness is considerably diminished.
Although the Starlight cultivar, which is low in certain glucosinolates, is always the least attractive, the level of glucosinolates does not seem to be the only reason for the observed difference in attractiveness.
Air entrainment of volatiles emitted from green buds and fully flowering racemes show a marked difference in chemical profiles with the turnip rape emitting volatile compounds from the buds, which are found only in the flowers of the two oilseed rape varieties.

OILSEED RAPE VOLATILES: RELEASE CARACTERISTICS AND BEHAVIOURAL RESPONSES IN ICHNEUMONID PARASITOIDS OF POLLEN BEETLES

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The pollen beetle, *Meligethes aeneus* (Coleoptera: Nitidulidae) is a serious pest on oilseed rape crops (Brassicacae) in northern Europe. Three of the most important larval parasitoids of pollen beetles are ichneumonids. Up to 50% parazitation of pollen beetle larvae by these ichneumonid parasitoids have been observed [1]. Female parasitoids search for larvae in the buds and open flowers.
This project investigates the role of plant volatiles in mediating tritrophic interactions in oilseed rape fields between the plant (*Brassica napus*), the herbivore and the parasitoids. We present results from experiments performed in southern Sweden during the years 2001-2002. These consist of behavioural studies, volatile collections and electrophysiological experiments. We have studied the female parasitoid behaviour to odours from infested and uninfested plants and to odours of plants in different growth stages. Olfactometer experiments show that the females are more attracted to oilseed rape in the bud stage compared to plants in the flowering stage. We have done volatile collections from infested and uninfested oilseed rape plants and from plants in different growth stages. We have found volatiles that are characteristic for infested plants and also volatiles that are typical for different growth stages of the plants. Electrophysiological experiments show that these parasitoids are able to detect several of these characteristic volatiles.

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ROLE OF SEMIOCHEMICALS IN CROP COLONISATION BY THE DIAMONDBACK MOTH PLUTELLA XYLOSTELLA

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Host plant selection by phytophagous insects can be seen as a continuum between two extremes: host plant recognition, when insects „choose" their host only after contact and host plant finding, when insects „choose" their host at a distance based on visual/chemical cues. Over the years, research has mainly focused on host-plant recognition whereas host-plant finding has received much less attention. A recent paper by Finch & Collier (2000) [1], has attempted to raise a general theory of host-plant selection by insects based on „appropriate/inappropriate landings" by pest insects of cruciferous plants. Doubt was expressed whether host plant volatile chemicals are truly attractants and play a major role in the host-selection process. We believe that semiochemicals may have a more important role than being simple arrestants [2] [3] and we are investigating this by a semi-field approach.
The cruciferous specialist, *Plutella xylostella* (diamondback moth), has been chosen as a model insect and Chinese cabbage and lettuce as host-plant and non host-plant respectively. These two plants emit distinctive different chemical cues but present similar visual cues under red-lights (similar architecture and „colour"). A Y-tube olfactometer was used to verify the attractiveness of cabbage odour and its preference over that oflettuce odour. „Field-simulator" experiments were then designed to simulate a situation of crop colonisation by a naïve female insect pest. The simulated crop consists of a square of 36 plants in pots (9 cm diameter) placed 10 cm apart in a Perspex tray which is covered with soil so that only the plants are apparent. Different monocrop and mixed-crop situations have been simulated and insects were released 70 cm away from the first row of plants. Preliminary results show that a lettuce monocrop does not trigger *P. xylostella* oriented flight and landing. However, when cabbage is present in the crop (3 rows of cabbage + 3 rows of lettuce) oriented flight and landing on plants are induced. However, there are more landings on cabbage only when they are placed in the front rows. This system now allows us to map the spatial patterns of alighting of the moth in different arrangements of host and non-host plants.

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EFFECT OF PLANT VOLATILES ON CODLING MOTH OVIPOSITION BEHAVIOUR

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The codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is an important pest in apple-growing areas all over the world. It is predominantly found on apple, but populations also occur on pear and walnut. The females oviposit on or close to the fruit, and the larvae feed on the fruit, causing great damage for the fruit growers. The moth has been controlled with insecticides, but it is necessary to find other, more environmentally safe control methods. During many years the sex pheromone system has been thoroughly investigated. Synthetic sex pheromones are now utilized in the control method called mating disruption. However, this method only affects males. It would be more efficient if also females were affected. Therefore, focus is now turned to the female behaviour.
It is important for the codling moth females to find suitable larval hosts and oviposition sites. Plant odours are used in the host-finding process. It is well known that the oviposition behaviour is stimulated by odours from apples. However, we have investigated whether odours from the other host species also stimulate oviposition. We also studied if the larval history affected the oviposition behaviour response to the plant odours.

OVIPOSITION STIMULANT FOR A MAGNOLIACEAE-FEEDING SWALLOWTAIL BUTTERFLY, *GRAPHIUM DOSON*: D-PINITOL FROM ITS MAJOR HOST PLANT, *MICHELIA COMPRESSA*.

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A number of oviposition stimulants have been reported for some papilionid butterflies. However, no publication has dealt with oviposition stimulants for Magnoliaceae-feeding swallowtail butterflies. *Graphium doson*, distributed in tropical to temperate Asian regions, utilizes *Michelia compressa* (Magnoliaceae) as a primary host plant in Japan. We attempted to identify oviposition stimulant(s) for the butterfly present in this plant. A MeOH extract (MC-1) from young leaves of *M. compressa* ,which showed potent stimulatory activity, was separated by solvent partition into three fractions: $CHCl_3$(MC-2), *i*-BuOH(MC-3) and water(MC-4). Strong activity was evoked by MC-3 and MC-4. Bioasay-guided further fractionation of MC-4 led to the isolation of one oviposition stumulant. The compound was identified as D-(+)-Pinitol from its NMR spectra and optical rotation. D-(+)-Pinitol showed moderate oviposition-stimulatory activity in itself. This compound was found to occur also in another host plant, *Magnolia grandiflora*.

CHEMICAL AND ECOLOGICAL RELATIONSHIP BETWEEN *PHYLA NODIFLORA* AND THE PHAON CRESCENT BUTTERFLY

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We are investigating the chemical ecology of the role of the larval host plant, *Phyla* (= *Lippia*) *nodiflora* for *Phyciodes phaon* (Edwards), a small nymphalid butterfly. The host plant and butterfly have a wide distribution across the southern United States.. The butterfly appears to be monophagous, laying its eggs in clusters on the lower surface of host leaves, and larvae complete their development on the plant. We have reared the butterfly to the adult stage on an artificial diet that contains no host plant material, but the adults do not lay eggs, although the ovaries develop and eggs in the ovaries have a normal appearance. Addition of 10% by weight of ground, freeze dried host leaves results in egg laying and hatching of larvae. We are currently fractionating the extracts of the host plant to determine the component (or components) needed to enable the adults to lay eggs.

HOST-PLANT USE AND HOST-RECOGNITION IN *HELLEBORUS*-FEEDING SAWFLIES.

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Sawflies in the tribe Phymatocerini (Hymenoptera: Tenthredinidae), which are known to be chemically defended against various predatory groups, are specialised on toxic plants in the orders Liliales and Ranunculales. Two species of *Monophadnus*, *M. monticola* and *M. latus*, have host plants in the genus *Helleborus* (Ranunculaceae). They have different primary hosts in the field, *H. viridis* and *H. foetidus* respectively. Laboratory host range testing with these two species has shown them both to be specific on genus level. Within the genus *Helleborus,* they accepted all species tested for oviposition and feeding, but preference and performance was better on the field host. We are carrying out a bioguided fractionation of *Helleborus* leaf extracts to isolate those components of the host plants' chemistry that are phagostimulatory to the larvae. Different secondary metabolites could be involved in this process since different fractions of the two *Helleborus* were preferred: the butanolic fraction of *H. foetidus* and the hexanic fraction of *H. viridis*. Preliminary analyses show that the butanolic fraction of *H. foetidus* is rich in simple glycosylated phenolics and gamma-lactones.
We hope that through this process we will be able to identify the particular chemicals that are involved in intra- and interspecific host recognition.

CHEMOSENSORY TUNING TO A HOST RECOGNITION CUE IN THE FACULTATIVE SPECIALIST LARVAE OF THE MOTH *MANDUCA SEXTA*

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Larvae of *Manduca sexta* are facultative specialists on plants in the family Solanaceae. Solanaceous reared larvae develop a strong preference for their host; otherwise, they remain polyphagous. The host-specific recognition cue for *Manduca* larvae is the steroidal glycoside, Indioside D, so far found only in the Solanaceae. Two pairs of taste receptors, the lateral and medial sensilla styloconica, are both necessary and sufficient for the feeding preferences of host-restricted larvae. In a previous study of solanaceous reared larvae, we found that the phasic portions (first 200 msec) of the responses of the lateral sensilla styloconica are tuned to Indioside D. Chemosensory tuning in *Manduca* can be defined as a decline in the receptor's sensitivity to nonspecific plant compounds, while maintaining a high sensitivity to Indioside D. The present study extends these findings to include the tonic portion of the response of the lateral sensilla and both phasic and tonic responses of the medial sensilla styloconica.
We conducted electrophysiological tip recordings from the lateral and medial sensilla of larvae reared on solanaceous foliage, or on wheat germ artificial diet. For each animal, 30 second long recordings of the responses to natural concentrations of Indioside D, tomatine, glucose and KCl, were compared. The lateral and medial sensilla styloconica of solanaceous reared larvae were tuned to Indioside D in the phasic and tonic portions of the responses, while non-solanaceous reared larvae were not. Feeding on solanaceous foliage therefore appears to result in a modification of the physiological responses of both the lateral and medial sensilla styloconica to cause them to be tuned to the host-recognition cue Indioside D. We propose that this tuning is the basis for the host-restricted larvae's strong behavioral preference for solanaceous foliage.

FEEDING STIMULANTS FOR COLORADO POTATO BEETLES IN SOLANACEOUS PLANTS

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We compared six species of Solanaceous plants for the presence of feeding stimulants for Colorado potato beetles. Earlier studies in our lab indicated the presence of feeding stimulants for Colorado potato beetles in the aq. extract of the foliage of potatoes (*Solanum tuberosum*, variety Allegeny). We were interested in knowing if the stimulants in different hosts are the same in all species. So we prepared aq. extracts of all the test plants and fractionated them as previously performed for potato foliage. We compared activities of extracts and fractions using choice test bioassays. Our results indicated that all aq. extracts (except for pepper and petunia extracts) were active, but activity differed slightly. Further fractionation of these extracts showed activity in different fractions for most different species. Pepper aq. extract was not active, but further fractionation of this extract gave some active fractions. Thus our results indicate the feeding stimulants for Colarado potato beetle are not identical in different Solanaceous species. Also some plants may also contain feeding deterrents, so that activity varies depending on the balance of stimulants and deterrents.

SYNERGISTIC EFFECT OF PHAGOSTIMULANTS IN THE MALE NUPTIAL SECRETION OF THE GERMAN COCKROACH, *BLATTELLA GERMANICA*

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In sequential courtship behavior of the German cockroach, *Blattella germanica* (L.), a male secretes a nuptial gift from the abdominal tergal glands. The glandular secretion functions as a courtship pheromone, which strongly stimulate the female to feed on the fluid, consequently arresting her into an appropriate position for the male to make a genital connection. Active components in the secretion were examined by a feeding bioassay using polyethylene glycol (PEG) as a medium, in which the test samples were mixed. Methanolic extracts of the male 8th tergal glands (TG-8) strongly induced the feeding response in the virgin females. The phagostimulants consisted predominantly of sugars and polar lipids. The sugar components were identified as a mixture of maltooligosaccharides and oligoglucosyl trehaloses [1, 2]. The activity of the sugars was strongly synergized by a series of phospholipid components, characterized here as phosphatidylcholine (PC) and phosphatidylethanolamine (PE). In addition, cholesterol in the secretion also synergized the activity of the sugars. Further survey revealed some synergistic effect between the sugars and an amphoteric fraction, from which a series of amino acids was identified as an additional factor contributing to the phagostimulant activity. These results indicate that the nuptial feeding behavior of the female cockroach is elicited by a complex synergistic effect among oligosaccharides, phospholipids, cholesterol and amino acids in the male tergal secretion. It appears that the male exploits the female's appetite for these gustatory cues in their precopulatory sequence, instead of utilizing specific pheromone compounds.

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FLIGHT REPONSES OF SOME PYRALID MOTHS TO ODOURS OF DIFFERENT STORED PRODUCTS

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Mass trapping with sex pheromone-baited traps has previously been tested for control of several species of moths that infest stored food products. The limitation of this method is that males only are attracted to the pheromone-baited traps. A few males escaping the trapping might suffice to fertilise most females in the population. As a result, efficient trapping of males may still not cause any significant reduction in larval numbers and damage. A complement to the pheromone-based mass trapping would be to use other substances, responsible for odour-mediated oviposition, to monitor and control females of a pest population. In the present study, we tested the attractive properties of different kinds of stored products for females of three pyralid moth species, *Ephestia cautella*, *E. kuehniella* and *Plodia interpunctella* in a flight tunnel. Mating status of the females had an drastic impact on female behaviour. Only mated females flew upwind, attracted by odours of a wheat-based laboratory breeding diet. Based on this observation only mated females were tested in the subsequent experiments. In the comparative study, females of both *E. cautella* and *P. interpunctella* where attracted to a variety of stored products, whereas none of the presented diet types attracted *E. kuehniella* females. Two types of chocolate sweets were the most attractive, inducing source contact in 40% of *E. cautella* and 60% of *P.interpunctella*. The results indicate a potential for utilization of food volatiles in pest control targetting females. Isolation and identification of volatiles emanating from the mashed chocolate sweets are now in progress.

IDENTIFICATION OF NON-HOST PLANT VOLATILES REPELLENT TO POLLEN BEETLES.

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A "push-pull" strategy for control of oilseed rape (OSR) pests is being developed at IACR-Rothamsted. This relies on the use of insect and plant-derived semiochemicals to manipulate pests and natural enemies. Pests are aggregated on highly attractive trap crops (pull), while simultaneously, a reduction in crop colonisation is achieved using repellent or anti-feedant stimuli (push). Biological control agents are also manipulated to achieve pest control within the trap crop areas.

An important aspect of pest manipulation requires the identification of non-host plant cues utilised by the pest insects. This work aims to identify non-host plant cues that act as repellents to the pollen beetle, *Meligethes aeneus* (Fab.), and to investigate the behaviour of these insects in order to establish the most effective use of repellent stimuli to disrupt their colonisation of oilseed rape crops.

Laboratory bioassays were used to test the effects of several essential oils from non-host plants in order to select the most effective repellent for *M. aeneus*. The most effective, especially at low concentrations, was lavender essential oil. Female *M. aeneus* were tested for their antennal responses to lavender oil using coupled gas chromatography-electroantennography (GC-EAG). The chemicals eliciting EAG responses were identified using coupled gas chromatography-mass spectrometry (GC-MS) followed by peak enhancement on GC using authentic samples. Identified chemicals were each tested using 4-way olfactometers for their effects on the behaviour of *M. aeneus*. Several of these showed significant repellent effects. The effectiveness of repellent plant-derived stimuli at the field scale will be investigated in future work.

SEARCHING FOR PINE WEEVIL ANTIFEEDANTS, STRATEGY AND SYNTHESIS.

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The pine weevil, *Hylobius abietis*, feeds on newly planted pine and spruce saplings, causing death of up to 80 % of the plants. Today the pyrethroid, Permethrin is used for protection of the plant but this insecticide is environmentally toxic and alternatives have to be found. We are searching for antifeedants, substances that either through taste or smell or both, when applied on the plants deter the pine weevil from feeding.
Our strategy has been to screen a library of substances for antifeedant effect. On the basis of the test results some of the substances with highest antifeedant activity becomes lead compounds. The structures of these compounds are chemically modified and a structure-activity study is performed. Based on the results from this study the optimal antifeedant can be chosen.

BEHAVIOURAL RESPONSES BY THE PINE WEEVIL *HYLOBIOUS ABIETIS* TO ODORANTS PRODUCED BY HOST AND NON-HOST PLANTS.

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The pine weevil, *Hylobius abietis*, causes great damage on seedlings of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) in reforestation areas in Northern and Middle Europe. The damage is caused by adult weevils feeding on the soft bark of the young plants. The influence of plant volatiles on the attraction to the plants has been demonstrated in laboratory and field tests, showing that the weevils are attracted to α-pinene [1] and to the mixture of α-pinene and ethanol [2]. By the use of gas chromatography linked to electrophysiological recordings from single receptor neurones (GC-SCR) and linked to mass spectrometry (GC-MS), it has been shown that the olfactory receptor neurones (ORNs) of the pine weevil are able to detect a large number of volatiles released by the conifer hosts as well as by non-host plants [3]. Out of 80 recorded ORNs, 30 functional types have been classified, characterized by narrow response spectra (molecular receptive range). One compound seemed to have a marked best effect and a few structurally similar compounds might activate each ORN at higher concentrations. In these studies enantiomers were not tested separately. The present study was carried out in order to test and compare the attractive effect of enantiomers of selected compounds among those having the strongest effect on the ORN types.

The weevils were tested in a round cage, the floor of which had 6 outlets, each ending in a glass tube [1]. Two were test tubes in which each had a glass capillary containing one test odorant, i.e. two odorants were tested against each other in all experiments. The other four tubes were controls, containing empty glass tubes. The weevils were placed in the center of the floor and left for at least 8 hours during the day or the night. Females and males were kept and tested separately.

The results showed that many of the selected compounds had an attractive effect. However, (-)- as well as (+)-α-pinene were stronger attractants than the conifer compounds (-)-β-pinene, (+)-3-carene, camphene and camphor. The two α-pinene enantiomers were also stronger attractants that the enantiomers of trans-verbenol, an aggregation pheromone of some bark beetles. By comparing the effect of (-)- and (+)-α-pinene tested in the same experiments, it was found a slight difference which might depend on the species of the food materials provided before the experiments. Other compounds tested against (+)-α-pinene were p-methylanisole, (+)-sabinene, bornyl acetate, (+)-limonene, (-)-limonene and verbenone. Interestingly the compound, p-methylanisole, had a stronger attractive effect than (+)-α-pinene. The three next showed a certain attraction, whereas the glasses with (-)-limonene and verbenone attracted only few beetles similar to the controls. These two latter compounds are shown to act as inhibitors in field experiments [4] and [5].

In future experiments it will be interesting to test the effect of mixtures containing the most attractive compounds tested in these experiments. The aim is to find the most and the least attractive blends of plant odorants of the pine weevil.

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ISOLATION OF VOLATILES AND OTHER PLANT COMPONENTS AFFECTING FEEDING OF MALADERA MATRIDA BEETLES.

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The scarab beetle *Maladera matrida* Argaman (Coleoptera, Scarabaeidae) was first detected in Israel as recently as 1983 and classified as a new species. Adult population and larvae of *Maladera matrida* Argaman are a serious polyphagous pest of various crops and ornamentals in Israel [1]. However, they avoid *Mangifera indica* leaves. In laboratory trial all beetles died of starvation but refused to eat *Mangifera indica* leaves.
Leaf extraction of *M. indica* leaves with chloroform was deterrent to feeding by the *M. matrida* beetle when applied to *Rosa* hyb. petals, the preferred food. A methanol leaf extraction was not deterrent to feeding, while the petroleum ether leaf extraction slightly reduced consumption when applied to petals. Chromatography on silica gel column with increasing polarity of solvents of a chloroform leaf extraction yielded 2 relatively non-polar fractions exhibiting higher specific activity among the 16 assayed.
Previously laboratory olfactometer bioassays [2] showed that peanut leaves (food) attracted *M. matrida* beetles. Our olfactometer bioassays and laboratory trials, similar to [3], were done in order to find deterrent components of *M. indica* leaves.
In this poster we will present the work that was done using NMR-spectroscopy, GC-MS in attempt to elucidate the structure of isolated components.

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LINKING METABOLIC PROFILES WITH BIOASSAYS TO IDENTIFY MAPLE (*ACER*) PHYTOCHEMICALS THAT INFLUENCE FOREST TENT CATERPILLAR BEHAVIOR.

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Maples (*Acer* spp., family *Aceraceae*) are among the most important hardwood species in North America. There are approximately 160 known maple species worldwide, and several are native to eastern Canada. The forest tent caterpillar (FTC) (*Malacosoma disstria* Hubner), a major pest of Canadian deciduous forests feeding mainly on poplar (*Populus* spp.), may also severely damage sugar maple *(Acer saccharum* Marsh). By contrast, red maple (*A. rubrum* L.) occupying a similar range is seldom attacked by FTC. We have analysed the crude alcoholic extracts of six species of maple by HPLC and conducted no-choice bioassys of leaf disks of these six species with FTC in order to examine the phytochemical basis that influences the insect's feeding behavior. HPLC chromatograms of red maple and silver maple (*A. saccharinum* L.), section Rubra Pax, are very similar and dominated by gallates, especially ethyl *m*-digallate. HPLC chromatograms of sugar maple, section Saccharina Pax, and Manitoba maple (*A. negundo* L.), section Negundo K. Koch, are similar in that they are dominated by proanthocyanidins, chlorogenic acid, ellagic acid and quercetin 3-*O*-rhamnoside. Mountain maple (*A. spicatum* Lamb.), section Spicata Pax, HPLC chromatograms differ from those of Manitoba maple mainly in having a lower amount of ellagic acid. The HPLC chromatogram of striped maple (*A. pensylvanicum* L.), section Macrantha Pax, is quite distinct from all the others. Thus both quantitative and qualitative differences in the HPLC chromatograms serve to identify each species. FTC fed readily on leaf disks of sugar and Manitoba maples while feeding was inhibited by red and silver maples. Ethanolic extracts of red maple inhibited feeding of FTC but those of sugar maple did not. These results suggest that the presence of several gallate compounds, acting together, may protect red maple and silver maple leaves from herbivores such as the FTC.

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CHEMICAL ELUCIDATION OF TRICHOME-BASED RESISTANCE OF *MEDICAGO SATIVA* L. TO A HEMIPTERAN PEST

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The potato leafhopper, *Empoasca fabae* (Harris), is one of the most important pests of alfalfa, *Medicago sativa* L., in the midwestern and eastern United States. Four years ago, leafhopper-resistant varieties of glandular-haired alfalfa were made available commercially. Little is known of the mechanism of resistance. Previous studies support that the leafhopper's biology [1] and behavior [2, 3] is affected by the glandular trichomes. Also, the trichomes secrete a viscous exudate [4]. We hypothesize that resistance is chemically-based and that biologically-active compounds are secreted by the glandular trichomes. Thus, our objectives were to assess alfalfa glandular trichome chemistry and determine how it affects the leafhopper‘s host-selection behavior. We used the highly resistant glandular genotype from Cal/West Seed Co., 'G98A', and the susceptible nonglandular clone 'Ranger'.

To identify major components, we isolated trichomes from stem sections of G98A and Ranger using liquid nitrogen, dry ice, and a vortex mixer. Isolated trichomes were then extracted with chloroform and analyzed using GC-MS. Several major peaks were unique to the trichomes of G98A, and some peaks were also present in higher concentrations. The exudate was comprised mainly of large, nonvolatile compounds, such as fatty acids. Histochemical analysis was also conducted using G98A glandular trichomes, with the alkaloid stains showing a strong positive reaction for the gland heads. Behavioral bioassays of adult nonpreference were conducted with the G98A and Ranger nonvolatile extracts. Trichomes were extracted with chloroform, acetone, and ethanol, and applied to the surface of a Parafilm-covered artificial diet sachet. Leafhoppers were then offered a two-way choice between Ranger vs. G98A, G98A vs. acetone control, Ranger vs. acetone control, and control vs. control. Time-course analysis showed that all three G98A solvent extracts deterred leafhopper settling, with increased activity resulting from increased solvent polarity. Thus, ethanol will be used in upcoming bioassay-guided fractionations. In contrast, volatile compounds were not detected in the gland exudate. However, volatiles were collected from the stem surface of G98A and Ranger, using vacuum-distillation. Results from GC-MS analysis indicated quantitative differences in volatile organic compounds among the resistant and susceptible clones. Behavioral bioassays revealed that these volatile compounds repel potato leafhoppers. Thus, the resistance mechanism may involve both olfaction of stem surface volatile and direct contact with the trichome exudate. In the exudate, the fatty acids may be a sticky, glue-like carrier for toxic alkaloids, or the fatty acids themselves may be responsible for resistance.

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PLANT WOUNDING AFFECTS OVIPOSITION OF THE CRUCIFER PEST *HELLULA UNDALIS* (F.) - THE IMPACT OF SECONDARY COMPOUNDS.

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Induced direct plant defence has been recorded for more than 100 plants in 34 families [4]. Crucifer plants damaged by insects, pathogens, or mechanical wounding haves been shown to modify not only the total glucosinolate (GS) content in various plant tissues but also the relative proportions of individual GS [2, 3]. We studied the changes in GS and their hydrolysis products after wounding in *Brassica campestris* L. ssp. *chinensis* (Black Behi) and their influence on the oviposition of *Hellula undalis* (F.) (Lepidoptera: Pyralidae).
H. undalis is considered a pest species on cruciferous crops in the tropics and subtropics. This species has recently become the most serious pest along with *Plutella xylostella* (L.) and *Spodoptera litura* (F.) in the Philippines [5]. Bioassays in the Philippines showed that mechanical wounding affects the oviposition of *H. undalis* females. Significantly fewer eggs were laid on previously wounded leaves. Chemical analyses of *B. chinensis* plants grown in fine mesh chambers in a green house showed significant changes in the GS content after wounding the plants. Three days after wounding the total GS content in plants was twice as high as in the controls. Furthermore, the proportion of indolyl GS such as glucobrassicin and neoglucobrassicin in tissues increased, whereas the aliphatic fraction decreased. An increase of indolyl GS after mechanical wounding or insect feeding is also reported for other *Brassica* species [1]. We detected as the main corresponding hydrolysis product of indolyl GS the slightly volatile cyanides. Our results suggest that hydrolysis products of GS may influence oviposition of *H. undalis*. In choice tests *H. undalis* females laid significantly fewer eggs on *B. chinensis* plants treated with 0.001 % 3-indolyl-methyl-cyanide than on control plants. Furthermore, the corresponding hydrolysis product of progoitrin (5-vinyl-oxazolidine-2-thione) at the same concentration has been proven to affect oviposition of this species. The cost and benefits of induced plant responses in the context of co-evolution with herbivores will be discussed.

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DIRECT AND INDIRECT EFFECTS OF *CIS*-JASMONE ON THE GRAIN APHID, *SITOBION AVENAE.*

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Plants infested with herbivorous insects, and some uninfested plants, produce stress-related semiochemicals that deter herbivores whilst attracting insects antagonistic to the herbivores. However, there is also increasing evidence for the existence of volatile signals that mediate interactions between plants, such that plants damaged by herbivores or pathogens induce defence chemistry in undamaged plants [1]. *Cis*-jasmone is potentially one such signal [2]. Although a well known component of flower volatiles it is also released when plants defences are induced during insect herbivory [3,4].
Laboratory and field simulation assays and field trials were used to investigate direct and indirect effects of *cis*-jasmone on the grain aphid, *Sitobion avenae,* a pest of cereal crops. Direct repellency was confirmed using an olfactometer, while indirect effects on colonisation and population development, resulting from changes in plant secondary metabolism of wheat exposed to *cis*-jasmone, were demonstrated in simulator and growth rate studies. Numbers of cereal aphids, predominantly *S. avenae*, were significantly reduced on plots of wheat treated with *cis*-jasmone compared to untreated plots.

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INTERACTIONS BETWEEN ROOT AND SHOOT INDUCTION OF GLUCOSINOLATES IN THREE SPECIES OF BRASSICACEAE

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So far, studies of induced defences have focussed almost exclusively on aboveground interactions. In addition to aboveground herbivores and pathogens, plants also have to deal with a wide variety of belowground phytophages. Because many induction signals are transported throughout the plant and elicit induced responses in undamaged organs, local induction often results in systemic induced responses. Root-feeding phytophages thus may affect the performance of shoot phytophages -and their natural enemies-, and *vice versa*.. Additionally, when a plant is attacked simultaneously by root and shoot phytophages, above and belowground induction processes may interact. Because such interactions may alter the net costs and benefits of induced responses, they may be of seminal importance for understanding the evolution of induced responses in plants.
For our experiments, we used three wild species of Brassicaceae, *Brassica nigra, B. oleracea* and *Barbarea vulgaris*, which all produce different types of glucosinolates. We induced either roots or shoots with jasmonic acid (JA) or salicylic acid (SA) solutions in water, as well as induced roots and shoots simultaneously with all possible combinations of JA and SA root-shoot treatments. Controls were treated with water that had a pH-value similar to that of the hormone solutions (pH ~ 3). After 7 days, roots and shoots were harvested, freeze dried and analysed for their glucosinolate content. Based on the results of our experiments we will address the following questions for all three species: 1) does JA or SA application induce glucosinolates both locally and systemically when applied to roots or shoots?; 2) Does simultaneous induction of roots and shoots result in -positive or negative- interactions between aboveground and belowground glucosinolate responses?; 3) does simultaneous induction with SA suppress the JA-induced response in the other organ?

COMPARISON OF THE GC-PROFILES OF THE ODOUR OF SIX PERSONS WITH DIFFERENTIAL ATTRACTIVENESS FOR MALARIA MOSQUITOES.

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Odours produced by human and animal hosts constitute the major cues used by mosquitoes during host seeking. Within the framework of malaria control, the present study is focussed on human odour. Six persons were selected for their differences in attractiveness to the malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) and their odours were investigated. The sampling was carried out by handling small glass beads. After the handling the beads were desorbed in a thermal desorption system (TDS) combined with a cryo-focussing system and the compounds were subsequently analysed by gas chromatography-mass spectrometry with an HP5-ms column. The quantities of each compound found in the odour of the six volunteers were compared. Several notable differences were observed between the human volunteers. Whether these chemical differences are linked to the differential attractiveness is a topic for further studies.

HOST-LOCATION KAIROMONE FROM *COCCUS HESPERIDUM* LINNAEUS FOR ENCYRTID PARASITOID *METAPHYCUS* NR. *FLAVUS*

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Parasitoids often use chemical stimuli, called infochemicals, as information from their environment when searching for their hosts. Accordingly, these infochemicals can be directly associated to their host. We used yucca leaves infested by soft brown scale (*Coccus hesperidum*); this scale emits attractants for the encyrtid parasitoid *Metaphycus* nr. *flavus*. Chemically mediated host location in *M. flavus* was investigated. Wasps were biossayed using a Y-olfactometer chamber. For each bioassay a single female was introduced into the Y-olfactometer and observed for five minutes with video tracking and motion analysis software (Xbug). We used yucca leaves infested by the *C. hesperidum* as a test. The scale age ranged from 26 to 30 days. On scale aged 26 to 29 days, the parasitoids spent significantly more time in the test arm than in the control arm; when the scale was 30 days old the wasps showed no significant preference for either arm. In addition, the parasitoids were tested with scale-infested leaves with all scales carefully removed (leaf unwashed) vs. scale-infested leaf, and scale-infested leaves with all scales removed (leaf washed) vs. a scale-infested leaf. In both tests, the wasps showed significant preference for the scale-infested yucca leaves with either *C. hesperidum* or their residue present.

PHORETIC-HOST SELECTION BY BROAD MITE, *POLYPHAGOTARSONEMUS LATUS* (ACARI: TARSONEMIDAE).

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Broad mite *Polyphagotarsonemus latus* (Acari), is a polyphagous pest in tropical and subtropical regions. Phoretic associations between broad mite and two genera of whiteflies (Insecta: Homoptera: Aleyroididae), namely *Bemisia* and *Trialeuroides*, have been reported from different parts of the world, raising the significance of phoresy in pest distribution.
We have studied the specificity of the association between the mite and its phoretic hosts and conducted preliminary experiments to investigate the nature of cues involved in the recognition of the phoretic host by the mite. Broad mite response was tested towards whiteflies of different genera and towards other winged insects that are common on the same host plants, namely thrips and aphids. *Bemisia tabaci* was used as a standard phoretic host in choice experiments. Insects frozen for 24 hours were used as potential phoretic hosts in all the experiments, while host acceptance was monitored by counting the number of mites attached to each insect. Preference tests were conducted between *Bemisia tabaci, Trialeuroides lauri, Dialeuroides citri and Aleyroides singularis* as well as between *Bemisia tabaci* and allate *Myzus persicae* and *Frankliniella occidentalis*. Our results show that broad mite readily attach to white flies from different genera, preferring some over the others. However, mites rarely attach to aphids and thrips, if at all. These results suggest that phoretic relationship between the broad mite and its insect hosts appears to be specific to whiteflies.
Attachment occurred equally well in dark and light conditions but, it was greatly reduced by washing the highly acceptable host *Bemisia tabaci* with various organic solvents. The results suggest the involvement of chemical cues in the recognition process of the phoretic host by the broad mite. The chemical nature of these cues will be discussed.

THE FATE OF BIRCH LEAF PHENOLICS IN DIGESTIVE TRACTS OF LARVAE OF THREE SAWFLY SPECIES.

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In higher plants the shikimate pathway produces various phenolic compounds, which are commonly regarded as important chemical defences of deciduous woody plants against insects. However, numerous studies correlating the foliar phenolic content with insect performance have brought about variable results. To clarify the situation, we investigated the fates of phenolic compounds of mountain birch (*Betula pubescens* ssp. *czerepanovii*) leaves in the digestive tracts of larvae of three sawfly species (*Amauronematus amplus*, *Pristiphora alpestris* and *Priophorus pallipes*) by comparing phenolics in leaves consumed and in faeces. Both leaf and faeces samples were extracted with 70% aqueous acetone, and analysed with HPLC-DAD and HPLC-ESI-MS as previously described [1], [2].

The phenolic composition of insect faeces was found to differ significantly, both in qualitative and quantitative terms, from that in the leaf material fed to larvae. Although the leaf diet contained more than 4.8 mg/g of galloylglucoses (GGs, subgroup of hydrolysable tannins), they were not found in the faeces. Instead, 2.5, 1.5 and 1.0 mg/g of gallic acid, the hydrolysis product of GGs, were detected in the faeces of *A. amplus*, *P. alpestris* and *P. pallipes*, respectively, reflecting extensive hydrolysis of GGs. Moreover, leaves contained only two caffeoylquinic acid isomers, but their number was increased to four, four and seven in the faeces of *A. amplus*, *P. alpestris* and *P. pallipes*, respectively. At the same time, the average excretion percents of ingested amounts of caffeoylquinic acids for the corresponding species were 68, 41 and 84%. The partial disappearance of *o*-diphenolic caffeoylquinic acids in digestive tracts of the first two species could be due to the phenolic catabolism or their oxidation to *o*-quinones through the action of polyphenol oxidases.

The presence of non-foliar, previously undetected phenolic products of larval metabolism were detected in the faeces of all species. On the basis of UV spectra, and mass spectral fragmentation, two of them were tentatively identified as galloylquinic acid and protocatechuic acid. The former was present in all faeces samples, but the latter only in faeces of *P. alpestris*.. Building blocks of galloylquinic acid could have derived from the hydrolysis of GGs (see above) together with the degradation of caffeoylquinic and/or *p*-coumaroylquinic acids, which all were present in the foliage. Protocatechuic acid in turn could originate e.g. from the dehydroxylation of gallic acid after the hydrolysis of GGs, or from the side chain reduction of caffeic acid after degradation of caffeoylquinic acids.

These preliminary results suggest that it is necessary to gain deeper understanding of the metabolic and/or catabolic processes occurring in digestive tracts of different insect species in order to clarify the role of phenolic compounds in plant-herbivore interactions.

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METABOLISM OF THYMOL AND ANETHOLE IN LARVAE OF *SPODOPTERA LITURA* AND *TRICHOPLUSIA NI* (LEPIDOPTERA: NOCTUIDAE).

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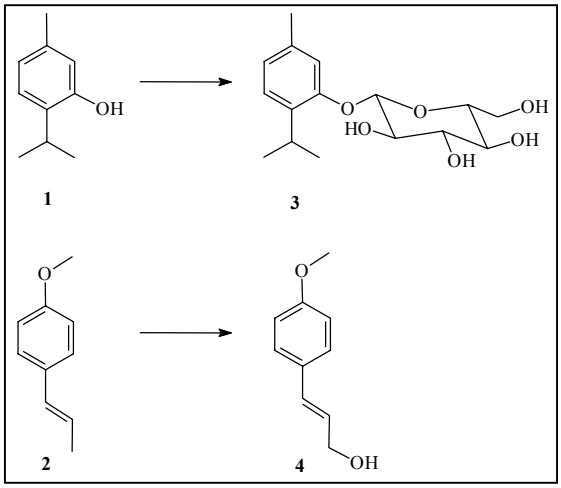

Plant essential oils are mainly mixtures of terpenes and phenylpropanoids. Many of them are acutely toxic to insects, and likely serve as defensive agents for the plants that produce them [1]. Metabolism of these substances in the insect can therefore act as system of detoxification, making it possible for the insect to tolerate these toxins in their diet.
The biotransformation of (+)- and (-)-limonene in *Spodoptera litura* was investigated previously by Miyazawa et al. [2]. Both monoterpenes were excreted after hydroxylation, which would render them more hydrophilic.
We have now investigated the metabolism of the phenolic monoterpene thymol (**1**) and the phenylpropane trans-anethole (**2**), the main constituents of the essential oils from thyme (*Thymus vulgaris*) and anise (*Pimpinella anisum*), respectively. After incorporation of the compounds into an artificial diet, metabolites of **1** and **2** were isolated from the frass of last instar larvae of *Trichoplusia ni*. The metabolite of **1** was additionally isolated from *Spodotera litura* after oral and *T. ni* after topical administration.
While the phenyl propane *trans*-anethole was hydroxylated at the propane side chain to give **4**, the carbon skeleton of the monoterpene thymol was left unchanged by both insect species. Both insects combined its phenolic hydroxy group with β-glucose and excreted it as thymol 3-β-O-glucoside (**3**). The isolated metabolites were identified on the basis of NMR and LC-MS analysis of the purified compounds.
Since **3** was also found in the frass of larvae topically treated with a 0.1 % solution of **1**, it is obvious that thymol penetrates the insect integument and is excreted by the Malpighian tubules into the hindgut. However the site of glucosylation remains unclear.

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NEW CHEMICAL METHODS FOR STUDYING POLYMERIC POLYPHENOLICS (TANNINS).

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Polymeric polyphenolics (tannins) continue to attract attention because of their potent activity as protein binding agents and as redox active compounds. Thus, they may play roles in plant defense; in nutrient cycling in leaf litter; and as biological antioxidants. Increased understanding of the complexity of polyphenolic chemistry has driven an interest in improving chemical methodology for these complex natural products.

Over the past few years, we have developed several new approaches to analysis of tannins. These new methods include improved functional group analyses; improved protein precipitation methods; methods for examining free radical chemistry; and methods for preparing radiolabeled polyphenolics. A brief overview of each new approach will be presented.

The potassium iodate method for determining gallotannins has been modified [1] so that it is conceptually analogous to the acid butanol method: In each analytical procedure, the formal monomer unit of the polymeric polyphenolic is released and reacted to form a chromophore. For the acid butanol method, the formal monomer is the anthocyanidin; for the potassium iodate method, the formal monomer is methyl gallate. This modified potassium iodate method is standarized with commericially available methyl gallate, and gives reliable results for a range of plant samples.

The radiochemical protein precipitation method for determining potential tannin bioactivity [2] has been modified to use tannin immobilized on paper disks. The plant extract is dispensed on filter paper disks. The disks are incubated with radiolabeled protein, washed, and directly counted. Because the precipitate is not isolated, the method can be performed even with minimal laboratory resources.

Electron spin resonance (ESR, EPR) methods can be used to characterize radicals. We have employed Zn^{+2} to stabilize polyphenolic radicals and are characterizing their reaction chemistry in the presence and absence of protein targets for redox reactions.

Radiolabeled polyphenolics have been prepared biosynthetically [3,4]but chemical synthesis is desirable for improved yield and specificity of labelling. We have devised a method for preparing uniformly labelled [^{14}C]pentagalloyl glucose in sufficient quantities for biochemical assays and feeding trials in small mammals [5].

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DOES THE INTRATHALLINE DISTRIBUTION OF PHENOLS IN LICHENS REFLECT OPTIMAL DEFENCE AGAINST HERBIVORES AND PATHOGENS ?

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Analysis of phenol content and phenol spectra in different tissue types of three foliose lichens showed that the concentration of phenolic substances is higher in reproductive (both sexual and asexual reproductive structures) than in somatic tissues.
The result is in accordance with optimal defence theory predicting higher allocation of phenols in structures that are most important for the fitness of an individual genet or ramet.

CHEMICAL COMPOSITION OF *MACARANGA TANARIUS* PLANT SURFACES

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Epicuticular wax crystals generally create slippery surfaces, thus representing physical barriers for ants and other insects. Nevertheless, diverse species of generalistic ants can adhere to the crystals on *Macaranga tanarius* surfaces. They climb up the stems, forage on the plants and protect them against herbivores. In order to fully understand this ant-plant-interaction, we investigated the chemical composition of the crystals and the resulting adhesion mechanism using GC-MS and SEM.
In the epicuticular wax layer on the leaf stalks and stems beta-Amyrin prevailed (60-70% and 70-80%), implying that this triterpenoid formed the thread-like crystals on these organs. Comparisons between various growth zones revealed that the crystal layer is torn into 50-60 µm long pieces in the course of surface expansion. The resulting crevaces are large enough to create footholds for ant claws.
In contrast, leaf waxes were dominated by aliphatic compounds. The epicuticular layer contained large amounts of primary alcohols (70%) that are likely to form the platelet-shaped crystals on this organ. Triterpenoids were restricted to inner parts of the leaf cuticle.

SEMIOCHEMICALS AT THE LEAF SURFACE: THE COMPOSITION OF CELERY (*APIUM GRAVEOLENS*) EPICUTICULAR WAXES

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At the very surface of plant leaves 'epicuticular waxes' form a thin film, necessarily acting as a substratum for herbivorous insects and pathogenic fungi when they first encounter the plant. During this critical stage in plant-insect or plant-pathogen interactions, host recognition is mediated by both semiochemicals and the surrounding species-dependent wax mixtures. Only recently, we were able to devise methods for selective probing and chemical analysis of epicuticular waxes.
These methods were now employed to quantify furanocoumarins and waxes at the surface of celery leaves. Furanocoumarins, induced after pathogen attack, have fungitoxic properties but also serve as oviposition stimulants for specialized insects (*Psila rosae*). We found that cuticles of uninduced celery leaves are devoid of furanocoumarins. After induction with the stress hormone jasmonic acid they appear in the epicuticular films of both adaxial and abaxial leaf surfaces. Subsequent evaporative loss of furanocoumarins from the epicuticular wax film can be monitored, hence enabling a complete assessment of their turnover kinetics. The constituents of the surrounding surface wax mixture are not changed by jasmonate induction or by evaporation. The epicuticular layer is extremely thin (app. 20 nm), differing significantly from the chemical composition of the underlying intracuticular waxes.
Based on this comprehensive description of the celery surface we can now interprete its ecological roles, the transient accumulation of furanocoumarins (spatially) allowing direct pathogen defense while (temporally) restricting host recognition by insects.

GLANDULAR TRICHOMES ON LOWER LEAF SURFACES OF THE ANT-PLANT *MACARANGA TANARIUS*

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Many trees of the palaeotropical ant-plant genus *Macaranga* form trichomes on their lower leaf surfaces. So far, nothing was known about the micromorphology, the chemical constituents and the ecological function of these structures. A method, originally developed for isolation of rose trichomes, was optimized for the separation of glandular and non-glandular trichomes of *Macaranga tanarius*. Microscopical analyses of the isolated glands showed that the glandular trichomes of *M. tanarius* have striking similarity to glands of the labiates *Mentha piperita* or *Ocimum basilicum*. UV-, IR-, mass- and NMR-spectroscopic methods were used to identify the glandular secretion as a non-volatile prenylflavonoid known as nymphaeol-C. In extraction experiments of lower leaf surfaces the amount of nymphaeol-C was strongly correlated with the trichome density, hence allowing to evaluate the amount of nymphaeol-C as ca. 0.03 µg per trichome. The ecological function of the glands and their constituent for the non-myrmekophytic *M. tanarius* will be discussed.

BIOSYNTHESIS OF GEOSMIN IN STREPTOMYCES SPP. AND THE LIVERWORT FOSSOMBRONIA PUSILLA

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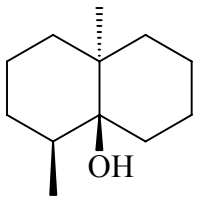
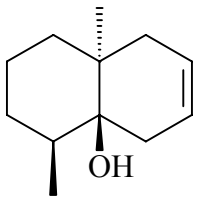
Geosmin(4,8a-dimethyl-octahydro-naphthalen-4a-ol) **1** [1] is a well known scent with a characteristic musty earthy odour. Geosmin is produced by Actinomycetes, Streptomyces, cyanobacteria, fungi and certain mosses. Due to its very low odour threshold (ca. 5-7 ng/l), geosmin **1** causes considerable problems as contaminant of drinking water.[2] On the other hand geosmin **1** is appreciated as aroma component in beetroot [3] and wine [4].



1 (-)-geosmin



2 (+)-dehydrogeosmin

Geosmin **1** is of terpenoid origin, but details of its biosynthesis are not known. We studied the mechanistic aspects of its biosynthesis by feeding labelled [5,5-$^2H_2$]-1-desoxy-D-xylulose, [4,4,6,6,6-$^2H_5$]-mevalolactone and [2,2-$^2H_2$]-mevalolactone to *Streptomyces* sp. JP95 and the liverwort *Fossombronia pusilla*. The microorganism produced geosmin via the methylerythritol-pathway (MEP-pathway), whereas the liverwort exclusively utilised mevalonate as the early precursor (MVA-pathway). Analysis of the labelling pattern in the resulting isotopomers of geosmin **1** by mass spectroscopy (EI/MS) revealed that geosmin **1** is synthesised in both organisms along the same sequence from Farnesyl diphosphate . Farnesyl diphosphate is first cyclised to a germacradiene-type intermediate. Further transformations *en route* involve an oxidative dealkylation of an *i*-propyl substituent, 1,2-reduction of a resulting conjugated diene, and bicyclisation of a germacatriene intermediate. The transformations largely resemble the biosynthesis of dehydrogeosmin **2** in cactus flowers[5] but differ with respect to the regioselectivity of the side chain dealkylation and 1,2-reduction.

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ISOPRENOID BIOSYNTHESIS IN MYCORRHIZAL MAIZE ROOTS: TRANSCRIPT AND PROTEIN ACCUMULATION OF 1-DEOXY-D-XYLULOSE 5-PHOSPHATE REDUCTOISOMERASE (DXR)

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Most terrestrial plants form arbuscular mycorrhizal symbioses with fungi from the order Glomales. In some of these plant species (e. g. in cereals), mycorrhization is correlated with the accumulation of apocarotenoids in the colonized parts of the roots. The precursors of these secondary metabolites originate from the non-mevalonate methylerythritol 4-phosphate (MEP) pathway to isopentenyl diphosphate (IPP). The enzyme 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) catalyzes the first committed step of the pathway by rearrangement and subsequent reduction of 1-deoxy-D-xylulose 5-phosphate (DOXP) to MEP. Using a heterologous probe from rice, transcript levels of this enzyme could be shown to be elevated in mycorrhizal maize roots compared to non-mycorrhizal controls [1].
The corresponding cDNA for a maize DXR was cloned from a mycorrhizal maize root λ-ZAP cDNA library. Expression analysis by Northern blot exhibited abundant DXR transcripts in mycorrhizal roots, leaves and seedlings of maize plants. To further characterize the enzyme, we cloned the coding sequence of the maize DXR (omitting the predicted signal peptide for plastid import) in frame with a N-terminal His-tag into an expression vector (pQE30, Qiagen, Hilden, Germany). Heterologous expression of this construct in *E. coli* resulted in a recombinant protein of the expected molecular weight. Purification of the recombinant His-tagged DXR by metal affinity chromatography yielded about 2 mg of the protein that was used for the production of a polyclonal antiserum. First Western blot experiments with maize protein extracts show the recognition of a single protein band with the correct molecular weight in leaves and roots. We are now initiating immunolocalization studies in mycorrhizal maize roots with the aim of establishing a spatial correlation between symbiotic structures and the occurence of the DXR protein. The overall aim of this project is to try and gain an insight into potential functions of the accumulating apocarotenoids or their respective precursors in the arbuscular mycorrhizal symbiosis.

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THE *IN VITRO* PRODUCTS OF THE MAIZE TERPENE SYNTHASE TPS4 AND TPS5 ARE CHARACTERISTIC CONSTITUENTS OF THE MAIZE VOLATILES

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Volatile organic compounds are emitted from plant foliage after herbivore attack. Since these volatiles have been shown to attract herbivore enemies, it has been suggested that volatile release represents an indirect defense mechanism to protect the plant from further damage. When maize is attacked by lepidopteran larvae like *Spodoptera littoralis*, the emission is dominated by monoterpenes and sesquiterpenes. These emissions attract parasitic wasps like *Cotesia marginiventris* that use them to locate their lepidopteran host for oviposition [1]. In order to understand the function of volatile emission in maize and its regulation by herbivore damage, we are investigating the molecular genetics and biochemistry of terpene formation in this species. We are currently identifying a gene family encoding terpene synthases, which catalyze a key regulatory step in terpene formation, the conversion of acyclic prenyl diphosphates to a wide variety of basic terpene skeletons.
The terpene synthase gene *tps4* was identified in the maize inbred line B73. To identify the function of this gene, we have determined its catalytic properties after functional expression in *E. coli*. The enzyme requires either Mg^{2+} or Mn^{2+} as a cofactor and accepts the substrates geranyl diphosphate (GPP) and farnesyl diphosphate (FPP) with a similar K_m, but V_{max}rel is greater with FPP. The faster formation of sesquiterpenes and the absence of a plastid targeting signal at the N-terminus of the peptide suggest that *tps4* encodes a sesquiterpene synthase. The enzyme forms multiple products, including 7-*epi*-sesquithujene, (S)-β-bisabolene and at least 13 minor sesquiterpenes that include several pairs of enantiomers and diastereomers. A proposed reaction mechanism will be discussed. The products of TPS4 are constituents of the terpene blend released by herbivore-damaged maize plants in different developmental stages.
The maize variety Delprim emits a similar terpene blend but shows a strongly altered distribution of the stereoisomers. From this variety, we identified the terpene synthase gene *tps5* which displays a 96% amino acid identity to *tps4* The main product of TPS5 is sesquithujene, a diastereomer of 7-*epi*-sesquithujene, and the minor products appear generally in altered stereoisomer ratios when compared to TPS4.

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HEBIVORE-INDUCED CHANGES IN THE TRANSCRIPTOME OF *NICOTIANA ATTENUATA*

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A host-plant-mediated cross-resistance was found with the two main herbivores of *Nicotiana attenuata*: prior infestation with the mirid *Tupiocoris notatus* results in slower growth of *M. sexta* larvae, reduced ovipostion of *M. sexta* moths, and increased predation of *M. sexta* eggs by the predatory bug *Geocoris pallens* [1]. There is evidence suggesting this plant vaccination effect to be mediated by mirid-induced secondary metabolites and mirid-induced VOC's serving as signals for *M. sexta* moths as well as predatory insects [1, 2]. Our work aims to characterize *N. attenuata's* transcriptional response to the two competing herbivores to 1) identify genes involved in this competitive interaction, and 2) unravel differences in gene induction after herbivory by different feeding guilds (sucking/piercing versus tissue-consuming insect). Moreover, we plan to include more species (caterpillars of closely or more distantly related species, aphids as a representative of phloem feeders) into the analysis to find common and specific response elements. The poster will report on two approaches to identify *T. notatus*- and *M. sexta*-induced genes without prior assumptions about the nature of these genes: a multiple Differential-Display-RT-PCR and a subtractive hybridization based on magnetic beads. Additionally, it will show some first results obtained with a cDNA micorarray composed of *M. sexta*-induced transcripts [3, 4] and hybridized with RNA from the following series of pair-wise comparisons: 1. control vs. *T. notatus* , 2. control vs. *M. sexta,* 3. control vs. *T.notatus* before *M. sexta,* 4. control vs. *M.sexta* before *T. notatus,* 5. control vs *T. notatus* and *M. sexta.*

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INDUCTION OF PROTEASE INHIBITOR MRNA AND ACTIVITY IN *NICOTIANA ATTENUATA* PLANTS.

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Nicotiana attenuata, a post-fire annual wild tobacco inhabiting the Great Basin Desert, expresses several constitutive and inducible chemical defenses. These include serine protease inhibitors (PI s) that reduced growth and survival of many insect herbivores. We characterize the relationship between PI mRNA expression and PI activity in plant tissues over time after different elicitation treatments to fully characterize this induced defense. *N. attenuata* plants were induced with four treatments: i) 150 µg methyl jasmonate (MJ), ii) wounding with a pattern wheel (W) which produced rows of punctures in the leaf lamina, iii) wounding with a pattern wheel with *Manduca sexta* oral secretion added to the puncture wounds (W+OS), or iv) first instar *M.sexta* caterpillar feeding for 24 h. Plants were harvested daily and the relative expression of PI mRNA was determined by RT-PCR (TaqMan) and PI activity by radial diffusion assay. The highest relative expression of PI mRNA after treatment with MJ was found 12 h after induction in the treated leaf, while the highest systemic response was found after 24 h. A similar response was found in the W+OS treatments but with lower relative mRNA expression levels compared to plants treated with MJ. In the W treatments, both the systemic and the local response reached a maximum 24 h after induction, with the highest relative accumulation of the transcript leveling the treated leaf. The effect of caterpillars on PI mRNA expression and PI activity was only detectable in the treated leaf and showed the smallest responses, suggesting that caterpillar attack down-regulated this induced defense. PI activity measures increased 3-4 days after the increases in PI mRNA for all treatments.

SEASONAL VARIATION IN ALLELOPATHIC POTENTIAL OF *ARTEMISIA PRINCEPS VAR. ORIENTALIS* ON PLANTS AND MICROBES

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Laboratory experiments were carried out to investigate the seasonal variation in allelopathic potential of *Artemisia princeps* var. *orientalis*. Aqueous extract and methanol extract prepared monthly (April-October) from *A. princeps* var. *orientalis* was tested for allelopathic potential on seed germination and seedling growth of *Lactuca sativa* and *Achyranthes japonica* and six strain microbial growth. The allelopathic potential varied with the time of sample preparation and concentrations. Seed germination of *L. sativa* was not inhibited but seedling (shoot/root) growth was inhibited, exhibiting significant seasonal variation. In case of *A. japonica*, seed germination was not inhibited at lower concentration (except August) but it was inhibited at higher concentration, depending on month. The degree of inhibition was highest in July. The inhibition of seedling growth was different by month and extract concentration. The root growth was more inhibited than shoot growth. The antimicrobial activity of ethyl acetate and water fractions of crude methanol extract from *A. princeps* var. *orientalis* sampled monthly were examined against three gram-positive bacteria, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, two gram-negative bacteria, *Escherichia coli*, *Pseudomonas fluorescens*, and *Lactobacillus plantarum*. The ethyl acetate fraction of crude methanol extract of *A. princeps* var. *orientalis* sampled in September showed remarkably potent antimicrobial activity against *B. cereus* and *B. subtilis*. The water fraction of *A. princeps* var. *orientalis* sampled in August and September showed remarkably potent antimicrobial activity against Gram-positive and negative bacteria. *L. plantarum* did not form clear zone at water fraction of *A. princeps* var. *orientalis* sampled monthly. The antimicrobial activity of ethyl acetate and water fractions of crude methanol extract from *A. princeps* var. *orientalis* sampled in April and October was lower than any other months.

INFLUENCE OF *MYRIOPHYLLUM SPICATUM* DERIVED TANNINS ON GUT MICROBIOTA OF ITS HERBIVORE *ACENTRIA EPHEMERELLA*

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The submerged living larvae of *Acentria ephemerella* were fed in the laboratory with either *M. spicatum* or *P. perfoliatus*, two of their host plants. Larvae exhibit a reduced growth when fed with *M. spicatum.* This freshwater angiosperm contains high amounts of tannins, secondary metabolites known for their herbivore-deterrent and antimicrobial properties. In this study, we investigated the influence of food derived tannins on gut microbiota. Bacterial densities in the guts did not differ between the food regimes, ranging from 2.8 to 13.3 x 10^6 cells per gut. Gut bacteria were characterized with cultivation techniques and subsequent identification of the strains by molecular methods. We isolated 17 bacterial strains belongig to all subdivisions, i.e. we identified α-, ß- and γ-proteobacteria, *Cytophyga/Flavobacteria* (CFB) and several gram positive bacteria. All exept one gram positive strains were found in the guts of larvae fed with *P. perfoliatus*. Gram-positive bacteria apparently are, together with those of the CF cluster, more sensitive to polyphenol-containing extracts of *M. spicatum* in an agar diffusion assay than strains of the α- or γ-proteobacteria subdivision. Our results suggest an influence of food-derived tannins on gut microbiota in *A. ephemerella*. Possible impacts of allelochemical interactions between tannins from food plants and gut microbiota in lepidopteran larvae are discussed.

METHYL JASMONATE AND CIS-JASMONE: INACTIVATION OR AMPLIFICATION OF THE OCTADECANOID PATHWAY?

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The octadecanoid pathway mediates the wound- and herbivore-induced elicitation of diverse defense reactions of *Nicotiana attenuata*. The decomposition or inactivation of the final product of this well-described lipid-based signal transduction pathway, jasmonic acid (JA), remains unknown. Two derivatives of JA, its methylester and *cis*-jasmone (cJ), are thought to be a means of disposing JA through volatilization at the plant surface. In *N. attenuata* neither of these substances could be recovered in the headspace of wounded plants, whereas levels of methyl jasmonate (MJ) and cJ increased 60- and 40-fold, respectively, in surface extracts of attacked leaves after feeding of *Manduca sexta* larvae. Inhibition of the wound-induced increase in JA with Indol-3-acetic acid revealed a biosynthetic link between the induced increases in MJ, cJ and JA. Over a period of four days after elicitation MJ showed a much higher and longer induction after *M. sexta* saliva application to mechanical wounds compared to mechanical wounding only. This enhancement could not be observed with cJ. The amount of cJ and MJ produced could only account for less than 9% of the JA burst elicited by herbivore attack and hence does not represent a major disposal pathway for the oxylipin cascade in *N. attenuata*. Since trichomes store a large proportion of the MJ-pool of the leaf, which could be released on to the leaf surface after elicitation, we suggest that the MJ stored in trichomes could function to amplify the octadecanoid pathway after herbivore attack.

CYCLIC COMPOUNDS IN CUTICULAR WAXES : ARE THEY EXPOSED AT THE PLANT SURFACE ?

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Primary plant organs are covered with cuticles, containing waxes within a cutin matrix (intracuticular wax) and on its surface (epicuticular wax). Only the epicuticular layer is exposed to insects on first contact, and so the composition of this outer film is important for initial host plant recognition.
Previously, total wax extracts consisting of both intra- and epicuticular waxes have been used to study the reaction of insects to wax components. In these bulk mixtures possible differences between both layers were not reflected and, hence, the infochemical relevance could not be tested.
Recently, distinct epi- and intracuticular wax compositions have been reported for some Rosaceae. Using the same methods, the leaf wax layering of several species from a variety of plant families was analysed in the present study.
In the leaf waxes of *e.g. Eichhornia crassiceps* and *Dizygotheca elegantissima* (no cyclic compounds) no significant differences between epi- and intracuticular wax could be detected.
In contrast, the waxes of *e.g.* O*reopamax guatamalense* (containing tocopherols), *Monstera deliciosa* (containing steroids), *Tetrastigma vionierianum* (tocopherols and steroids), *Coffea arabica* (steroids, triterpenoids, and the alkaloid caffein) were distinctly layered. In all cases, the cyclic compounds were restricted to the intracuticular wax, whereas the epicuticular film consisted of aliphatics, and the cyclic constituents can therefore not serve as infochemicals.
Only in *Garcinia spicata* β-amyrin could be detected in both layers, while tocopherols, steroids and the other triterpenoids were restricted to the intracuticular wax.
We hypothesize that cyclic compounds are partitioned between intra- and epicuticular layers due to their specific molecular geometries and/or polarities.

ESTERASE MEDIATED CAULERPENYNE TRANSFORMATION IN SIPHONOUS GREEN ALGAE OF THE ORDER CAULERPALES

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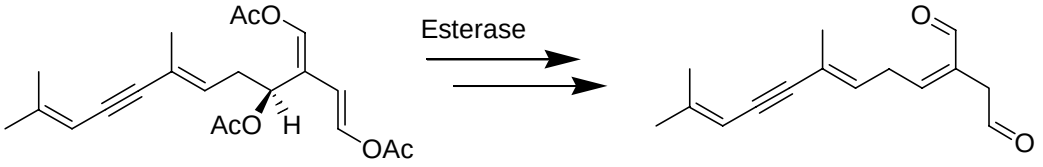
The invasive green alga *Caulerpa taxifolia*, spreading rapidly after its introduction into the Mediterranean and the North American Pacific, reacts upon wounding with the transformation of its major metabolite, the sesquiterpene caulerpenyne. This wound-activated reaction involves an esterase-mediated saponification of the bis-enol acetate moiety of caulerpenyne releasing reactive 1,4-dialdehydes after enolization [1].



Since caulerpenyne is also found in other *Caulerpa* spp. the question arose if this defensive mechanism is restricted to the invasive *C. taxifolia*, or if it is, moreover, a general feature of this genus. Comparison of the two invasive species, *C. taxifolia* and *C. racemosa*, and the indigenous non-invasive *C. prolifera* from the Mediterranean confirmed that all produce caulerpenyne in varying amounts. We show here that due to the fast transformation mechanism the caulerpenyne content has been underestimated in previous studies. Using a modified extraction procedure esterase activity can be suppressed and the caulerpenyne content in intact tissues can be corrected to the following. *C. taxifolia* and *C. prolifera* contain almost the same amount of caulerpenyne (6 mg per g of the algal fresh weight) while the caulerpenyne content in *C. racemosa* reached only half this value. In all three species caulerpenyne transformation occurred rapidly after wounding and severe tissue damage caused degradation of more than 50% of the stored caulerpenyne within one minute. Speed and mechanism of the wound-activated transformation as well as the caulerpenyne content in the intact tissue of invasive and non-invasive *Caulerpa* spp. are comparable and thus this caulerpenyne based defense, despite being highly efficient, is not the key for the success of the two invasive species [2].
As shown [1] the transformation of caulerpenyne results in a complex mixture of downstream metabolites that degrade or react very. Therefore investigations of the transformation mechanism in the presence of wounded algal material are difficult. To facilitate studies, model systems have been established allowing to monitor caulerpenyne transformation *in vitro*. A range of esterases has been used for caulerpenyne transformation in presence and absence of esterase inhibitors.

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CHEMODIVERSITY IN THE CALEDONIAN FOREST

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The Ballochbuie forest, located on the Balmoral Estate in north-east Scotland, is considered to be a mature remnant of the Scots pine (*Pinus sylvestris* L.) Caledonian forest. The total area under study is approximately 4 km^2. On site, individual trees have been chosen to form a grid with trees being spaced 200m apart. In addition, for 20of these trees an additional three satellite trees - within a 45° angle at distances 30, 70 and 100m – have also been sampled.
Needle samples of the previous year's growth were taken during the dormant season. A hexane extraction of the needles was performed and the extracts were filtered through silica gel. GC-FID analysis, using both a BPX 5 and a carbowax column, gave the concentration of individual monoterpenes. The main constituents of the needle hexane extracts were α-pinene (range 272 to 9040 μg gDW^{-1}), β-pinene (range 51 to 3647 μg gDW^{-1}), and Δ^3-carene (range 0 to 2776 μg gDW^{-1}). Other compounds present, identified based on retention times of known standards, were camphene, and in minor amounts (<0.1% of the total) α- and γ-terpinene and limonene. The variation in monoterpene concentration and composition for all trees was considerable, e. g. the ratio of α-pinene to Δ^3-carene ranged from 0.3 to 24.0, omitting 14 trees for which Δ^3-carene was absent.
Statistical analysis was conducted on the four main monoterpenes and two unknown terpenoids . Concentrations were logarithmically transformated following replacement of β-pinene,Δ^3-carene values less than 0.01, 0.1 (units) by 0.005, 0.05 (units) respectively. Regressions of log-concentrations on co-ordinates and altitude revealed no strong trends across the study area, and semi-variogram plots revealed no evidence that trees close together to have more similar values than trees far apart. The log-concentrations were only weakly correlated, with no pairwise correlation exceeding 0.5. A principal components analysis on the covariance matrix of log-concentrations allocated 51, 21, 11 and 10% of the variance to each of the first four axes respectively. The first axis was dominated by β-pinene, and the second by Δ^3-carene, to such an extent that the plot of the scores indicated a clear separation between trees with low or absent β-pinene, trees with low or absent Δ^3-carene, and trees with moderate to high values of both.
Forrest[1] analysed cortical resin samples of distinct Scots pine populations in Scotland and, despite highly variable monoterpene compositions within populations he identified genotypic variation between populations. Even though the concentration and composition of needle samples, as used in this study, compared to resin samples[1] was expected to be different,[2,3] we were surprised by the presence of at least three distinct chemotypes within the small area of the Ballochbuie forest, and the lack of spatial correlation. Hence, a mature Scots pine forest seems to be characterised by extensive chemodiversity. For reforestation programs, some of which require specific chemotypes, the results of this study suggest that less strict guidelines could be used. Additionally, the chemodiversity on-site is expected to influence ecosystem processes and future research is directed towards investigating these.

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EFFECTS OF PLANT/PLANT COMMUNICATION IN BARLEY ON BIOMASS ALLOCATION

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Patterns of biomass allocation between different plant organs have often been used to explain the response of plants to variations in resource availability. This study reports how aerial allelopathy (plant/plant communication) affects biomass allocation *i.e.* the trade off between root, stem and leaves, and also relative growth rate (RGR, increased in biomass per unit biomass per unit of time, mg g^{-1} d^{-1}) and its components. Based on previous experiments [1,2], communication between two barley (*Hordeum vulgare* L.) cultivars (Alva and Kara) was used for the present study. Kara exposed to volatiles from Alva allocated significantly more biomass to roots compared with Kara exposed to volatiles from Kara or to clean air. There was no significant difference between plants of Kara exposed to volatiles from Kara and those exposed to clean air. Changes in total dry weight (TDW), RGR and unit leaf ratio (ULR, increase in biomass per unit time and leaf area, kg m^{-2} d^{-1}) were not significantly affected by plant/plant communication. However, there was a significant increase in specific leaf area (SLA, leaf area per leaf dry weight, m^2 kg^{-1}) in Kara when exposed to volatiles from Alva. The results show that aerial plant/plant communication does not affect total biomass production but does significantly affect biomass allocation in individual plants. There may be differences in the volatile profiles of Kara and Alva that induce increased biomass allocation to roots in the Kara exposed plants to volatiles from Alva.

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A GENE CLUSTER ASSOCIATED WITH PRODUCTION OF INSECTICIDAL LOLINE ALKALOIDS IN THE FUNGAL ENDOPHYTE *NEOTYPHODIUM UNCINATUM.*

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Grass plants can acquire chemical defenses against insect and vertebrate herbivores by living in symbiosis with fungal endophytes of the genus *Epichloë* (anamorphs, *Neotyphodium*). In several *Epichloë*/*Neotyphodium*-grass associations, insecticidal 1-aminopyrrolizidine (loline) alkaloids are produced, which hold promise as natural plant protectants. Since very little is known about loline alkaloid biosynthesis, the objective of this study was to identify the genes involved. *N. uncinatum* can produce lolines under certain culture conditions, and a culture system was developed for regulated loline expression in this fungus. With this system, genes differentially expressed during loline accumulation were isolated by subtractive hybridization. Two genes isolated, *lolA* and *lolC*, were similar to genes encoding aspartate kinases and homocysteine synthase, respectively, enzymes in methionine biosynthesis. *lolA* and *lolC* were highly expressed during loline production in culture, and the two genes were present only in endophytes with loline-producing phenotypes. Close linkage of *lolA* and *lolC* in *N. uncinatum* was found by PCR, corroborated by data from a genomic library survey of the closely related endophyte *E. festucae*. At least seven more open reading frames were clustered with *lolA* and *lolC*. All but one of these putative genes are significantly related to known genes, mainly from other fungi, encoding decarboxylases, P450- and FAD-monooxygenases, oxidoreductases, aminotransferases and epoxidases. In other fungal systems such genes are frequently found in secondary-metabolite gene clusters.

METABOLITE PROFILING OF THE ARBUSCULAR MYCORRHIZAL SYSTEM *MEDICAGO TRUNCATULA/GLOMUS MOSSEAE*

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Fungi from the order Glomales are integral components of mutualistic interactions with plant roots of most terrestrial plants (arbuscular mycorrhizas) increasing the plant-soil interface which improves supply of nutrients and stress tolerance of plants. For functional genomics of a biological system high-throughput multi-parallel techniques have been developed for transcriptome, proteome and metabolome analyses. As the first two approaches do not provide direct information about how alterations in mRNA and protein patterns are associated with changes in biological function, a project within the German Mycorrhiza Priority Program was started, targeting to the analysis of the metabolome of the arbuscular mycorrhizal system between the model plant *Medicago truncatula* and *Glomus mosseae*. Due to the chemical complexity of the metabolome, no singular experimental technique exists that is capable in profiling all primary and secondary metabolites concurrently. To address this issue, the metabolome has to be subdivided into manageable parts of lower complexity that can be separated by chromatographic procedures coupled with mass spectrometers. These techniques provide data on both the abundance and specific properties of metabolites that can be utilized in chemical identification. The work focuses on recording alterations in metabolite profiles during the functional establishment of the mycorrhizal symbiosis. From the results suggestions may be derived concerning the role of metabolite changes in functioning of the plant-fungus symbiotic interaction. The observed changes in metabolite profiles of a given tissue (roots, stems, leaves) will be correlated with the corresponding mycorrhiza-specific gene expression profiling data. A search for natural products of alfalfa (*Medicago sativa*), a major forage legume closely related to *M. truncatula*, assigned 168 compounds, mainly (>70%) in the groups of flavonoids and terpenoids. For comprehensive analyses of metabolite patterns in non-mycorrhizal, mycorrhizal and transgenic plants, various methods (extraction, solvent partitioning, derivatization) have to be elaborated. In a first approach, lyophilized roots (*M. truncatula*/*G. mosseae*) and corresponding non-mycorrhizal controls of different developmental stages were sequentially extracted with dichloromethane, acetone and 80% aqueous methanol. In these extracts more than three hundred compounds were detected by RP-HPLC-PDA. A multitude of them showed pronounced mycorrhiza-specific alterations, e.g. the contents of both the isoflavone glucoside ononin and the corresponding malonyl conjugate increased in mycorrhizal roots. Similarly, first analyses of the acetone extracts by LC/ESI-MS showed qualitative and quantitative differences between control and mycorrhizal root samples. Finally, capillary GC/EI$^+$-TOF-MS, a method with high mass resolution and accuracy, was applied to the extracts derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide. Comparative analyses showed good reproducibility concerning retention time and total ion current, a necessary prerequisite for obtaining statistically significant data. Palmitelaidic acid and the corresponding monoglyceride were found exclusively in the unpolar extract of mycorrhizal roots, whereas in the polar extract of the same tissue an increased content of proline was observed, which may play a role as osmoprotectant, possibly involved in an elevated stress tolerance of mycorrhizal plants.

AGGREGATION FACTORS OF THE GERMAN COCKROACH ARE PRODUCED BY GUT MICROBIAL SYMBIONTS.

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Based on previous findings that aggregation behavior in the German cockroach, *Blattella germanica*, is mediated by a mix of volatile carboxylic acids [1], we hypothesized that symbiotic microbes might be involved. Aggregation assays in dishes and olfactometer assays in Y-tubes revealed significant differences in the attractiveness of fecal extracts of German cockroaches reared under sterile or non-sterile conditions. Eleven species of bacteria were aerobically cultured and isolated on broad-spectrum and selective artificial media from feces of non-sterile cockroaches and identified by phenotype and sequencing of 16S rDNA. Sterile first instars cockroaches inoculated orally with individual or mixtures of bacterial isolates produced feces that was significantly more attractive to conspecifics than the feces of sterile cockroaches. These results show that gut microbes are involved in the production of *B. germanica* aggregation attractants and arrestants. Analysis of carboxylic acids from feces extracts by GC-MS revealed significant differences between sterile and non-sterile feces.

[1] Scherkenbeck, J. et al.; J. Chem. Ecol., **25**.; (1999), 1105–1119.

THE ROLE OF LIPID TRANSFER PROTEINS IN WILD TOBACCO (*NICOTIANA ATTENUATA*)

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Glandular trichomes of *Nicotiana attenuata* contain a number of chemicals that are toxic or deterrent to herbivorous insects. Using molecular and analytical chemistry tools, we explored which constituents of the trichome are influenced by insect herbivory. To determine which constituents are produced in the trichome, we constructed a trichome-specific cDNA library and sequenced 192 different sized clones. The most prevalent sequence had 98% homology to a *Nicotiana glauca* lipid transfer transcript. Lipid transfer proteins have putative defense related functions and are known to exchange and/or transfer polar compounds that may play a role in plant resistance to herbivores. In order to determine their possible role, we evaluated changes in the putative lipid transfer protein MALTI-TOF spectrum and, with RT-PCR (*Taq*Man), the amount of LTP mRNA transcribed after herbivore damage and induction by methyl jasmonate.

EXPRESSION OF TRANSCRIPTS CODING FOR PLASTID LOCATED PROTEINS IN ARBUSCULAR MYCORRHIZAL ROOTS FROM *MEDICAGO TRUNCATULA*

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Arbuscular mycorrhiza is an ancient and wide-spread symbiosis between fungi from the order Glomales and most terrestrial plants. *Medicago truncatula* has been chosen as a model plant for the molecular study of this interaction. During the colonization of root cortical cells by highly branched fungal hyphae (arbuscules), the plant cell is responsible for the establishment of parts of the symbiotic structure. In addition, the plant cell has to generate energy for transport processes and to supply metabolites to the fungus. Furthermore it has to process metabolites it receives from the fungus and to degrade the remnants of decomposing arbuscules. Root cell plastids are involved in a number of these processes and are subjected to a dramatic cytological reorganization during the colonization of a given cell [1].. To characterize the biochemical changes corresponding with this reorganization, we are currently analysing the expression level of plastid-located proteins using real time PCR. First results indicate an induction of transcripts referring to biosynthetic pathways, e.g. fatty acid, pyrimidine and carotenoid biosyntheses.

[1] Fester T.; Strack, D.; Hause B.; Planta, **213**; (2001), 864–868.

SIMULTANEOUS MONITORING OF PLANT VOLATILE EMISSION BY PHOTOACOUSTIC SPECTROSCOPY AND AN ULTRA FAST GC

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Plants produce a wide range of volatiles as part of their metabolic activity, in reaction to abiotic stress, or as a defense against attacks by other organisms. To follow the time course of such induced volatile emissions or to monitor the release of volatiles from flowers and other sources, non-invasive and, at the same time, highly sensitive methods are important. [1].
This poster presents two complementary techniques for the detection of different classes of volatiles emitted by plants. Small hydrocarbons (especially ethylene) are detected with a photoacoustic spectrometer (PAS) using the infrared fingerprint of a compound and a tunable laser for selective excitation. For monitoring higher molecular weight compounds, a fast and ultra-sensitive sampling system (the zNose^{TM}) [2] is used.
Diurnal ethylene emission rhythms using PA and diurnal volatile emission were studied using the zNose^{TM} and herbivore damaged lima bean leaves (*Phaseolus lunatus*) or leaves previously elicited with ion-channel forming peptides (e.g. alamethicin from the plant parasitic fungus *Trichoderma viride*).
Simultaneous use of the methods provides high time-resolution along with quantitative data on the released volatiles.
The special advantages of trace gas detection by PAS are shown for isoprene, ethylene, and ethane. An example of an isotopomer-selective excitation of molecules is shown for isoprene. This technique facilitates simultaneous detection of labeled (deuterium) and unlabeled isoprene allowing a detailed study of isoprene biosynthesis.
Summary: Ultrafast GC and infrared PA detection are powerful tools for the monitoring of volatile emission with high time resolution. Their fast response along with their unique sensitivity makes them well suited to study the temporal response of plants to external factors like herbivore feeding, pathogen attacks or abiotic stress. The automated long-term monitoring capability of the instrument greatly facilitates the analysis of circadian rhythms in volatile emission. Simultaneous monitoring of volatiles and ethylene by the zNose^{TM} and PAS is expected to provide time-resolved information on the synergistic interaction of the octadecanoid- and the ethylene pathway.

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[2] www.estcal.com

SALICYLIC ACID AND APHID-PLANT INTERACTIONS

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When the polyphagous aphid *Myzus persicae* feeds on Arabidopsis, genes associated with salicylic acid (SA)-dependent induction are transcribed to particularly high levels (Moran & Thompson, 2001, *Plant Physiology* 125: 1074-1085). However, the biological relevance of such SA-dependent responses has not been demonstrated. In order to explore the effects of SA-dependent induction on aphid biology, we have investigated behaviour and reproductive performance of *M. persicae* on transgenic Arabidopsis showing constitutive expression of the bacterial gene for salicylate hydroxylase (*nah*G). This enzyme oxidises SA into catechol, so *nah*G plants cannot accumulate SA and are unable to induce local or systemic acquired resistance to pathogens. Our results show that *nah*G plants also have enhanced susceptibility to aphids. The intrinsic rate of reproductive increase (r_m) of *M. persicae* is significantly higher on *nah*G Arabidopsis than on wild-type Columbia (Col-0), suggesting that SA-mediated changes in plant chemistry/physiology following aphid feeding may constrain insect fitness on wild-type plants. Electrical recording of stylet activities (EPG technique) showed that high-frequency stylet movements (waveform F) often occur on Col-0, indicating difficulty in penetrating cell walls. By contrast, on *nah*G plants the stylet pathway to the phloem is rarely hindered by such penetration difficulties. The clear difference in stylet penetration patterns demonstrated by EPG analysis indicates that SA mediates apoplastic defences against aphids.

ODORANT-BINDING PROTEINS FROM THE DAMPWOOD TERMITE, *ZOOTERMOPSIS NEVADENSIS NEVADENSIS*

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Odorant-binding proteins were isolated and cloned for the first time from a primitive termite species, the dampwood termite, *Zootermopsis nevadensis nevadensis* (Isoptera: Termopsidae). With a protein-based approach, a major antennae-specific protein was detected by native PAGE along with four other minor proteins, which were also absent in the extract from control tissues (hindlegs). Multiple cDNA cloning led to the full characterization of the major antennae-specific protein (ZnevOBP1) and to the identification of two other antennae-specific cDNAs, encoding putative odorant-binding proteins (ZnevOBP2 and ZnevOBP3). N-terminal amino acid sequencing of the minor antennal bands and cDNA cloning showed that olfaction in *Z. n. nevadensis* may involve multiple odorant-binding proteins. Database searches suggest that the OBPs from this primitive termite are homologues of the pheromone-binding proteins from scarab beetles and antennal-binding proteins from moths.

NEW RESULTS IN THE DEFENSIVE CHEMISTRY OF CREMATOGASTER ANTS

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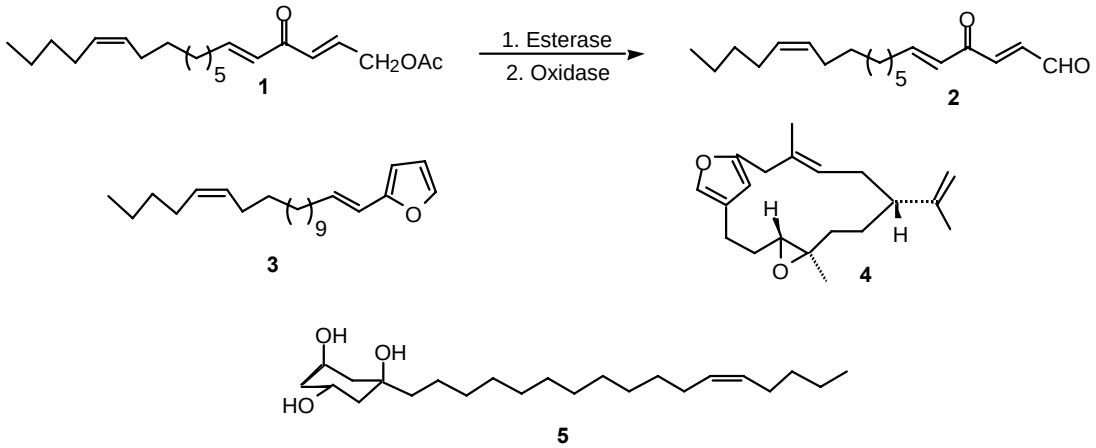
Ants of the genus *Crematogaster* are able to raise their abdomen forwards and over the thorax and head. In many *Crematogaster* species, the venom is emitted as a froth that accumulates on the spatulate portion and at the basis of the sting, and thus can be easily applied to the integument of enemies. In the three European species of *Crematogaster* ants, the Dufour gland contains complex mixtures of long chain derivatives bearing a (*E*,*E*)-cross-conjugated dienone linked to a primary acetate function (e.g. **1**). When the venom is emitted, these compounds are transformed into highly electrophilic and toxic 4-oxo-2,5-dienals (e.g. **2**) by an esterase and an oxidase stored in the poison gland [1]. On the other hand, the Dufour gland of one *Crematogaster* species from Papua-New Guinea produces long chain furan derivatives such as **3**, [2], whereas two Brazilian species produce furanocembrene diterpenes (e.g. **4**) [3].
To further investigate the defensive mechanisms in this genus and to assess whether the composition of the Dufour gland secretion could be used for taxonomic purposes, we have now studied two other *Crematogaster* species. The Dufour gland of *C. nigriceps* from Africa contains a mixture of 1-alkyl-1,3,5-cyclohexanetriols (e.g. **5**). In *C. montezumia*, collected in Argentina, the defensive mechanism is based on the production of triacylglycerols bearing polyunsaturated fatty acids containing (*Z*,*Z*,*Z*)-conjugated trienes or (5*E*,8*Z*,10*Z*,12*Z*)-tetraenes, which are responsible for the strong sticking properties of the secretion.



1 **2** **3** **4** **5**

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[2] Leclercq, S., Daloze, D., Kaisin, M., Braekman, J.C., Detrain, C., de Biseau, J.C., Pasteels, J.M.; J. Nat. Prod., **60**, (1997) 1143-1147.
[3] Leclercq, S., de Biseau, J.C., Braekman, J.C., Daloze, D., Quinet, Y., Luhmer, M., Sundin, A., Pasteels, J.M.; Tetrahedron, **56**, (2000), 2037-2042.

ANTIFEEDANT ACTIVITY IN *HYLOBIUS* WEEVILS – FROM BASIC RESEARCH TO FOREST PROTECTION

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In small, stressed conifers like the seedlings on a forestry clear-cut, the quantitative defense is weak. These seedlings have been protected by insecticides like DDT and pyrethroids against the feeding by adult pine weevil *Hylobius abietis*. However, Permetrin will be banned in 2003. Our work follows two lines: 1) The screening of synthetically available anti-feedant compounds and related structures, mainly of plant origin. 2) The search for anti-feedants in non-host plants.
Along line 1, we have screened >100 compounds and found some groups with high activity. The structure-activity based on dose-response tests for several compound groups will be described and compared to a putative ODP component.
Along Line 2, pine and 29 other woody plants were presented in a no-choice assay. The hypothesis is that the barks from non-host plants contain antifeedants, causing the insects to starve rather than feed. Inner bark was significantly less preferred than pine in 20 cases and outer bark was less preferred in 7 cases. The cases where the bark was partly consumed but the phloem remained show that there are positive signals in non-host plants. The weevils regard these non-hosts as palatable, but are inhibited from further feeding when reaching the phloem.

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