Chemical Ecology and Phytochemistry in Forest Ecosystems

Joint Meeting

International Society of Chemical Ecology, 20th annual meeting

Phytochemical Society of North America, 39th annual meeting

University of Ottawa, Canada

July 24 to 28, 2004

Hosted by

University of Ottawa, Canada

Canadian Forest Service, Great Lakes Forestry Centre

Scientific Committee	M.M. Abou-Zaid J.T. Arnason V. de Luca C. Nozzolillo B.J.R. Philogène
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PSNA President	Daneel Ferreira
Local Committee	Erin Lamont Mark Primavera, Elaine Liepins, Karen Jamieson University of Ottawa graduate students

The organizing committee gratefully acknowledges support from:

University of Ottawa
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Program

Saturday afternoon, July 24

3:00 to 9:00 Registration in the Unicentre, University of Ottawa

7:00 to 9:00 Welcome barbecue on the terrace of the Unicentre

Sunday morning, July 25

8:30 to 12:00 Registration in the Unicentre, set-up of posters

8:30 to 12:00 **Marion Hall Auditorium**

8:30 to 9:15 **Opening ceremonies and official welcome**
Chair: **B.J. R. Philogène**
Christian Detellier, Dean of Science, U. of Ottawa
Geoff Munro, Canadian Forest Service, DG-Science Division
Tom Baker, ISCE , President
Clint Chapple, PSNA, President-elect

9:15 to 10:00 **ISCE silver medal address**
J. McNeil introduced by **Joceyln Millar**
Putting ecology back in chemical ecology

10:00 to 10:30 **Refreshment break in Bioscience I**

10:30 to 12:00 **Main symposium**
Chair: **Clint Chapple**

10:30 to 11:15 **Jonathan Gershenzon**
Molecular regulation of induced chemical defenses in Norway spruce: tools for testing function. **S1**

11:15 to 12:00 **Geraldine A. Wright**
Odor recognition and the features of naturally occurring odors. **S2**

12:00 to 1:30 **Lunch in the Unicentre cafeteria** (second floor)

Sunday afternoon, July 25

1:30 to 5:30 **Concurent sessions M1 and M2 and C1 and C2 in Montpetit Hall**
Session M1 and C1, De Celles 202, Chair: **V. de Luca**

1:30 to 4:30 **Minisymposium 1, The Arthur Neish Minisymposium**
Organized by **V. de Luca**
"Out of the Forest and into the Diet: Wine Grape Genetic Diversity and Secondary Metabolism in *Vitis* species"

1:30 to 2:00 **P. Pollefeys**
Molecular genetic diversity of the French-American grapevine hybrids cultivated in North America. **M1.1**

2:00 to 2:30 **Jihong Wang**
'Foxy' methyl anthranilate potential of Concord grape'. **M1.2**

2:30 to 3:00 **Diane Martin**
Vitis vinifera terpenoid synthase genes and wine grape metabolite profiling. **M1.3**

3:00 to 3:30 **Refreshment break**

3:30 to 4:00 **Jane Coventry**
Red red wine - enhancement of berry colour and quality in Niagara wine grapes. **M1.4**

4.00 to 4.30 **Debra L. Inglis**
Yeast stress responses during icewine fermentation and their impact on icewine quality. **M1.5**

4:30 to 5:30 **Contributed papers, C1**

4:30 to 4:45 **D.R. Gang**
Micropropagation and genomics investigations of ginger and turmeric. **C1.1**

4:45 to 5:00 **John C. D'Auria**
Identification and characterization of an acyltransferase capable of producing the green leaf volatile, Z-3-hexen-1-yl acetate in *A. thaliana* **C1.2**

5:00 to 5:15 **A. Schmidt**
Characterization of prenyltransferases from Norway spruce - critical branch-point enzymes in terpene resin biosynthesis. **C1.3**

5.15 to 5.30 **R. Jetter**
Very-long-chain polyketides in the cuticular wax of *Taxus* needles. **C1.4**

Sunday afternoon, July 25, cont'd

1:30 to 5:30 **Session M2 and C2, De Celles 203,** Chairs: **C. Keeling and E. Plettner**

1:30 to 4:30 **Minisymposium 2**
Organized by **C. Keeling and E. Plettner**
Hymenopteran semiochemicals

1:30 to 2:00 **Keith N. Slessor**
Sociochemicals: the complicated pheromone arsenal of honey bees. **M2.1**

2:00 to 2:30 **Tanya Pankiw**
Pheromone regulation of honey bee foraging ontogeny. **M2.2**

2:30 to 3:00 **Gene E. Robinson**
Pheromone regulation of division of labor in honey bee colonies: from behavior to genes. **M2.3**

3:00 to 3:30 **Refreshment break**

3:30 to 4:00 **Robert K. Vander Meer**
Multiple functions of the fire ant, *Solenopsis invicta*, alarm pheromone. **M2.4**

4:00 to 4:30 **Jocelyn G. Millar**
Sex and bondage: queen sex pheromone of the slave-making ant *Polyergus breviceps.* **M2.5**

4:30 to 5:30 **Contributed papers, C2**

4:30 to 4:45 **F.P. Drijfhout**
Chemical basis for aggression behaviour in the invasive ant, *Lasius neglectus* and relation with *L. turcicus.* **C2.1**

4:45 to 5:00 **S. Jarau**
Hexyl decanoate, a trail pheromone component identified from labial gland secretions of a stingless bee, *Trigona recursa* (Apidae, Meliponini). **C2.2**

5:00 to 5:15 **Todd Barsby**
A defense dgainst whom? Investigating the chemical defense of the sea pansy *Renilla reniformis* using multiple predator types. **C2.3**

5:15 to 5:30 **R.L.Genna**
Identification of semiochemicals involved in sea lice host location, and their potential use in pest control. **C2.4**

Sunday evening July 25 Unicentre

7:00 to 9:00 **Poster session P with "degustation –les vins de l'Ontario". Presenters with odd numbered posters should be at their posters.**

Monday morning, July 26

8:30 to 12:00 **Montpetit De Celles 202**

Main symposium
Chair: **J. Delisle**

8:30 to 9:15 **Norman Lewis**
Seeing the forest and not the trees: a global perspective on lignin and lignan biosynthesis for structural support and defense function. **S3**

9:15 to 10:00 **Erika Plettner**
Pheromone binding proteins: insights into the mechanism of ligand recognition. **S4**

10:00 to 10:30 **Refreshment break**

10:30 to 11:15 **Joerg Bohlmann**
Biochemistry and functional genomics of herbivore induced defences in spruce and poplar. **S5**

11:15 to 12:00 **Hanna Mustaparta**
How is plant odour information handled by the olfactory system in insects? **S6**

12:00 to 1:30 **Lunch in the cafeteria**

Monday afternoon, July 26

Concurrent Sessions M3, M4 and society annual general meetings in De Celles 202/203, Montpetit Hall

1:30 to 4:30 **Minisymposium 3, De Celles 202**
Organized and chaired by **S. MacKinnon**
Marine Chemical Ecology

1:30 to 2:00 **Georg Pohnert**
The rapid bioactivation of secondary metabolites in the wound response of *Caulerpa taxifolia*. **M3.1**

2:00 to 2:30 **Gunilla Toth**
Brown algal phlorotannins: secondary compounds of primary importance. **M3.2**

2:30 to 3:00 **Mark E. Hay**
Chemical defense of marine animals: effects on color, mobility, overtness, and local and geographic patterns of distribution. **M3.3**

3:00 to 3:30 **Refreshment break**

3:30 to 4:00 **Julia Kubanek**
The role of red tide toxins and other metabolites in competitive and predator-prey interactions. **M3.4**

4:00 to 4:30 **Shawna MacKinnon**
Studies of the toxigenic dinoflagellate *Alexandrium ostenfeldii* and its production of a novel class of marine toxins known as the spirolides. **M3.5**

4:30 **ISCE annual general meeting, DeCelles 202**

Monday evening, July 26, Unicentre

7:00 to 9:00 **Poster session (P). Presenters with even numbered posters should be at their posters.**

Monday afternoon, July 26 (cont'd)

1:30 to 3:00 **Minisymposium 4, De Celles 203**
Organized and chaired by **B. Kimball** and **D. Nolte**
Chemically Mediated Behavior in Wildlife: Examination and Application

1:30 to 2:00 **T.P. Clausen**
Has browsing by mammals caused continent-scale variation in the chemical defenses of woody plants? **M4.1**

2.00 to 2.30 **F.D. Provenza**
Linking herbivore experience, varied diets, and plant biochemical diversity. **M4.2**

2.30 to 3.00 **T.P. Sullivan**
Weasels, voles, and crop protection: whither the future? **M4.3**

3.00 to 3.30 **Refreshment break**

3.30 to 4.00 **D.L. Nolte**
Chemical ecology and managing forest resources. **M4.4**

4:00 to 4:30 **B.A. Kimball**
That's disgusting: deer responses to seedlings treated with proteins. **M4.5**

4.30 **PSNA annual general meeting, De Celles 203**

Monday evening, July 26, Unicentre

7:00 to 9:00 **Poster session (P). Presenters with even numbered posters should be at their posters.**

Tuesday morning, July 27

8:30 to 12:00 **Montpetit Hall, De Celles 202**

Main symposium
Chaired by **Mark Bernards**

8:30 to 9:15 **Ken Raffa**
Interactions among conifer terpenoids and bark beetles across multiple levels of scale: an attempt to understand links between population patterns and physiological processes. **S7**

9:15 to 10:00 **Takashi Yoshida**
High molecular weight polyphenols: prospect in their functions. **S8**

10:00 to 10:30 **Refreshment break**

10.30 to 11.15 **C. Peter Constabel**
Molecular biology and biochemistry of induced insect defense in poplar. **S9**

11:15 to 12:00 **Claus Tittiger**
Insights into the remarkable metabolism of the bark beetle midgut. **S10**

12:00 to 1:30 **Lunch in the Unicentre cafeteria**

Tuesday afternoon, July 27

Cruise on the Ottawa River (or afternoon free)

Tuesday evening, July 27

6:30 to 9:30 **Cocktail (cash bar) and banquet at the historic Chateau Laurier Hotel, Adams Room**
After dinner speaker, **Neil Towers**
The role of natural products chemistry in natural history.

Wednesday morning, July 28

8:30 to 9:15 **Montpetit Hall, De Celles 202**
Main symposium
Chair: **J. McNeil**

Murray B. Isman
Tropical forests as sources of natural insecticides. **S11**

9:15 to 12:05 **Concurrent sessions M5 and C3, De Celles 202/203**

Pheromone Minisymposium M5, De Celles 202
Organized by **J. McNeil**

9:15 to 9:35 **K.F. Haynes**
Aggressive chemical mimicry by a bolas spider. **M5.1**

9:35 to 9:55 **A.J. Mordue Luntz**
The role of pheromones and kairomones in mate location of the salmon louse. **M5.2**

9:55 to 10:25 **Refreshment break**

10:25 to 10:45 **M. L. Evenden**
Can we combine pheromone-based attracticide formulations for more than one species: a case study using the oriental fruit moth and the codling moth (Lepidoptera: Tortricidae)? **M5.3**

10:45 to 11:05 **D. Eliyahul**
Pheromone stereochemistry and sexual mimicry: a close look at the sex life of cockroaches. **M5.4**

11:05 to 11:25 **Y. Hillbur**
Pheromones for management of gall midges. **M5.5**

11:25 to 11:45 **B. Frerot**
Reproductive isolation and host plant specialization in two *Ostrinia* pherotypes in France. **M5.6**

11:45 to 12:05 **M. Ayasse**
The role of semiochemicals in the pollination of sexually deceptive orchids. **M5.7**

Wednesday morning, July 28 , cont'd

9:20 to 12:05 **Concurrent contributed papers session C3, De Celles 203**
Chair: **Cecilia MacIntosh**

9:20 to 9:35 **A.M. Rimando**
Elucidation of the biosynthesis of sorghum phytotoxins by ^{13}C-NMR spectroscopy. **C3.1**

9:35 to 9:50 **J. Murata**
New tools to understand alkaloid factories at the cellular level. **C3.2**

9:50 to 10:20 **Refreshment break**

10:20 to 10:35 **F.S. Menezes**
Brazilian palms as source of biologically active molecules. **C3.3**

10:35 to 10:50 **Wilhelm Boland**
Mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. **C3.4**

10:50 to 11:05 **V. Thoss**
Individual variation in Scots pine phytochemistry and its effects on associated ecological processes in a Caledonian forest. **C3.5**

11:05 to 11:20 **C. Müller**
Growth and defense in the invasive crucifer species *Lepidium draba.* **C3.6**

11:20 to 11:35 **T.A. Arnold**
Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink-source model of plant defense. **C3.7**

11:35 to 11:50 **J. Weidenhamer**
Allelopathy as a mechanism for resisting invasion: the case of the Florida scrub. **C3.8**

11:50 to 12:05 **Emma Despland**
How does food nutrient content influence forest tent caterpillar feeding? **C3.9**

12:00 to 1:30 **Lunch in the Unicentre cafeteria**

Wednesday afternoon, July 28

1:30 to 3:00 **Montpetit Hall, DeCelles 202,** Chair: **Jocelyn Millar**

1:30 to 2:15 **Silverstein-Simeone Award winner address**
Richard Vogt, introduced by **Tom Baker**
Do odorant-binding proteins, odor degrading enzymes and other orphan sensory proteins comprise biochemical networks which influence odor presentation and perception?

2:15 to 3:00 **Robin Marles**
Boreal forest ethnobotany, conservation and sustainability. **S12**

3:00 to 3:30 **Refreshment break**

3:30 to 5:00 **Concurrent contributed paper sessions C4 and C5**

3:30 to 5:00 **Session C4, De Celles 202,** Chair **C. Cespedes**

3:30 to 3:45 **A. Zhang**
Identification of sex pheromone component of the blueberry leafminer, *Caloptilia porphyretica*. **C4.1**

3:45 to 4:00 **J.R. Aldrich**
Semiochemistry of the goldeneyed lacewing *Chrysopa oculata* (Neuroptera: Chrysopidae): attraction of males to a male-produced pheromone. **C4.2**

4:00 to 4:15 **T. Hartmann**
The polyphagous arciid *Estigmene acrea* sequester pro-t oxic pyrrolizidine alkaloids from any plant source for protection, synthesis of idiosyncratic alkaloids and pheromone formation. **C4.3**

4:15 to 4:30 **Imene Saïd**
Interactions between a plant volatile and the pheromone in *Rhynchophorus palmarum*: electrophysiological and behavioral evidence. **C4.4**

4:30 to 4 :45 **P.H.G. Zarbin**
Chemical ecology of the papaya weevil *Pseudopiazurus papayanus* (Coleoptera: Curculionidae). **C4.5**

4:45 to 5:00 **M. Haribal**
Role of preen gland secretions in feather protection: are feather mites mutualistic, commensalistic or parasitic? **C4.6**

Wednesday afternoon, July 28, cont'd

3:30 to 5:00 **Session C5, De Celles 203**
Chair **H. Niemeyer**

3:30 to 3:45 **M A.-G.Bagnères**
The chemical signature of subterranean termites: an adjunct for phylogeographic study. **C5.1**

3:5 to 4:00 **James E. Oliver**
Stereoselective synthesis and absolute configuration of gaur acid**. C5.2**

4:00 to 4:15 **Dietland Müller-Schwarze**
Where even goats' feet cannot tread: plant defense on mammal-free rock pillars in a Mediterranean oak forest. **C5.3**

4:15 to 4:30 **Dietland Müller-Schwarze**
A woodland mammal's response to compounds from an unpalatable spurge, *Euphorbia lathyris*. **C5.4**

4:30 to 4:45 **L. L. Tibbles**
The glucosinolate-myrosinase defence system in the cabbage aphid, *Brevicoryne brassicae*. **C5.5**

Main Symposium Abstracts

S1

Molecular regulation of induced chemical defenses in Norway spruce: tools for testing function.

Axel Schmidt[1], Katja Witzel[1], Gazmend Zeneli[1], Ulrike Temp[1], Trevor Fenning[1], Diane Martin[1,2], Jörg Bohlmann[1,2], Paal Krokene[3], Trygve Krekling[3], Erik Christiansen[3], Ari Hietala[3], Carl G. Fossdal[3] and **Jonathan Gershenzon[1]**, [1]Max Planck Institute for Chemical Ecology, Jena, Germany, [2]Current address: Department of Botany, University of British Columbia, Vancouver, BC, Canada, [3]Norwegian Forestry Research Institute, Aas, Norway

The study of conifer chemical defense has been dominated by investigations of oleoresin and its components. However, the actual function of resin components in plant defense and their mode of action is still uncertain, and the role of other defense compounds is relatively unexplored. We are studying the biochemical and molecular bases of chemical defenses, including terpenes, phenolics and chitinases, in Norway spruce (*Picea abies*) to learn more about how the accumulation of defense compounds is regulated, with the long-term goal of manipulating defense levels to test their function. Manipulation can be crudely accomplished by treatment with methyl jasmonate, which often mimics the general increases in defenses seen following herbivore or pathogen attack. Such treatment was shown to increase resistance to a fungal associate of bark beetles. To more conclusively test function, isolated genes of defense biosynthetic pathways are being transformed into Norway spruce to produce plants whose defense profiles are altered more precisely.

S2

Odor recognition and the features of naturally occurring odors.

G.A. Wright, Mathematical Biosciences Institute, Ohio State University, Columbus, OH 43210, USA

Animals use odor signalling for several functions that are important to their fitness, including: attracting mates, identifying kin, finding food, and avoiding predators. To recognize functionally important odors, the olfactory system must allow animals to separate such stimuli from unimportant ones. Naturally occurring odors may, however, be difficult to recognize since they are complex mixtures of odorants that vary from one experience to the next. Some statistical characteristics of odors, such as variability in concentration or correlations among odorant compounds, are likely to be features that animals use to identify stimuli. Using honeybees as a model system for studying the olfactory system, I will discuss both the types of features observed in naturally occurring odors and how the olfactory system of the honeybee uses these features to identify odor stimuli.

S3

Seeing the forest and not the trees: a global perspective on lignin and lignan biosynthesis for structural support and for defense functions.

N.G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA, USA

Heartwood-forming woody tissues are some of the most valuable yet recalcitrant forms of plant life being, for example, more resistant to degradation/decay. This presentation focuses upon the evolution and development of such heartwood-forming tissues, with particular emphasis being placed upon the lignin/lignan biochemical pathways. Comparisons, where appropriate, to the biochemical machinery also present in non-woody plant forms leading to these same pathway metabolic products, such as in *Arabidopsis*, are discussed. The applications of functional genomic, metabolomic, and proteomic approaches at the whole tissue and single cell level are summarized in order to dissect how and where such pathways are organized and to determine their metabolic significance. Determination of X-ray crystal structures of various proteins/enzymes involved in the lignin/lignan pathways are also discussed.

S4

Pheromone-binding proteins: insights into the mechanism of ligand recognition.

E. Plettner, N. S. Honson, Y. Gong
Department of Chemistry, Simon Fraser University, Burnaby, B. C. V5A 1S6 Canada

Pheromone-binding proteins (PBPs) are small, soluble proteins, members of the family of insect odorant-binding proteins (OBPs). PBPs are located in the lymph of sensory hairs involved in pheromone detection. PBPs appear to be involved in transport of the hydrophobic pheromone through the aqueous phase of the lymph, in buffering at high doses of pheromone and possibly in odorant recognition. Evidence from several research groups around the world indicates that the pheromones bind to PBPs, that PBPs differ in their ligand selectivity, but also that a PBP can bind a selection of ligands. The structures of several insect OBPs and PBPs have been determined, and some insights into the relationship of structure and function have been gained. We study the two pheromone-binding proteins from the gypsy moth, *Lymantria dispar*. These two proteins differ in their affinity for the enantiomers of disparlure (*cis* (7,8) epoxy-2-methyloctadecane). A paradox has been the apparently contradictory roles of PBPs as both ligand transporters and scavengers. We hypothesize that both functions can be carried out by the protein, depending on the dose of ligand: at low concentrations the protein is a transporter and at high concentrations, a transporter and a scavenger. We have studied the multimerization (which has a potential role in ligand buffering and scavenging), the pH profiles for various ligands (which give insight into ionizable residues that contribute to ligand binding) and the three disulfide bridges of these proteins (which are conserved, but whose role is not understood). We employ various structural and biochemical techniques to understand the ligand recognition by PBPs and the potential transport *vs*. scavenging function of PBPs.

S5

Biochemistry and functional genomics of herbivore-induced defenses in spruce and poplar.

Joerg Bohlmann, GI Arimura, J Fäldt, K Godard, D Huber, N Kolosova, DM Martin, B Miller, R Philippe, S Ralph, DK Ro, J Robert, Biotechnology Laboratory, University of British Columbia, 237-6174 University Boulevard, Vancouver V6T 1Z3, B.C., Canada, Email: bohlmann@interchange.ubc.ca

Scientists at the University of British Columbia, Vancouver Canada, are leading a 3.5-year forestry genome project (Treenomix, http://www.treenomix.com) funded by Genome Canada / Genome British Columbia (http://www.genomebc.ca) and by the Province of British Columbia. The project is developing and providing access to genomics resources for species of spruce, a conifer, and poplar, an angiosperm model for tree biology. Research of the Treenomix project targets two areas important for forestry on a national and international scale: Forest health and wood/fibre formation. In poplar and spruce, we have completed to date much of our original goal of sequencing 200,000 3'-ESTs from a diverse collection of standard, normalized, and full-length cDNA libraries. The poplar ESTs are used, together with those from other groups, by the International Poplar Genome Consortium (IPGC, http://www.ornl.gov/sci/ipgc) to support gene finding and genome annotation. We are sequencing a large number of full-length cDNAs to further support the IPGC's effort of ORF discovery in the poplar genome. End-sequencing and fingerprinting of 48,000 poplar BAC clones are providing a physical map resource that complements the DOE/JGI's shot-gun sequencing strategy for the polar genome. Within our mandate of forest health research, we have developed and applied spotted cDNA microarrays for studies of the spruce and poplar transcriptome in response to various insect pests and, in collaboration with other groups, transcriptome analysis of poplar pathogen response. Research of poplar defense to insects addresses direct and indirect defenses. Indirect defenses involve the systemic emission of phenolic and terpenoid volatiles for possible attraction of natural enemies of defoliating insects, forest tent caterpillars. Our research in spruce genomics complements and much extends an ongoing program on constitutive and induced terpenoid defenses in conifers.

Arimura G-i, Huber D, and J Bohlmann (2004) Forest tent caterpillars induce systemic and diurnal emissions of terpenoid volatiles in hybrid poplar: cDNA cloning, functional characterization, and patterns of gene expression of (-)-germacrene D synthase, PtdTPS1. *The Plant Journal* 37: 603-616.

Martin D, Fäldt J, and J Bohlmann (2004) Functional characterization of nine Norway spruce *TPS* genes and evolution of gymnosperm terpene synthases of the *TPS-d* subfamily. *Plant Physiology*, in press.

S6

How is plant odour information handled by the insect olfactory system?

Hanna Mustaparta, Norwegian University of Science and Technology, Department of Biology, Neuroscience Unit, Trondheim, Norway.

As shown by molecular biological studies, odorants are detected by a large and species-specific number of receptor neurone types in insects as well as in other animals. By the use of gas chromatography linked to single cell recordings, we have identified relevant plant odorants and studied the molecular receptive ranges of the receptor neurones (RNs) in species of beetles and moths. The RNs can be classified into many distinct types, each tuned to one or two primary odorants with weaker responses to a few chemically related, secondary odorants that are often found in the same pathway in the biosynthesis. The primary odorants might be minor components present in many plant species, hosts as well as non-hosts. Oligophagous and polyphagous species of related heliothine moths have RN types showing similar structure-activity relationships. In contrast, some RNs with the same primary odorant in distantly related species respond differently to the secondary odorants. Relevant odorants are included in studies concerning encoding and integration of plant odour information in antennal lobe interneurones. The behavioural significance of the odorants is studied in wind tunnel and the ability to learn and to discriminate between them is studied in conditioning experiments using the proboscis extension response.

S7

Interactions among conifer terpenoids and bark beetles across multiple levels of scale; an attempt to understand links between population patterns and physiological processes.

Ken Raffa, Department of Entomology, University of Wisconsin, U.S.A.

A major challenge confronting chemical ecologists involves transferring information from specific components of a plant – herbivore interaction, to population and landscape - level phenomena. Bark beetles- conifer interactions comprise a valuable model for addressing this issue, because host compounds are known to affect multiple components of their relationships. In particular, terpenoids play important roles in host acceptance, beetle aggregation, host defense, establishment of microbial symbionts, exposure to and avoidance of predators, and other functions. Moreover, some bark beetle species undergo dramatic population eruptions in which they convert from relatively stable to outbreak dynamics. These eruptions both play major roles in ecosystems processes, and pose significant economic and natural resource management challenges. A wealth of information has been developed for each individual component of bark beetle - fungal - conifer interactions. However, we have limited ability to scale across multiple layers of biological organization, which is essential for an integrated understanding of the system and for judicious management decisions. We propose that focusing on one group of compounds that plays an important role at each stage of colonization and whose effects are density –dependent, can provide an approach to achieving such integration. We also identify biological thresholds, whose outcomes are qualitative but whose determinant inputs are quantitative, as a major challenge to either the mechanistic or landscape approaches currently employed.

S8

High molecular weight plant polyphenols (tannins) — prospect in their function.

T. Yoshida
Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan

Remarkable progress on the chemistry of high molecular weight plant polyphenols referred to tannins during the last two decades has made possible to explore the physiological properties of individual polyphenols with defined structures. In our continuing study on the biologically active polyphenols from medicinal plants and beverages, we have found a wide range of the physiological activities of tannins including antioxidant, anti-tumor, anti-ulcer, anti-diabetes, immunomodulating and anti-bacterial activities as well as inhibitory effect on polygalactulonase related with defense from herbivores. Among them, interest in their potent antioxidant effect towards reactive oxygen species (ROS) is particularly growing in recent years because the life-style diseases such as cancer, diabetes, arteriosclerosis and cardiac infarction, are recognized to be associated with accumulated cellular damage by ROS. In this paper, new antioxidative polyphenols recently isolated from walnuts and cacao liquor, and also some selected examples on the development of new functions of tannins such as anti-bacterial effects against *Helicobacter pylori*, synergistic effects with beta-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA) and anti-tumor promoting effects, which have been found in our laboratory, are reviewed.

S9

Molecular biology and biochemistry of induced insect defense in poplar.

C. Peter Constabel

The genus *Populus* (poplars and aspens) is ecologically and economically significant in North America and worldwide, and also contains a diversity of phytochemicals. Salicin-based phenolic glycosides and flavonoids are typical phytochemicals found in poplar and aspen, and many of these are important for defense against herbivorous insects. Protection from herbivores in poplar is also mediated by an inducible defense response, and molecular work from a number of labs has identified a suite of genes upregulated by herbivore damage. These studies have provided a snapshot of inducible gene expression in poplar and new insights into defense mechanisms; it suggests that both defensive proteins and phytochemicals are components of this dynamic process. For example, multiple genes encoding protease inhibitors and chitinases are induced, together with enzymes of phenylpropanoid metabolism and condensed tannin biosynthesis. Genes encoding nutrient-related and other enzymes are also upregulated, which provides avenues for further research into the physiology of plant defense.
Recently, poplar has become a model for tree molecular biology and genomics, and is the first tree species to have its entire genome sequenced. Therefore, powerful molecular and genomics techniques can now be applied to major questions in poplar biology and tree physiology, which will contribute to a deeper understanding of herbivore defense and other ecological interactions.

S10

Insights into the remarkable metabolism of the bark beetle midgut.

Claus Tittiger, Biochemistry Department, University of Nevada, Reno, Reno, NV, USA 89557

As with all animals, the insect midgut is an important site of digestion, and is also subject to hormonal regulation. Adult bark beetle midguts are unusual in the animal Kingdom because they can produce monoterpenes de novo via the mevalonate pathway. The sometimes-high levels of monoterpene production must incur a significant metabolic load in addition to digestion for some beetles when they attack a host tree. In addition, the midgut is a likely detoxification site because the beetles ingest poisonous host resins while feeding. The combined roles of digestion, endocrine signal reception, and detoxification make the midgut a very busy tissue. Comparative expression profiling of different insects, including bark beetles, shows conservation of some classes of genes and quite divergent representations of others. Differences in expression profiles apparently correlate with differences in diet compositions and metabolic roles. Functional genomics can also aid identification of unknown genes involved in pheromone production and endocrine regulation.

S11

Tropical forests as sources of natural insecticides.

Murray B. Isman, Faculty of Agricultural Sciences, University of British Columbia, Vancouver, BC, Canada

Beginning in the 1980's, standardized seed kernel extracts from the Indian neem tree (*Azadirachta indica*, Meliaceae), based on the active principle azadirachtin, became the new paradigm for botanical insecticides. Further phytochemical exploration of the Meliaceae (mahogany family) led to the discovery of wood extracts from Malaysian *Azadirachta excelsa* and seed extracts from Kenyan *Melia volkensii* as sources of limonoids related to azadirachtin with potent bioactivity against insects. The genus *Trichilia*, with its center of diversity in Brazil, is also a potential source of botanical insecticides. Screening of *Trichilia* species native to Costa Rica led to the identification of *T. americana* as one possible source, based on its outstanding antifeedant effect against lepidopteran pests. The Annonaceae (custard apple family) is another large family of tropical and subtropical trees producing phytochemicals (acetogenins) with potentially useful insecticidal and anti-cancer activities. Extracts of seeds from the edible sweetsop tree, *Annona squamosa*, collected in Indonesia, are insecticidal to lepidopteran pests. Selected members of the African walnut family (Olacaceae) also have demonstrated bioactivity against pest insects. In this paper I will review the chemistry of these tropical tree species, compare their bioactivity against pest insects, and discuss their suitability for the development of botanical insecticides.

S12

Boreal forest ethnobotany: conservation and sustainability.

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Traditional and modern uses of boreal forest plant species are considered in the context of historical, ecological, economic, and socio-cultural issues surrounding the balance between conservation and sustainable development. Ethnobotanical research methods are described that promote socio-cultural sustainability by involving host communities from the start in project planning and the teaming of Aboriginal research assistants with university students. Interviews with the elders are conducted in their own languages and voucher specimens, artifacts, written and audiovisual records are prepared to fully document the research. Objectives of the research include cultural heritage documentation, health promotion through evaluation of traditional foods and medicines for their potential modern roles, research skill transfer, and identification of non-timber forest resources. Ecological sustainability is related to Good Agricultural and Collecting Practices. Economic sustainability involves local value-added processing and guidance on Good Manufacturing Practices. The new Natural Health Products Regulations enhance the sustainability of an ethnobotanically-derived natural health product industry by promoting consumer confidence in the products. This is accomplished through the requirement of product licences supported by evidence of the product's safety, efficacy, and quality, and the requirement of site licences for importers, manufacturers, packagers, and labellers supported by compliance to Good Manufacturing Practices.

Minisymposium 1

The Arthur Neish Minisymposium: (V. deLuca)

"Out of the Forest and into the diet: Wine Grape Genetic Diversity and secondary metabolism in Vitis species"

(This minisymposium was established in 1999 by the PSNA to honour the distinguished Canadian scientist Arthur Neish for his many contributions to polyphenolic chemistry. It is presented by promising young researchers.)

M1.1

Molecular genetic diversity of the French-American grapevine hybrids cultivated in North America

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French-American hybrid grapevines are most popular in eastern and mid-western North America: they are hardy cultivars derived from crosses between the European *Vitis vinifera* and North American wild vines. The aim of this study was to characterize their genetic background using 6 microsatellite (SSR) markers and a set of 33 diagnostic RAPD markers. . Estimates of genetic diversity derived from SSRs were generally higher and the average similarity between cultivars was generally lower than values reported for subgroups of *V. vinifera*, in accordance with expectations for hybrid cultivars. The probability of multiple occurrence for the DNA profile of each cultivar was much lower with multi-locus SSR data than with multi-fragment RAPD data. The phenetic relationships depicted by UPGMA (unweighted pair-group method with arithmetic averaging) and neighbor-joining analyses of microsatellite data were congruent and, to a large extent, in agreement with the known pedigree or history of each cultivar. A major dichotomy was observed between one group where the known genetic background was dominated by the North American *Vitis riparia* and *Vitis labrusca*, and another one where the genetic background was dominated by the European *V. vinifera*. Two Kulhmann varieties thought to be synonymous were found to be different, though closely related.

M1.2

'Foxy' methyl anthranilate potential of Concorde grape

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Methyl anthranilate is the characteristic volatile compound produced by *Vitis Labrusca* cv. Concord grapes and it is also found as a minor component in the floral scent of some flowers such as Jasmine and in certain citrus species. Synthetic methyl anthranilate is used in the food industry for the characteristic grape flavor, and it has also been widely used as a bird repellent for the protection of lawn and turf. However, little is known about how methyl anthranilte is synthesized in plants. In our study of methyl anthranilate biosynthesis in Washington Concord grapes, we found that unlike other carboxyl-methylated volatiles found in plants, such as methylbenzoate, methylsalicylate, methyljasmonate, methyl anthranilate is not synthesized by a carboxyl methyltransferase. Instead, its formation is catalyzed by an alcohol acyltransferase. An enzyme anthraniloyl-coenzymeA(CoA) : Methanol Acyltransferase (AMAT) which catalyzes the formation of methy anthranilte was purified from Concord grape berries and the cDNA encoding this protein was isolated. The cDNA encodes a protein of 449 amino acids whose sequence showed 58-60% identity with the benzoyl-coenzymeA(CoA):benzyl alcohol benzoyl transferase (BEBT) from Clarkia and Tobacco. Like other members of this family, AMAT can also use a broad range of acyl –CoA and alcohols as substrates. During grape berry development, AMAT gene transcripts started to appear at veraison stage, increased to maximal levels over the following two weeks, and transcript levels remained relatively stable with a slight decline over the rest of berry development. AMAT protein levels also followed the transcript pattern and these were consistent with the accumulation of anthranilic acid and methyl anthranilate in the grape juice during the berry development. In contrast, the berries of Vitis vinifera, that do not accumulate methyl anthraniliate, had neither AMAT protein nor AMAT enzyme activity.

M1.3

Vitis vinifera terpenoid synthase genes and wine grape metabolite profiling

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Monoterpenes and sesquiterpenes contribute flavor and aroma qualities to fruits of many species, including wine grapes. In an effort to understand the formation of terpenoids in grapevine, *Vitis vinifera* L., a commercial full-length EST database created from the Gewürtztraminer cultivar was screened *in silico* for putative terpene synthase (TPS) genes. We isolated and functionally characterized four TPS cDNAs, the first to our knowledge in any grapevine species. These TPS cDNAs encode two identical monoterpenol synthases (VvTer) that produce primarily (-)-α-terpineol and two sesqui-TPS, (+)-valencene synthase (VvVal) and (-)-germacrene D synthase (VvGerD). Based upon amino-acid sequence alignments, we determined that these *V. vinifera* TPS genes are phylogenetically similar to other angiosperm mono-TPS and sesqui-TPS genes. RNA blot analyses were used to determine that all three TPS transcripts tested were detectable in floral buds, however, only the transcripts of VvVal were present late in the development of ripening berries. Additionally, VvGer transcripts were present in vegetative tissues and in flowers at fruit onset. A future direction of our research in *V. vinifera*, will involve metabolite profiling of a number of grape constituents throughout berry development in the Cabernet Sauvignon cultivar, including flavonoids, terpenoids, norisoprenoids, esters, sugars and organic acids in conjunction with gene and protein expression profiling in order to globally characterize the genomic basis for aroma and flavor production in wine grapes grown in different vineyard microclimates.

M1.4

Red red wine - enhancement of berry colour and quality in Niagara wine grapes

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The microclimate of the Niagara wine region of Ontario produces excellent grapes and award- winning wines, however, this region is at the northern fringe of adaptability for *Vitis vinifera* and in some growing seasons many varieties do not achieve optimal berry quality before the impending winter forces harvest. Grape berry quality is a result of the genetics of the vine and the environment in which it is grown. Approaches involving both of these factors have been initiated in our lab with the aim of improving berry quality of Ontario-grown grapes, particularly colour and stilbene levels. Reflective mulch has the potential to improve berry quality through its effect on the microclimate within the canopy and in particular light penetration into the fruiting zone. We conducted a multi-varietal experiment in 2003 to explore the effect of reflective mulch on berry quality and the expression of genes involved in flavonoid and stilbene biosynthesis. Analysis of this experiment is still in progress, however we have determined that mulch advanced the onset of ripening and had a positive effect on several quality characteristics including the development and harvest levels of anthocyanins, flavonols, and stilbenes. This indicates that there is potential for reflective mulch to improve berry quality in Niagara grown grapes, particularly in a cool, backward/late growing season. Crosses between a highly coloured teinturier variety and classic red and white *V. vinifera* wine varieties provide the material to study the genetics of colour in grapes. A target gene approach using partial DNA sequences of a number of Myb genes and specific alleles of genes involved in the anthocyanin biosynthetic pathway is being applied to these F1 populations. HPLC has been used for biochemical characterization of this material, however a capillary electrophoresis system to separate anthocyanins, flavonols and stilbenes is the subject of a current PhD project in our lab. Progress in this ongoing study will be presented.

M1.5

Yeast stress responses during icewine fermentation and their impact on icewine quality.

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Icewines contain elevated levels of acetic acid and glycerol due to the metabolic response of wine yeast fermenting this hyperosmotic media. The transcriptional response of genes encoding cytosolic aldehyde dehydrogenases (*ALD3* and *ALD6*) and glycerol-3-phosphate dehydrogenase (*GPD1*) in wine yeast during icewine fermentation was investigated to relate metabolites in icewine to expression patterns of these genes known to be upregulated in laboratory strains of *Saccharomyces cerevisiae* under hyperosmotic salt-induced stress. No difference in the *ALD6* expression pattern (NADP+-dependent aldehyde dehydrogenase) was observed in a commercial wine yeast during fermentation of icewine juice (38.8 Brix), diluted icewine juice (21.3 Brix) and the diluted juice with an addition of 100 g/L of glucose and 100 g/L of fructose to match the sugar in the icewine juice. In comparing peak gene expression levels, *ALD3* (NAD+-dependent aldehyde dehydrogenase) showed a 6.2-fold induction and *GPD1* (NADH-dependent glycerol 3-phosphate dehydrogenase) showed a 2.5 fold induction in the commercial yeast during icewine versus the diluted fermentation. Acetic acid production increased 7.1-fold and glycerol production increased 1.8-fold in the icewine fermentation over that in the diluted juice fermentation. *ALD3* was not glucose repressed when the additional sugar was added to the diluted juice, but was upregulated 7.0-fold. The additional sugar added to the diluted juice stimulated acetic acid and glycerol production but to a lesser extent to that found during icewine fermentation, indicating that solutes in icewine juice in addition to sugar contribute to the metabolic response evoked in the wine yeast.

Minisymposium 2

Hymenopteran semiochemical Minisymposium (C. Keeling and E. Plettner): honouring the career work of Keith N. Slessor in chemical ecology, particularly in honey bees, (sponsored by Simon Fraser University and the Canadian Association for Professional Apiculturalists.)

M2.1

Sociochemicals: the complicated pheromone arsenal of honey bees

Keith Slessor, Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6

The pheromone communication system of the honey bee appears to be a series of highly complex mixtures, resulting in separate behavioural or physiological actions being derived from subsets of the semiochemical blend. Synergistic interaction of components is a dominant theme and primer components promoting physiological processes require releaser components for attraction and distribution of the bioactive mixture. An overview of queen, worker and brood pheromone actions will demonstrate the involved nature of this complex communication system.

M2.2

Pheromone regulation of honey bee foraging ontogeny

Tanya Pankiw, Department of Entomology, Faculty of Neuroscience, Texas A&M University, College Station, TX 77843-2475, USA

Pheromones are communication chemicals used among members of a species. The evolution of sociality has configured communication chemicals, called primer pheromones that play key roles in mediating the organization of social insect life. Primer pheromones exert relatively slow effects that fundamentally alter developmental, reproductive, physiological, and neural systems. Only recently has it been directly demonstrated that adult worker honey bees, the most populous members of a colony, produce pheromones that regulate a behavioral development process, the age of foraging onset. In general, when workers are in about their third week of adult life, they cease performing tasks within the nest and begin foraging outside the nest. Compounds extractable from the surface of foraging and young pre-foraging workers were added to colonies and age of foraging onset was measured in known aged cohorts of bees. Forager extracts increased age at foraging onset versus controls, whereas extracts of decreased foraging age. This represents the first known direct demonstration of adult worker derived primer pheromone activity in the honey bee (*Apis mellifera*). The honey bee is a model organism for the study of social insect behavior and of enormous economic importance to agriculture. A more complete identification of the chemical components that influence behavior development and the individuals from which the components are derived are fundamental in advances our understanding of the functions that primer pheromones play in social insect life.

M2.3

Pheromone regulation of division of labor in honey bee colonies: from behavior to genes

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Honey bees (*Apis mellifera*) show complex social organization that is controlled to a large extent by pheromones. It is well known that pheromones are involved in the regulation of the basic reproductive division of labor between queens and workers; pheromones are also increasingly being implicated in the regulation of division of labor among workers for activities related to colony growth and development. In this talk I will review our understanding of pheromone regulation of worker honey bee behavioral maturation, with a particular focus on studies of the age at onset of foraging and associated changes in gene expression in the brain, as revealed by microarray analysis.

M2.4

Multiple functions of the fire ant, *Solenopsis invicta*, Alarm Pheromone

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As with most social insects, the red imported fire ant, *Solenopsis invicta*, utilizes complex chemical signals to regulate the activities of the colony. Several of these pheromones, including the trail pheromone and queen recognition pheromones, have been identified. However, the isolation and identification of the alarm pheromone has proven to be more elusive. Laboratory bioassays suggest *S. invicta's* alarm pheromone performs several functions, from signaling the presence of a threat, to inducing worker activity during mating flights, and attracting eavesdropping parasitoids. Ant alarm pheromones have been identified from several glandular sources, e.g. Dufour's, mandibular, and anal glands. Behavioral studies point to the mandibular gland as the source *S. invicta's* alarm pheromone. In *S. invicta*, the mandibular gland consists of only a few cells. This along with the ephemeral nature of the active components has complicated the identification of this pheromone. We employed solid phase micro extraction (SPME) and purge and trap techniques to collect and analyze headspace contents above workers exhibiting alarmed behavior. We identified a tri-substituted pyrazine as a component of the fire ant alarm pheromone. The lowest significant alarm response to the pyrazine was at a headspace concentration of 0.03 pg/μL. This pyrazine has now been isolated from the mandibular glands of workers, as well as female and male sexuals, who use the alarm pheromone to initiate mating flights. Additionally, the alarm pheromone serves as a kairomone – attracting phorid fly parasites.

M2.5

Sex and bondage: Queen sex pheromone of the slave-making ant *Polyergus breviceps*

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Slave-making ants are obligate social parasites that depend entirely on workers of slave species to carry out the tasks that enable a colony to survive. One such species is *Polyergus breviceps*, which inhabits the western United States where it exclusively parasitizes ants in the genus *Formica*. Slave raids are conducted in early summer around the onset of summer rains, with raiding columns and winged males emerging from nests in mid- to late afternoon. Virgin queens accompany workers in the raiding columns, and during these raids, a queen releases a pheromone that immediately attracts swarms of flying males, one of which quickly mates with her. The newly mated *Polyergus* queen invades and takes over a colony of a *Formica* species, killing the resident *Formica* queen. The subjugated *Formica* workers then rear her brood as their own. We will describe the identification, synthesis, and field testing of the two-component queen-produced sex pheromone of this species. Remarkably, this is appears to be only the second queen sex pheromone to be fully identified from ants, from the more than 10,000 described ant species.

Minisymposium 3

Marine Chemical Ecology: (Shawna MacKinnon)

M3.1

The rapid bioactivation of secondary metabolites as wound response of *Caulerpa taxifolia*

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Caulerpa taxifolia is an invasive tropical green alga that spreads rapidly after its introduction into the Mediterranean thereby causing massive alterations in the natural ecosystem. This alga has recently also been detected at the American Pacific coast and is now subject of intensive eradication efforts. The siphonous *C. taxifolia* consists of one single giant cell with numerous nuclei. This assembly requires an efficient wound response to protect the cell against abiotic environmental influences and herbivores. We investigated the wound response of *C. taxifolia* and other caulerpales with focus on the fate of secondary metabolites. A rapid transformation of the major terpenoid metabolite caulerpenyne was observed after wounding of the cell. We could show that the mechanical disruption of the compartimentation of *C. taxifolia* leads to the activation of an esterase, which attacks caulerpenyne. This metabolite is then transformed in a multi step sequence within minutes resulting in a reactive 1,4-dialdehyde. Interestingly this dialdehyde has been previously been reported from specialized mollusks feeding on Caulerpa spp. The mechanism and the function in wound healing of this transformation is presented.

M3.2

Brown algal phlorotannins – secondary compounds of primary importance

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Phlorotannins (polyphenolics) have been suggested to have multiple primary and secondary functions in brown seaweeds. The concentration of phlorotannins can vary on a wide rage of spatial and temporal scales, indicating that both genetic and environmental factors can influence their production. Phenotypic and genetic correlations show negative trade-offs between phlorotannin concentration and growth, suggesting that phlorotannins are costly for the seaweeds to produce. We have focused primarily on the protective and defensive properties of phlorotannins and how the concentration of these compounds can be induced by different external factors. Our results show that the production of phlorotannins can be induced by several abiotic and biotic external factors, and that there is a genetic variation both in the constitutive and inducible production of phlorotannins. Furthermore, phlorotannins can absorb UV-B radiation and deter herbivores and fouling organisms, showing that they have multiple secondary functions in brown seaweeds. From the results of our work and work done by others on the primary functions of phlorotannins, we propose a model for how phlorotannins can function both as primary and secondary metabolites on different scales within a seaweed plant.

M3.3

Chemical defense of marine animals: effects on color, mobility, overtness, and local and geographic patterns of distribution

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Many studies address how sessile marine prey growing on hard substrates (e.g., sponges, seaweeds) use chemical defenses to deter consumers. Mobile and more behaviorally complex species that may integrate escape and refuging behavior with chemical deterrents are less investigated. To correct this imbalance, we investigated palatability and defenses of marine worms from the temperate through tropical Atlantic and Caribbean. Of 81 species surveyed, 30 were unpalatable, with many of these being chemically defended. Most unpalatable worms were brightly colored, sedentary, exposed to epibenthic predators, lived on more structured habitats, and occurred in tropical latitudes. Most palatable species were drab, mobile, sheltered from epibenthic predators, inhabited unconsolidated sediments, and were from more temperate latitudes. For worms with morphologically distinct body parts, exposed parts were often unpalatable, while sheltered parts were usually palatable. Defended species were less constrained by consumers and foraged on resource-rich surfaces, even when consumers were common. Palatable species were more constrained by consumers and fed more on resource-poor, sub-surface sediments where they were less exposed. Chemical defenses interact with behavioral and physical traits of these mobile species to have a large impact on the basic ecology and evolution of the group.

M3.4

The role of red tide toxins (and other metabolites) in competitive and predator-prey interactions

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Some phytoplankton form virtually monospecific blooms on a seasonal basis, disrupting marine community structure and threatening human health and local economies. Why these red tide species produce deadly neurotoxins is still unknown. In lab experiments, we tested whether the outcome of competition between the Florida red tide dinoflagellate, *Karenia brevis*, and 12 co-occurring marine phytoplankters could be explained by allelopathic effects of compounds released by *K. brevis*, and whether brevetoxins are involved. Most competitors were suppressed by *K. brevis*; about half of these cases appeared to be caused by allelopathy, and brevetoxins accounted for the allelopathic effects against only one competitor – suggesting the involvement of other *K. brevis* compounds. We also used feeding experiments to ask how *K. brevis* escapes predation by a common phytoplankton grazer. We found that the negative fitness effects of consuming *K. brevis* are mostly caused by its nutritional inadequacy, rather than acute or chronic toxicity, or deterrence. Overall, chemically-mediated interactions appear to be of importance in the plankton, although most chemical cues and their ecological consequences still await discovery.

M3.5

Studies of the toxigenic dinoflagellate *Alexandrium ostenfeldii* and its production of a novel class of marine toxins known as the spirolides.

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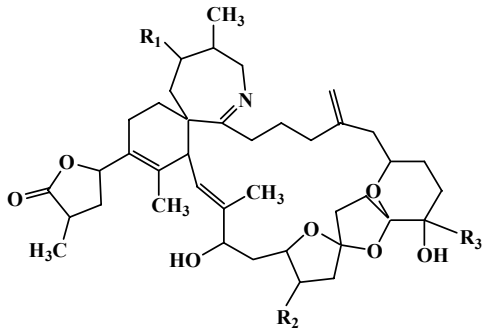
The macrocyclic imines known as spirolides were first identified in extracts of the digestive glands of mussels and scallops from the Atlantic coast of Nova Scotia, Canada in the early 1990s. The source of this class of toxins was later identified to be the marine dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen. To date our marine toxin group has isolated and structurally characterized the structures of over nine pirolides from shellfish extracts and cultured dinoflagellate isolates obtained from Nova Scotia, Denmark, and Norway. While this structural class of marine toxins is known to elicit a "fast acting response" in the mouse assay, very little is known about the importance of these compounds to the dinoflagellate. Earlier work with *A. ostenfeldii* indicates that spirolides may play a role in predator defense against the ciliate *Favella ehrenbergi*. *A. ostenfeldii* blooms have not to date been linked to fish kills at aquaculture sites.



Minisymposium 4

Minisymposium on Chemically Mediated Behavior in Wildlife: Examination and Application (Bruce Kimball and Dale Nolte)

M4.1

Has browsing by mammals caused continent-scale variation in the chemical defenses of woody plants?

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In the North American taiga/boreal forest complex there is a continental pattern of wildfire. We propose that this wildfire pattern correlates directly with biogeographic variation in snowshoe hare abundance. As a result, selection for anti-browsing defense is greatest where fire has burned the greatest area over evolutionary time. To test this hypothesis we examined biogeographic variation in the defenses of Alaska paper birch and the canoe birch. We obtained the coordinates of 46 years (1956 - 2002) of lightning-caused wildfire from the Alaska Fire Service and the Canadian Forest Service. These coordinates were overlaid with coordinates of 1353 Alaska paper birch collections and 725 canoe birch collections. A spatial t-test indicated a greater ($P < 0.01$) wildfire frequency (mean □ SE) in 1-degree latitude x longitude cells containing Alaska paper birch (25.4 □ 1.6) than cells containing the more palatable canoe birch (4.9 □ 0.5). Thus, the most chemically defended birch (Alaska paper birch) is the most closely associated with wildfire. We then examined the chemical defenses of 18 Alaska paper birch populations from Alaska and northwestern Canada by counting resin glands on CAG internodes of seedlings grown in a common garden, and by assaying resin and papyriferic acid in CAG internodes of saplings growing in nature. These measures of defense also yielded significant positive correlations with fire (gland density $r = 0.73$, $P < 0.01$; resin content $r = 0.88$, $P < 0.0005$; papyriferic acid content ($r = 0.87$, $P < 0.001$). These data sets provide definitive evidence that selection by an herbivore (snowshoe hare) has resulted in a continental scale biogeographic pattern in antiherbivore defense.

M4.2

Linking herbivore experience, varied diets, and plant biochemical diversity.

F.D. Provenza, Department of Forest, Range, and Wildlife Sciences, Utah State University, Logan, UT, USA.

Diets and habitats that allow animals to select among alternatives enable individuals to better meet needs for nutrients and to better cope with toxins. All plants contain toxins, and the amount of toxin an animal can ingest depends on the kinds and amounts of nutrients and toxins in the forages on offer. Nutrients and toxins both cause animals to satiate, and excesses of nutrients, nutrient imbalances, and toxins all limit food intake. Thus, individuals can better meet their needs for nutrients and regulate their intake of toxins when offered a variety of foods that differ in nutrients and toxins than when constrained to a single food, even if the food is "nutritionally balanced." Food intake and preference also depend on differences in how individual animals are built morphologically and how they function physiologically, and marked variation is common even among closely related animals in needs for nutrients and abilities to cope with toxins. Transient food aversions compound the inefficiency of single-food diets by depressing intake among individual animals, even if they are suited "on average" to that nutrient or toxin profile. Finally, experiences play a crucial role in an animal's propensity to learn to eat different foods. When herbivores are allowed to eat only the most preferred plants, they are not likely to learn to mix foods high in nutrients with foods that contain toxins. Conversely, herbivores encouraged to eat all plants in an area are more likely to learn to eat mixes of plants that mitigate toxicity. Experienced animals that have learned to eat a variety of foods that differ in nutrients and toxins do so even when nutritious alternatives are available, whereas naive animals familiar only with the nutritious alternatives eat only that subset of familiar foods.

M4.3

Weasels, voles, and crop protection: whither the future?

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The use of mustelid semiochemicals to disrupt populations of voles (*Microtus* spp.) has been suggested as a means of reducing feeding damage to coniferous tree seedlings planted on cutover forestland, and to agricultural crops. Several experiments have been conducted in forest and agricultural study areas near Summerland, British Columbia, Canada. Two synthetic compounds (2-propylthietane and 3-propyl-1,2-dithiolane) from the anal-gland of the short-tailed weasel (*Mustela erminea*) seemed to produce an avoidance response in montane voles (*M. montanus*) and reduced feeding damage to apple trees in small-scale (< 1 ha) field bioassays. A replicated (n=4) field experiment with high populations (50-200 voles/ha) of montane and meadow (*M. pennsylvanicus*) voles exposed to these same weasel compounds over a 1-year period resulted in little change in numbers of voles. Survival of voles was lower in treatment areas, and those trees near the odour survived better than those further away. However, tree mortality was still unacceptably high. Another replicated (n=4) field experiment measured the population responses of meadow and long-tailed (*M. longicaudus*) voles to these same odours dispersed over 9-ha treatment areas of recently planted forest land during a fall-winter period of high vole numbers. There were few changes in numbers or survival of voles on control and treatment areas. The larger scale of this experiment was designed to attract weasels, and perhaps other mammalian carnivores, to the treatment areas. However, very few mustelids were captured on any of the areas. Despite the increase in scale of treatment with these mustelid semiochemicals, it appears that high (> 50/ha) populations of voles are relatively unaffected by the odour or the presumed attraction of predators to treatment sites. A more prudent approach would be to test this method during years of low vole numbers to try to prevent or reduce the buildup of peak populations of voles and consequent damage to crops.

M4.4

Chemical ecology and managing forest resources.

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Forest ecosystems include multiple wildlife species. Landowners recognize these species as integral parts of the natural ecology, and appreciate the many desirable recreational and aesthetic opportunities proffered by wildlife. Unfortunately, herbivores can impede efforts to establish trees on timber sites after a fire or harvest. For example, planting seedlings in areas with high mountain beaver population is futile. Ungulate browsing on young seedlings is widespread across forest plantations. Older saplings are vulnerable to girdling by bears. Invariably, damage is inflicted by foraging animals. Thus, outside the adage of "dead animals do not eat", the potential for developing new management strategies requires a better understanding of their foraging ecology. We investigated how plant phytochemistry impacts diet selection for several pertinent species. These studies suggest a promising role for using chemical ecology to protect forest resources. Understanding herbivore responses to phytochemistry enables us to predict which trees are likely to be eaten, while understanding phytochemical responses to silvicultural and genetic manipulation provides promising avenues to develop protective strategies.

M4.5

That's disgusting: deer responses to seedlings treated with proteins.

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Contact herbivore repellents have historically been classified as irritants, taste modifiers, flavor aversion agents, or fear inducers. Irritants cause pain by acting on the trigeminal system. Taste modifiers and flavor aversion agents alter the palatability of the food. Sulfur containing volatiles released from animal-based repellents (e.g. egg, blood, urine) are thought to invoke a "fear response" through their chemosensory association with predators. However, we propose that avoidance of animal-based repellents is mediated by altered palatability, rather than fear. To test this, we offered western redcedar (*Thuja plicata*) seedlings treated topically with a variety of proteins to captive deer (*Odocoileus hemionus*) and monitored browsing for three weeks. In each pen, twelve seedlings of each treatment were planted in 4 x 3 grids with 1 m tree spacings. Treatment plots were separated by a minimum of 3 m and the design was replicated in eight pens, each containing three deer. Our results suggest that the protein fraction of animal-based repellents deters herbivory by altering palatability of the treated food and that avoidance is correlated with methionine content of the protein. We conclude that avoidance of materials that do not produce negative postingestive effects or pain is more consistent with the concept of "disgust" rather than "fear".

Minisymposium 5

Pheromone Minisymposium (J. McNeill)

M5.1

Aggressive chemical mimicry by a bolas spider

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Late instar and adult female bolas spiders capture male moths by ensnaring them with a modified web that consists of a sticky ball hanging at the end of a thread. These moths do not simply blunder into the few centimeter range of a swung bolas; rather they are attracted by an allomone that mimics the sex pheromone of the moth species. Adult female *Mastophora hutchinsoni* are known to capture four species of moths. In one case, we found this bolas spider produces a blend of (*Z*)-9-tetradecenyl acetate and (*Z,E*)-9,12-tetradecadienyl acetate that is similar to the blend produced by the adult females of *Lacinipolia renigera*. The other documented prey species are not attracted to these compounds, suggesting the spider may produce other pheromone mimics to attract these species. In fact, the pheromone blend of *L. renigera* interferes with attraction of males of another prey species, *Tetanolita mynesalis,* to its own pheromone blend. Aspects of the hunting behavior of the spider are influenced by the wing vibrations of moths. Spiders that have already produced a bolas will swing it toward a small speaker broadcasting recorded wing vibrations. Furthermore, spiders that have not started to hunt will produce a bolas in response to wing vibrations. It is apparent that this spider has several interacting adaptations that make this unusual method of prey capture more effective and efficient.

M5.2

The role of pheromones and kairomones in mate location of the salmon louse *Lepeophtheirus salmonis* (Crustacea; Caligidae)

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Lepeophtheirus salmonis is a major pathogen of farmed salmon causing £20 million damage annually in treatments and losses. It has a10-stage life cycle comprising three planktonic stages and seven stages associated with its salmonid host. There are two pre-adult mobile stages before the highly mobile adult stage all of which move between hosts for feeding and mate location. Host attraction of the mobile stages results in settlement at preferred sites on the fish with females dorsal and anterior to males. Adult males find the females and take up a pre-copulatory position predominantly with pre-adult II females. Copulation occurs between the adult male and recently moulted adult female. Mate recognition occurs by short and long range chemical cues males showing specific behaviours in the presence of pre-adult II and adult virgin females. The role of olfaction and diffusible pheromones in mate location have been assessed with Y-tube behavioural bioassays. Adult male sea lice display activation and directional responses to seawater conditioned with pre-adult II females and also to solid phase extraction (SPE) extracts of the conditioned water. Distillation under vacuum produced volatile semiochemicals that were attractive to males. Our research provides evidence that small lipophilic organic molecules are used by sea lice as sex pheromones to locate a member of the opposite sex.

M5.3

Can we combine pheromone-based attracticide formulations for more than one species? A case study using the oriental fruit moth and the codling moth (Lepidoptera: Tortricidae).

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A strength and potential shortcoming of pheromone-based control tactics is their specificity to only one or a few target insect pests. Here we test the impact of combining attracticide formulations for the Oriental fruit moth and the codling moth (Lepidoptera: Tortricidae) on the attraction of both species in the field. In addition, we examine upwind flight behaviour and toxicity of the formulations to male Oriental fruit moths in a wind tunnel.
Capture of codling moth males in traps baited with the mixed formulation was reduced, while capture of oriental fruit moth males was increased in comparison to traps baited with each species' pheromone alone. Mixed formulations increased source contact rates by male oriental fruit moths in a wind tunnel. Oriental fruit moth males remained on mixed formulations longer than on formulations containing only its conspecific pheromone and this was correlated with increased toxicity of the mixed formulation. A combined attracticide formulation will have different effects on each of the targeted species. Due to decreased attractiveness, a mixed formulation may be less effective against the codling moth. However, due to increased attractiveness and toxicity, a mixed formulation may be more effective against the oriental fruit moth. Our results have also led to inferences about synergism of upwind flight response to pheromone in the oriental fruit moth and the potential for interspecific chemical communication between these two species.

M5.4

Pheromone stereochemistry and sexual mimicry: A closer look at the sex life of cockroaches

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The German cockroach male exhibits a complex courtship behavior upon contact with a sexually mature female. The behavior, which includes raising of the wings, can be elicited by contact of the male's antennae with an isolated female antenna or with a male antenna treated with 3,11-dimethylnonacosan-2-one, the main component of the female contact sex pheromone. This compound has four stereoisomers, but their relative effectiveness at releasing courtship in males has not been delineated through dose-response studies. The four stereoisomers of the C29- methyl ketone were synthesized and behaviorally assayed, and we show here that (3*S*,11*S*), the natural isomer, is surprisingly, the least effective of the four stereoisomers. This is the first evidence of an unnatural stereoisomer being a better releaser of behavior than the natural isomer. It suggests that geographically separated populations of the German cockroach might have diverged in their use of pheromonal stereoisomers.

The German cockroach male exhibits sexual behavior also in response to newly emerged females, males and nymphs. However, unlike males, who lose their ability to stimulate courtship behavior in mature males within several days after the adult molt, nymphs retain this capacity throughout the last instar. We are using chromatographic approaches to isolate and identify this pheromone. Thus far, this pheromone appears to be different from the female pheromone. The adaptive significance of this communication system in nymphs and young adults will be discussed.

M5.5

Pheromones for management of gall midges

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The swede midge, *Contarinia nasturtii* (Diptera: Cecidomyiidae), occurs in many brassica growing regions in Europe where it often causes serious yield losses in broccoli, cauliflower and Brussels sprouts. Relatively recently the occurrence of *C. nasturtii* was reported in Canada, where it is now a quarantine pest. One reason for the high damage levels caused by *C. nasturtii* is the lack of a good monitoring method. Growers therefore tend to use scheduled insecticide sprayings throughout the entire crop growing period. Because of inadequate timing the treatments are frequently inefficient. Furthermore due to the scheduled sprayings against *C. nasturtii*, supervised control of other brassica pests cannot be considered an option.
With the purpose of developing an efficient pheromone-based monitoring method, we have identified the sex pheromone of *C. nasturtii*. Electrophysiological data and results from behavioral tests in the wind tunnel, comparing the attractancy to *C. nasturtii* males of female extract and different synthetic pheromone blends, will be presented. In addition, the most attractive synthetic blend was tested in a field trapping experiment with released laboratory reared midges. The effects of dispenser type and trap positioning will be discussed.

M5.6

Reproductive isolation and host plant specialization in two *Ostrinia* pherotypes in France.

B. Frerot,

M5.7

The role of semiochemicals in the pollination of sexually deceptive orchids

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Orchids of the genus *Ophrys* are pollinated by means of sexual deception, mostly by male bees and wasps. Lured by the odor and insect-like shape of the flower, the pollinator alights on the flower and attempts to copulate with it. In several allopatric and sympatric *Ophrys* species, pollinated by either *Andrena nigroaenea or A. flavipes,* we compared male attracting odor compounds of female bees and *Ophrys* flowers. We performed behavioral tests, quantitative chemical analyses and electroantennogram assays. The aim was to get information about species- and individual-specific recognition signals.
We could show that *Ophrys* flowers and females of their pollinators show a great agreement in behavior releasing chemical substances on all examined *Ophrys*- pollinator relationships, i.e. pollinator females and orchids attract the males with identical compounds. We found 51 GC-EAD active compounds, mainly saturated and unsaturated hydrocarbons with a chain length of 21 to 29, aldehydes, esters and acids. We compared the investigated species based on the relative proportions of all GC-EAD active compounds. Our results show that *Ophrys* species with the same pollinator - independent of their phylogenetic relationship - use the same odor compounds in the same compound composition for pollinator attraction. Differences between the species mainly involve different odor bouquets that are responsible for the specific attraction of pollinators. In congruence with previous results we found that alkanes and alkenes are most important for pollinator attraction.
This study was financially supported by the FWF Austria

Contributed papers C

C1.1

Micropropagation and genomics investigations of ginger and turmeric

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Members of the Zingiberaceae, in particular ginger (*Zingiber officinale* Rosc.) and turmeric (*Curcuma longa* L.), accumulate important pharmacologically active metabolites at high levels in their rhizomes. These plants are excellent model species to study rhizome specialized metabolism and biology. Investigations into the biosynthesis and function of these compounds in the plants are currently limited by the ability to reproduce valuable stock lines. Because of this, we have developed an *in vitro* micro-propagation method, which is a straight-forward and highly effective means of producing new plants in large numbers from a single bud from an original rhizome. An average of 10.5 shoots per bud was obtained after three to four weeks of culture. A high amplification rate of well-developed plantlets (10.5 shoots per bud) with a 7.5 cm shoot length and a 8.5 cm root length was also obtained. The amplification rate did not decrease in continuous subcultures on the same medium. We have also produced 8 EST databases (comprising over 48,000 ESTs) from root, rhizome and leaf tissue from red turmeric and from white ginger and yellow ginger. These databases indicate that the phenylpropanoid and terpenoid pathways are highly expressed in the aromatic portions of these plants, and have provided candidate genes for important enzymes in pathways leading to the production of such metabolites. We are performing metabolic profiling and DNA microarray-based expression profiling of the propagated plants to further characterize the mechanisms responsible for regulation of these pathways in these plants.

C1.2

Identification and Characterization of an Acyltransferase Capable of Producing the Green Leaf Volatile, Z-3-hexen-1-yl acetate in *A. thaliana*

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Green leaf volatiles (GLV) are common constituents found in mechanically and herbivore-damaged plants. These products of the lipoxygenase pathway are thought to be important for the prevention of the spread of bacteria and fungi in addition to serving as cues for
herbivores and their predators and parasitoids. More recently, GLVs have been shown to act as plant signaling compounds that can induce several important plant defense pathways. We have identified a member of the BAHD acyltransferase gene family in *Arabidopsis thaliana* that specifies the ability to catalyze the formation of the green leaf volatile ester Z-3-hexen-1-yl acetate. The enzyme it encodes, acetyl-CoA:cis-3-hexen-1-ol acetyl transferase (CHAT), has been purified from transgenic plants expressing the CHAT gene under the 35S promoter as well as from *E. coli* heterologously expressing the protein. Kinetic data show that both the plant-purified and *E. coli*-produced CHAT enzymes have a high affinity for acetyl Coenzyme A as well as the ability to accept several medium-length chain aliphatic and benzyl-derived alcohols. CHAT transcripts are detectable in an ecotype-specific manner with *CHAT* transcript present in flowers of undamaged plants. Upon damage by mechanical wounding, CHAT gene transcript levels increase in a time-dependent manner with the highest levels detected between 3 and 6 hours after wounding. CHAT enzyme activity and protein levels correlate with transcript levels, with CHAT enzyme activity levels peaking between 10 and 12 hours after damage. Transgenic plants expressing CHAT under the constitutive 35S promoter have an altered volatile profile in the floral headspace of undamaged plants. In addition, the volatiles produced after mechanical damage to vegetative parts show an enrichment of Z-3-hexen-1-yl acetate.

C1.3

Characterization of prenyltransferases from Norway spruce – critical branch-point enzymes in terpene resin biosynthesis

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Conifers are susceptible to attack by herbivores and pathogens such as the bark beetle *Ips typo-graphus* and associated fungi. As a result of such infection, an increase in stem oleoresin can be observed, involving the differentiation of new resin ducts. The main components of stem oleoresin are terpenes; monoterpene (C10) and sesquiterpene (C15) hydrocarbons and diterpene (C20) carboxylic acids. To understand how metabolic flux is channeled among these terpene types, we are investigating prenyltransferases (PT) the enzymes that catalyze the assembly of C5 precursors into C10, C15 or C20 intermediates, in *Picea abies* (Norway Spruce). To investigate the induction of PTs, we used methyl jasmonate (MJ) for mimicking the responses of *P. abies* to herbivore/pathogen attack (Martin et al., *Plant Physiol*. 129, 2002). Two cDNA-libraries were constructed from bark and wood of MJ-treated spruce saplings. By screening these cDNA-libraries with an homology based PCR approach, cDNAs encoding a farnesyl diphosphate synthase and a geranylgeranyl diphosphate synthase were isolated, expressed in *E. coli* and characterized. In addition, a cDNA encoding a PT, showing both geranyl diphosphate and geranylgeranyl diphosphate synthase activity was expressed and characterized. The substrate specificity of this unusual PT was investigated by attempting to alter the distribution of enzyme products by side-directed mutagenesis. The expression of the isolated PT genes in 40-year-old MJ-treated trees was studied by semi-quantitative RT-PCR.

C1.4

Very-long-chain polyketides in the cuticular wax of *Taxus* needles

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All primary, above-ground plant surfaces are covered by a cuticle, consisting of cutin and cuticular waxes. These mixtures of homologous mono-functional aliphatics are formed by elongation of fatty acyl-CoA precursors and successive modification of the functional group. Surface waxes perform multiple functions in protecting the plant against the biotic and abiotic environment. Several novel compounds were isolated from the needle wax of yew trees, *Taxus baccata* and *T. brevifolia*, and investigated by GC-MS. Diverse homologues of □-lactones, 5-hydroxyaldehydes, and alkanediols were identified. These bi-functional compounds were accompanied by 2- and 10-alcohols as well as 2-ketones. We hypothesize a polyketide elongation pathway leading to these components, in contrast to previous models that assumed hydroxylation steps in the pathway. The coverages of all constituents on yew needles were quantified (μg/cm²). Besides, the localization of individual compounds in the epi- and intracuticular wax layers of both needle surfaces was investigated. Secondary alcohols were found to accumulate in microscopic crystals on the adaxial surface, while the abaxial surface showed high concentrations of diols.

C2.1

Chemical basis for aggression behaviour in the invasive ant, *Lasius neglectus* and relation with *L. turcicus*.

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Many ants are territorial and vigorously defend their nest against non-nestmates of their own species. Ant populations are thus structured into distinct, mutually aggressive colonies. Invasive ants, however, have completely lost this intra-specific aggression, leading to a network of hundreds or thousands of cooperating nests in a single population. To test whether the observed loss of territorial aggression in invasive ants is due to a reduction of the information content of recognition cues on the ants cuticles, we compared cuticular hydrocarbon profiles of the invasive garden ant, *L. neglectus*, in its native range (where aggression is present) and its introduced range (where aggression is absent). GC/MS revealed a clear separation of both groups. Only little to none n-alkanes were present in both groups, but a broad spectrum of compounds that are expected to have a high information content for the ants such as alkenes, alkadienes, methyl branched alkanes and interestingly a fairly large amount of methyl branched alkenes. Most compounds are large hydrocarbons (C34-C36).
The potential use of cuticular hydrocarbon patterns to differentiate *L. neglectus* from its sister species *L. turcicus* will be discussed.

C2.2

Hexyl decanoate, a trail pheromone component identified from labial gland secretions of a stingless bee, *Trigona recursa* (Apidae, Meliponini)

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It is generally assumed that the trail pheromones of stingless bees are secreted by their mandibular glands, although convincing experimental proof for this conjecture was never provided. We studied the trail marking behaviour and chemical communication of *Trigona recursa* with the aim of identifying the trail pheromone and the gland producing it. We could show that the trail pheromone is produced in the labial glands. Foragers deposit the secretions by rubbing the extended tongue on the substrate. In bioassays, the bees were attracted to feeders baited with labial gland extract but repelled from feeders baited with mandibular gland extract. In addition, significantly more bees followed artificial scent trails composed of labial gland extract than control trails using pure solvent. There was no difference, however, in the number of bees that followed control- or mandibular gland extract trails. By contrast, significantly more bees followed artificial trails made of hexyl decanoate, the main compound identified from labial gland extracts, than the control trails. Because the labial gland extract trails still were the most attractive ones we consider hexyl decanoate an important component of the trail pheromone of *T. recursa*. It is the first compound identified from the trail pheromone of any species of stingless bees. (The study was partially supported by a grant from the Austrian Science Foundation, FWF).

C2.3

A defense against whom? Investigating the chemical defense of the sea pansy *Renilla reniformis* using multiple predator types

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The soft bodied, umbrella-shaped sea pansy, *Renilla reniformis*, occurs on inter/subtidal sandy beaches along the coast of the Southeastern United States. Phylogenetically related to sea pens and soft corals, sea pansies are brightly colored, their body is conspicuously exposed to predators at the sand-water interface, and they are a rich source of diterpene secondary metabolites that are common to their phyla (Cnidaria). *R. reniformis* was a low preference food for several common consumers, suggesting that this sea pansy might be chemically defended. We used bioassay-guided fractionation with the predatory fish, *Fundulus heteroclitus*, and the lesser blue crab, *Callinectes similis*, to lead to several new, and one known, briarane diterpenoid. Assays with the above consumers, as well as with the hermit crab, *Clibanarius vittatus*, show that the numerous different briarane diterpenoids produced by the sea pansy are differentially effective against different consumers and suggests that consumer diversity could be selecting for compound diversity. Or results using multiple predatory types underscore the advantage of producing numerous analogues of a defensive chemotype as opposed to investing in a single compound, perhaps providing a mechanism for keeping the biosynthetic machinery of secondary metabolism "un-tuned" relative to primary metabolism.

C2.4

Identification of semiochemicals involved in sea lice host location, and their potential use in pest control

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The sea louse, Lepeophtheirus salmonis Krøyer 1838, is an important, highly specific ectoparasite of salmonid hosts, causing substantial economic losses to global production of Atlantic salmon. The infective stage of the life cycle is the larval copepodid, its role being to successfully find and settle on a suitable host fish to ensure development to the adult stage. Mechanisms of host location and identification by copepodids are believed to involve responses to host-derived chemical cues, as identified in the adults.
Copepodid behaviour on exposure to chemical cues were investigated in a vertical arena bioassay, with changes observed on exposure to waterborne odours from water entrained with, or chemicals derived from, host and non-host fish species. Copepodid movement was digitally tracked using Ethovision® to allow quantitative comparisons.
Control copepodids performed a repertoire of bouts of sinking interspersed by swimming, during which they displayed linear, looping and spiral movements. Exposure to host odours and isophorone resulted in highly significant changes in non-oriented behaviour. Host odours and isophorone induced copepodids to perform local search behaviour, dominated by hop swimming, and increased turning, loops, somersaults and helices relative to control behaviour.
Non-host odours (cod, turbot) induced partial activation and different swimming patterns, whilst other odours did not induce an effect. The implications of these findings are discussed in terms of behavioural search patterns during host location and the potential use of such chemicals in pest management strategies for farmed salmonids.

C3.1

Elucidation of the biosynthesis of sorghum phytotoxins by ^{13}C-NMR spectroscopy

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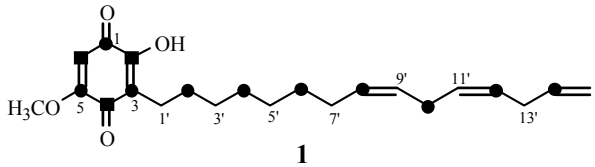
Sorghum species exude phytotoxic constituents from the roots, the major component being sorgoleone (**1**). Analogues of **1** have been identified, as well as novel resorcinolic lipids. These constituents inhibited photosystem II electron transport. The biosynthesis of **1** was investigated using labeled precursors. Incorporation of labeling was determined by ^{13}C-NMR spectroscopy. When fed with [2-^{13}C]acetate, an increase in signals of C-2, 4 and 6 (**1**,■) was observed. Feeding with [1-^{13}C]acetate led to increased signals from C-1 and 5 but not C-3. When fed with [2-^{13}C]glucose, labeling was observed in C-1, 3, 5, 2', 4', 6', 8', 10', 12' and 14' (**1**,•). These results supported the hypothesis that **1** is synthesized from a starter C_{16}-fatty acid, with the quinone moiety being formed by cycloaddition of three acetate molecules, mediated by a polyketide synthase. A minor constituent, 4,6-dimethoxy-2-[(8′Z,11′Z)-8′,11′,14′-pentadecatrienyl]-1,3-benzenediol (**2**) was found to also incorporate [2-^{13}C]acetate (**2**,■). Biosynthetic studies provided evidence that **2** shares the same biosynthetic route as **1** through a common intermediate. Compound **2** was more phytotoxic than **1** in a lettuce germination assay. This study opens an avenue to manipulating the pathway favoring synthesis of **2**.



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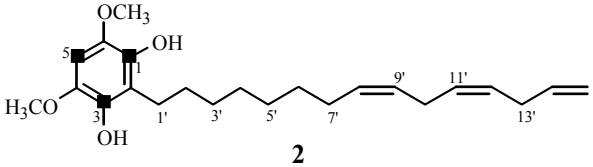

2

C3.2

New tools to understand alkaloid factories at the single cell level

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Catharanthus roseus produces a variety of valuable monoterpenoid indole alkaloids, including vinblastine (VBL) and vincristine (VCR) that have been very valuable as anticancer agents over the last 30 years. Recent renewed interest in a new generation of more powerful and less toxic alkaloid drugs has prompted more studies on the elucidation of the pathways leading to the biosynthesis of alkaloids such as vindoline and catharanthine, which comprise the dimeric alkaloids VBL and VCR. In the case of the vindoline pathway, its biosynthesis is very active in basal parts of the young leaves and involves multiple specialized cells, requiring the transport of biosynthetic intermediates between cell types. More than ten pathway genes have been cloned and some of the genes have been localized by *in situ* hybridization (Plant Cell 11:887; Plant Journal 38:131). The present study describes that expression profiling of indole alkaloid pathway genes is possible at the single cell level using a laser capture microdissection (LCM) technique to harvest RNA from single cells. RT-PCR of single cell RNA successfully localized expression of genes such as tryptophan decarboxylase and deacetylvindoline acetyltransferase to the same cells identified by *in situ* hybridization in the previous studies. We conclude that RNA from single cells obtained by LCM can be used as a versatile tool to understand how particular cells become combinatorial cell factories for specialized natural product biosynthesis.

C3.3

Brazilian palm as source of biologically active molecules

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Brazil is one of the greatest centers in the world concerning the biodiversity. Because of that, together to the fact that this biodiversity could be lost in a short period of time lacking knowledge about its chemistry and pharmacological information, this great study was started. This big project encompasses plants from the family Palmae: *Euterpe oleracea, Syagrus oleracea, Mauritia vinifera, Orbgnya speciosa* and *Copernicia cerifera*. Regarding the pharmacology, several models have been used, like antinociceptive, anti-inflammatory, antioxidant, molluscicidal, anti-microbiane and nitric oxide production inhibition. Obtained results showed that the ethnopharmacological information is a very interesting way to search for new bioactive molecules. In this way, several classes of metabolites were found such as steroids, terpenoids, phenylpropanoids, fatty acids and also constituents from essential oil of each species. It is noteworthy to mention the activity from the Açaí fruit extracts in the inhibition of nitric oxide production. It was also possible to identify extracts responsible for the antinociceptive and anti-inflammatory activities in *O. speciosa.* This specie was also very interesting when assayed as anti-leukemia cells. From *S. oleracea* fruits it was isolated HMF in great amount showing that these fruits could have any toxicity. *M. vinifera* is a great source of vitamins like retinols and tocopherols. Palmae species, rich in fatty acids and steroids led to enriched extracts that could be responsible for the anti HPB activities like the standardized extracts from *Serenoa repens*, belonging to the same family. Afterwards, in a general way, all species were very antioxidant due to their contents in molecules able to donate hydrogen radical, especially in the DPPH assay. Summing up, Brazilian Palms have been shown as an import source of bioactive molecules. Acknowledgements: FUJB, FAPERJ, PRONEX, CNPq, CAPES.

C3.4

Mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission

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Herbivore feeding elicits defense responses of the infested plants, typically the emission of a blend of volatile organic compounds (VOCs) which mediate indirect defense reactions. The contribution of plant tissue wounding during the feeding process in the elicitation of defense responses is not clear up to now. Thus, a mechanical caterpillar has been designed which closely resembles the herbivore-caused tissue damage in terms of physical appearance and a long lasting wounding period on defined leaf areas. This mode of treatment was sufficient to induce the emission of a VOCs' blend as known from real herbivore feeding. In all test plants the same volatiles as induced by certain herbivores (*Spodoptera* spp.) were emitted albeit with different quantities. Moreover, both the duration and the area that has been mechanically damaged, contribute to the induction of the volatile response. Based on those two parameters, time and area, which can replace each other to some extent, a damage level could be defined. That damage level exhibits a close linear relationship with the accumulation of fatty acid-derived volatiles, while methyl salicylate and terpenoid volatiles respond in a different manner. The results strongly suggest that the impact of plant tissue wounding on the induction of defense responses during herbivore feeding was underestimated up to now. A controlled and reproducible mechanical damage that strongly resembles the insect's feeding represents a novel and valuable tool to analyze the role of the various signals involved in the induction of plant defense reactions against herbivory.

C3.5

Individual variation in Scots pine phytochemistry and its effects on associated ecological processes in a Caledonian forest

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The Caledonian forest is dominated by Scots pine (*Pinus sylvestris* subspecies *scotica*) whose phytochemistry is particularly varied in monoterpenes, with needles containing up to 20 mg gDM^{-1}. Terpenoids occur at lower concentrations with the main monoterpenoid bornyl acetate contributing around 2% (w/w) of the total monoterpenes. Phenolic secondary metabolites are dominated by prodelphinidin-containing condensed tannins[1]. Quercetin and its derivatives are commonly occurring flavanoids in Scots pine and have been reported to occur in the Scottish subspecies in concentrations of less than 0.06 mg gDM^{-1} [1]while for Scots pine of other origins concentrations of 2 mg gDM^{-1} were observed[2].
Due to the abundance and variability of monoterpenes and the presence of two distinct chemotypes, Δ^3-carene present or absent, within the study site[3], a comparison of the effect of the major monoterpenes in the field on diversity of key ecosystem components and ecological processes was possible, in particular:

- Ground vegetation
- Crown insects and arthropods
- Mycorrhizal growth
- Pine looper caterpillar growth

To detail just one aspect, ground vegetation diversity did not correlate with individual monoterpenes. However, converting the individual relative monoterpenes concentrations in needles per tree into its 'chemodiversity' resulted in a significant relationship with the vascular plant community species richness located underneath each tree.

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C3.6

Growth and defense in the invasive crucifer species *Lepidium draba*

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The success of invasive weeds has been attributed to escape from natural enemies and a subsequent evolutionary change of allocation from defense to growth and reproduction. Several parameters of this hypothesis of evolution of increased competitive ability (EICA) were tested in the hoary cress, *Lepidium draba* L. This perennial mustard was introduced to North America from Europe in the late 19th century and spread since then throughout the western and the north-eastern U.S. states and three Canadian provinces. Plants originating from 10 European (native) and 10 American (non-indigenous) populations were grown under the same conditions in a greenhouse in Germany. After three months, plants were harvested and growth parameters (hight, number of ramets, above and below-ground biomass) measured. Levels of glucosinolates and myrosinase activity were analysed in shoots and roots as these are important defense parameters for members of the plant family of Brassicaceae. Plants showed a high within population variation but there were almost no significant differences between populations from different continents, neither in growth nor in defense. The success of *L. draba* in the introduced range is thus likely due to other factors than a selection for genotypes with improved competitive abilities and reduced resource allocation to herbivore defence.

C3.7

Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink-source model of plant defense.

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We examined the importance of induced sink strength for the development of constitutive and inducible levels of phenylpropenoids in hybrid poplar (*Populus nigra* x *P. deltoides*) foliage. Phenylpropenoid concentrations and the expression of related genes were determined for developing foliage after CHO flow, measured as the translocation of ^{13}C from labeled sources, was disrupted. We found that phenolic metabolism in both un-wounded and induced sink leaves is directly and positively linked to rates of CHO import. Alterations in rates of translocation yielded different results, depending on how CHO import was affected: the removal of competing sinks rapidly and dramatically increased leaf phenolic contents as much as 300%, whereas phenolic levels (and their inducibility) tended to be reduced when import to wounded leaves was interrupted. Results indicate that induced sink strength, i.e. elevated activities of cell wall invertase and increased CHO flow, provides resources for phenolic biosynthesis in developing poplar leaves. Accordingly, events that disrupt CHO import also disrupt phenolic-based wound responses.

C3.8

Allelopathy as a mechanism for resisting invasion: the case of the Florida scrub.

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Recent papers linking the success of certain invasive plants to allelopathy have led to the proposal that allelopathy may be an important mechanism in exotic plant invasions. This claim is reviewed in light of work done on allelopathic mechanisms in the Florida sand pine scrub. This community contains a number of perennial shrubs which are allelopathic toward invasive grasses of the adjacent sand hill community. Scrub vegetation is vulnerable to fire, and grasses would provide fuel for fires if they became established. Field and laboratory chemical studies with the woody shrub *Polygonella myriophylla* support a role for gallic acid and hydroquinone. Recent work indicates that non-microbial and microbial oxidation is important in activating and in degrading these allelochemicals. Studies with other scrub perennials also point to the importance of environmental and microbial degradation processes in activating phytotoxins. Environmental stress factors such as nutrient limitation have been implicated in contributing to the toxicity of scrub allelochemicals. A combined approach coupling laboratory and field studies has provided insight into the apparent mechanisms that keep grasses out of the Florida scrub. Furthermore, these studies suggest that the role of allelopathy as a mechanism in plant invasions is more complex than has been appreciated. While in some cases, allelopathy may allow exotic invaders to succeed, the Florida scrub provides a counter-example in which allelopathy appears to play a primary role in preventing invasion.

C3.9

How do host plant supplements influence forest tent caterpillar behaviour and performance?

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Plant secondary metabolites can influence herbivorous insects via effects on the taste or the nutritive value of foliage. These two properties of a compound, phagodeterrence and toxicity, are not always correlated: to understand the effects of a plant allelochemical on a herbivore, both feeding behaviour and performance must therefore be examined. We compared growth and development, as well as meal initiation and food choice, of the forest tent caterpillar (*Malacosoma disstria*) on three diets: early-spring trembling aspen foliage (*Populus tremuloides*, the preferred host plant), Addy's diet (a meridic artificial diet for forest tent caterpillars), and Addy's diet supplemented with leaf powder prepared according to standard protocols of freezing, lyophilizing and grinding. Caterpillars performed best on foliage, followed by Addy's diet, and did very poorly on the supplemented artificial diet. However, caterpillars appeared to prefer the supplemented artificial diet to plain Addy's diet. These findings suggest that the preparation of foliage alters its chemistry in a way that degrades its nutritive value, but not its taste. Aspen foliage constitutively expresses high levels of condensed tannins and phenolic glycosides (which decrease forest tent caterpillar performance) and exhibits induced expression of trypsin inhibitors and polyphenol oxidase in response to leaf damage. It is not clear how any of these defences could increase in dried leaf powder relative to fresh foliage.

C4.1

Identification of sex pheromone component of the blueberry leafminer, *Caloptilia porphyretica*.

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Coupled gas chromatographic-electroantennographic detection (GC-EAD) of both gland extracts and effluvial collections from female blueberry leafminer, *Caloptilia porphyretica* Braun (Lepidoptera: Gracillariidae), showed that the females produced a single EAD active compound. The amount of natural pheromone collected from virgin female *C. porphyretica* was below the GC-flame ionization detector (FID) and mass spectrometry (MS) detection thresholds, even with highly concentrated gland extracts (~150 female equivalent). (*E*)-11-Hexadecenal (*E*11-16:Al) was identified as the pheromone component by comparison of EAD-active compounds' peak retention times with authentic standards on both polar and non-polar capillary columns and micro-reaction-GC-EAD analyses. The GC-EAD experiments proved that synthetic *E*11-16:Al exhibited extraordinary high electrophysiological activity, resulting in significant male antennal responses at as low as the 10 femto-gram (10^{-12}) level. Field tests demonstrated that traps baited with *E*11-16:Al alone were attractive to males. Addition of 1 or 3 percent of its geometric isomer, *Z*11-16:Al, to *E*11-16:Al did not significantly increase trap captures compared to *E*11-16:Al alone, but significant inhibitory effect was observed at the 10 percent level. The influence of two kinds of rubber septa on trapping efficiency was also evaluated in two separate field tests. Data from these studies indicated that male moth captures, except at the lowest (3-µg) and the highest (1000-µg) doses tested, were significantly greater in traps baited with red natural rubber septa compared to gray halo-butyl rubber septa at 30-300-µg loading. Monitoring of adult flight activity with 3-µg dose of *E*11-16:Al indicated three distinct flight periods throughout the 2003 season.

C4.2

Semiochemistry of the goldeneyed lacewing *Chrysopa oculata* (Neuroptera: Chrysopidae): Attraction of males to a male-produced pheromone

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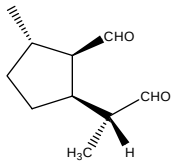
Antennae of males and females of the goldeneyed lacewing, *Chrysopa oculata*, consistently responded by GC-EAD to four compounds extracted from the abdominal cuticle of males: nonanal, nonanol, nonanoic acid, and 1*R*,2*S*,5*R*,8*R*-iridodial. No volatiles were detected from the abdominal cuticle of the females. *Co. oculata* adults were most sensitive to 1*R*,2*S*,5*R*,8*R*-iridodial at an EAD-response threshold between 0.1 and 1 pg, which was up to 10000 times lower than thresholds for other compounds tested. 1*R*,2*S*,5*R*,8*R*-Iridodial was the only male-specific compound that exhibited significant attraction to *Co. oculata* males in the field. A weak attraction of males was also found to 1*R*,4a*S*,7*S*,7a*R*-nepetalactol (an aphid sex pheromone component), which may be due to the 5% 1*R*,2*S*,5*R*,8*R*-iridodial present in the synthetic sample as an impurity. An herbivore-induced plant volatile, methyl salicylate, showed a significant synergistic effect on male attraction to 1*R*,2*S*,5*R*,8*R*-iridodial. No females were caught in the entire study. SEMs revealed numerous male-specific, elliptical epidermal glands on the 3rd-8th abdominal sternites of *Co. oculata*, which are likely the pheromone glands. Another lacewing, *Chrysoperla rufilabris*, did not produce male-specific volatiles or possess the glands presumed to produce pheromone in *Co. oculata* males.



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

C4.3

The polyphagous arciid *Estigmene acrea* sequester pro-toxic pyrrolizidine alkaloids from any plant source for protection, synthesis of idiosyncratic alkaloids and pheromone formation.

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The highly polyphagous caterpillars of the arctiid *Estigmene acrea* are able to sequester pro-toxic pyrrolizidine alkaloids (PAs) of any known structural type. In the insect the PAs are detoxified by specific *N*-oxidation and are stored or transmitted as non-toxic *N*-oxides. Part of the plant acquired PAs are hydrolyzed yielding the free necine bases. The necine bases are specifically converted into idiosyncratic necine esters (insect PAs). The respective retronecine O^9-monoesters, the creatonotines, are assumed to be the common precursors for the formation of the male courtship pheromone, hydroxydanaidal. Larvae of *Estigmene acrea* appear to utilize PA containing plant only as "drug source" but not for alimentation. Highly sensitive sensory recognition of PA sources accompanied by transient loss of sensory responsiveness enables caterpillars to find PA sources for temporary visits between substantial meals. This can be especially important in natural environments where PA plants are rare. The integration of plant acquired PAs into the insect's defense biology is discussed with respect to specific physiological, biochemical and behavioral adaptations.

C4.4

Interactions between a plant volatile and the pheromone in *Rhynchophorus palmarum*: electrophysiological and behavioral evidence

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Abstract*:* Aggregation in the palm weevil is mediated by a male-produced pheromone ((5*S*)-2-methyl-(5*E*)-hepten-4-ol, rhynchophorol) and plant volatiles (PVs) acting synergistically. Based on the identification of the components of natural volatile emitted by the host plants, multicomponent synthetic blend were designed and proved to be a good substitute for natural plant material in field trials. Catches increased significantly when this blend was mixed with rhynchophorol in the traps [1]. Using single sensillum recording on the antennae of *R. palmarum* we characterized specific olfactory receptor neurons (ORNs) tuned to rhynchophorol and to some components of the PV blend. Recordings showed simultaneous responses to PVs and rhynchophorol with different spike amplitudes suggesting that some sensilla housed ORNs tuned to rhynchophorol and to PVs. Furthermore, some ORNs showed more complex response patterns, as they were co-activated by rhynchophorol and an essential component (3-hydroxy-2-butanone, acetoin) of the attractive blends in the field [2]. In a four-choice olfactometer, male and female weevils were attracted more to a blend of rhynchophorol, and acetoin than to pure compounds. ORNs responses to a mixture of rhynchophorol and acetoin and to each component presented singly were recorded. Several types of interactions were observed between the two compounds when they were tested in blend. This provides evidence of a modulation of the detection of one compound by the other at the peripheral level of the sensory system.

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C4.5

Chemical ecology of the papaya weevil *Pseudopiazurus papayanus* (Coleoptera: Curculionidae)

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The papaya weevil *Pseudopiazurus papayanus* Marshall, 1922 (Coleoptera: Curculionidae) is a pest of economic importance for the producing areas of the northeast region of Brazil. Laboratory bioassays employing host plant alone, volatiles released from males and females conspecifics alone, and a combination among the insect volatiles and host plant suggested a male-produced aggregation pheromone in the species. GC analysis of the airborne volatiles released by males and females showed the existence of three male-specific compounds, that were identified as grandisal, grandisol, and a new compound that we named as papayanol. The synthetic compounds had their biological activity testes in Y olfactometer. Males and females were significantly more attracted to a combination of grandisal plus host plant, in a dosage over 20 ug. Experiments to evaluate the response of insects to a binary and tertiary mixture are now in progress.

C4.6

Role of preen gland secretions in feather protection: Are feather mites mutualistic, commensalistic or parasatic?

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Most species of birds, posses preen gland and the secretion from this gland is applied to feathers to keep them in good conditions. Several functions such as antibacterial, antifungal, and antiectoparasatic activities have been attributed to preen gland secretions, as birds are hosts to numerous kinds of arthropods, fungi, bacteria and other organisms. Some of these occur on feathers and are known to be detrimental to the fitness of the individuals. Feather mites, belonging to the genus *Proctophyllodes* are prevalent in House Finches (*Carpodacus mexicanus*) most of the year. Not much is known about the interaction between feather mites, the hosts and preen gland secretions. Several scholars consider feather mites as commensalistic species that feed on preen gland secretions and others have shown that the infestations by the mites affect the host fitness. House Finches possess fairly large preen gland and produce mostly long chain wax esters. We found that there is seasonal variation in their composition. We analyzed the chemical components of secretions of mite infested and non-infested House Finches and also conducted bioassays to test, if the secretions are stimulants or deterrents to the mites. We found that there is lot of variation among the individuals' secretions in relation to mite infestation and bioassays suggested that the variation in chemical composition is responsible for variation in bioactivity.

C5.1

The chemical signature of subterranean termites: an adjunct for phylogeographic study

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Little is known about the evolution process underlying the development of insect chemical signatures. However, considering the importance of such signatures in recognition between social insects, we decided to use them in conjunction with molecular tools to study the evolutionary history of subterranean termites in Europe. Samples were collected from 100 different colonies in France, Spain, and Portugal. Phylogeographic determination of species was "cross-checked" by comparing multiple DNA sequences (2 mitochondrial genes and 1 noncoding nuclear sequence) and cuticular hydrocarbon variations. Data demonstrated that an ancestor species spreading from the south of Spain north gave rise to two distinct species, i.e., *Reticulitermes grassei* and *R. banyulensis*. Chemical analyses showed several chemical phenotypes seemingly correlated with geographic location. As they progressed northward, different populations of *R. grassei* probably retained different DNA and chemical phenotypes. Cross-checking with chemical signature data enabled us to better understand the current distribution of those species. Use of analytical chemistry in phylogeny opens a new area in chemical ecology. We have used similar double cross-checking approach with other intraspecific markers (microsatellites) to study colonial range.

C5.2

Stereoselective synthesis and absolute configuration of gaur acid

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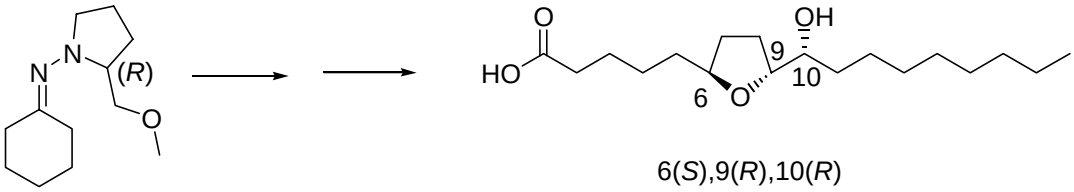
The mosquito deterrent "gaur acid" [5-(1-hydroxynonyl)-2-tetrahydrofuranpentanoic acid], isolated during a bioassay-guided fractionation of gaur skin lipids, was concluded to have the 6*S*,9*R*,10*R* configuration. This assignment was confirmed by stereoselective synthesis. RAMP Hydrazone alkylation and Sharpless dihydroxylation were employed to install chiral centers of known configuration.



6(*S*),9(*R*),10(*R*)

C5.3

Where even goats' feet cannot tread: Plant defense on mammal-free rock pillars in a Mediterranean oak forest.

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A group of rock pillars in Northern Greece is inaccessible to livestock, but surrounded by oak forests and heavily browsed "antipastoral" spiny vegetation. Leaves of spiny kermes oak, *Quercus coccifera*, were collected over 4 years on the rock pillars, reached by technical rock climbing, and compared with leaves in the surrounding landscape that is accessible to livestock and wild herbivores. We tested for (1) phenolics levels in the leaves, (2) number of leaf spines, and (3) the response of kermes oak to experimental defoliation ("pseudobrowsing") to test for induced defense. While phenolics levels in leaves tended to be lower on the pillars than on the range in other species (*Pistacia, Cistus)*, no such trend was found in Kermes Oak. This spiny species appears to rely more on mechanical defense. While numbers of spines did not significantly differ between the pillar summits and the general range, the numbers varied more widely on the pillars, as evidenced by the Coefficient of Variation ("relaxed defense"). Pseudobrowsing led to increased numbers of spines both on the pillars and on the range, but such "induced defense" occurred only on shrub specimens with low numbers of spines. Large numbers of spines could not be increased further. Continuously livestock-browsed shrubs appear to be at the maximum of their defense potential.

C5.4

A woodland mammal's response to compounds from an unpalatable spurge, *Euphorbia lathyris*.

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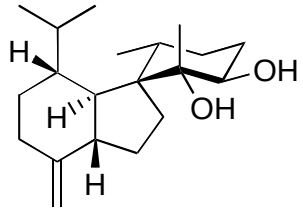
José Giner, Department of Chemistry; College of Environmental Science and Forestry, State University of New York, Syracuse, New York 13210. dmullers@esf.edu; jlginer@syr.edu.

Abstract—Mammalian herbivores nearly never attack gopherweed, *Euphorbia lathyris* (Euphorbiaceae). To find the active principle responsible for the deterrent effect, we prepared extracts, and field-tested these extracts, plus specific isolated compounds, for antifeedant activity in response-guided bioassays. We treated winter-dormant apple twigs with extract solutions and presented them to free-ranging eastern cottontails, *Sylvilagus floridanus*, at 3 different home ranges in Upstate New York during late winter over 4 years. We recorded twig tips cut and amount of bark removed. Extracts from all plant parts reduced feeding, but root extracts were most active. The bioassay led to isolation of two compounds in the root extract, docosanyl ferulate and gopherenediol, a new compound. Both had some had anti-feeding activity. The bioassays are continuing.

Gopherenediol



C5.5

The glucosinolate-myrosinase defence system in the cabbage aphid, *Brevicoryne brassicae*.

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Cruciferous plants contain glucosinolates that are hydrolysed by myrosinase enzymes within the plant to produce volatile isothiocyanates which act as repellents for generalist pests but attractants for specialist crucifer feeders including the cabbage aphid, *Brevicoryne brassicae*. Specialist aphids also produce a myrosinase enzyme and assimilate high levels of plant glucosinolates. However, the biological role of the aphid enzyme has not been explored. It is likely that the aphid myrosinase and sequestered glucosinolates play a defensive role against predators and parasitoids. Aphid myrosinase is found only within muscle tissue of the head and thorax while the glucosinolates are most likely located in the haemolymph, a spatial arrangement suggestive of a defensive role. Recent research has concentrated on the dynamics of the aphid myrosinase and glucosinolate system particularly: glucosinolate uptake; released volatiles and the biological role of this defensive system in *B. brassicae*

C5.6

Separation of male haemolymph fractions containing sex pheromonal components derived from methyl eugenol in fruit fly, *Bactrocera papayae.*

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Pharmacophagy of methyl eugenol (ME) - a highly potent male attractant of the pest fruit fly, *Bactocera papayae* results in the hydroxylation of ME to sex pheromonal components, 2-allyl-4,5-dimethoxyphenol (DMP) and *E*-coniferyl alcohol (CF). These compounds are sequestered in the rectal gland prior to release during courtship period at dusk. Chemical and behavioural analyses have shown that these sex pheromonal components are present in the haemolymph and transported to the male rectal gland of ME-fed *B. papayae*. Separation of ME-fed male haemolymph using native polyacrylamide electrophoresis (PAGE) gel and subsequent homogenisation of the PAGE gel fractions were conducted. Thereafter, when the homogenized gel fractions were exposed to conspecific male flies, no flies were observed to be attracted to the fractions. No differences were observed in the haemolymph protein patterns of ME-fed and deprived males from both native and sodium dodecyl sulphate (SDS)-PAGE analyses. However, protein assays showed higher haemolymph protein content in ME-fed male as compared with ME-deprived male. This result is further substantiated from the elution profile of *B. papayae* male haemolymph following FPLC® column chromatography using gel filtration on Superdex 200. The haemolymph elution profile of ME-fed male showed a significant increase in protein absorbance as compared with that of ME-deprived male. Using male attraction bioassay of the individual fractions collected, two fractions were highly attractive. These bioactive fractions were found to be located at the peak of protein absorbance in the ME-fed male haemolymph elution profile. The molecular mass of the pooled bioactive fractions as determined by gel filtration was in the peptide range of 3.3 to 5.5 kDa. GC-MS analyses further confirmed the presence of the sex pheromonal components, DMP and CF, the bioactive fractions. The appearance of four peptide bands in the bioactive fractions i.e. peptides I (4.1 kDa), II (4.8 kDa), III (5.9 kDa) and IV (8.0 kDa), was confirmed in subsequent peptide gel electrophoresis of the bioactive fractions, using 16.5% gel in tris-tricine SDS-PAGE. These findings suggest that the bioactive peptides function as pheromone binding peptides (PBPs).

POSTERS

P1

Comparative volatile analysis and terpene biosynthesis in *Arabidopsis thaliana* and its close relative *Arabidopsis lyrata* ssp. *petraea*

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Terpene secondary metabolites exhibit important ecological functions in plant-organism interactions. Low molecular weight terpenes like monoterpenes (C_{10}) and sesquiterpenes (C_{15}) volatilize easily under ambient temperature and are emitted from floral and vegetative tissues, where they play significant roles in the attraction of pollinators or predators/parasitoids of herbivorous insects. *Arabidopsis thaliana* has proven to be a valuable model system for the biochemistry and function of terpene secondary metabolism. The Arabidopsis genome contains a large gene family of terpene synthases (TPSs), enzymes that catalyse the formation of terpenes from central linear precursors in the terpene biosynthesis pathway. Employing terpene volatile metabolite profiling and TPS gene expression profiling, we have shown that *A. thaliana* constitutively emits mono- and sesquiterpenes from flowers and roots, and we have indentified several TPS genes responsible for the biosynthesis of these compounds (Chen *et al.* 2003; Chen *et al*. 2004). Relatively little is known about differences in constitutive and stress induced volatile emissions and the mechanisms controlling these differences among related plant species with significantly different life histories. *A. thaliana* and the closely related *A. lyrata* ssp. *petraea*, provide a promising model for such studies since, compared to the mainly self-pollinating annual species *A. thaliana*, *A. lyrata* ssp. *petraea* is a perennial that is strictly outcrossing. We have analysed floral volatile blends from different *A. lyrata* ssp. *petraea* populations and found that the floral scent of all lines contains mostly benzenoid derivatives and almost no terpenes. This is clearly different from the floral volatile blend of *A. thaliana*, and indicates a strict divergence of floral volatile emission in the evolution of these two closely related species. We also compared induced volatile emission profiles from rosette leaves of *A. thaliana* and *A. lyrata* ssp. *petraea* after feeding by larvae of the specialist lepidopteran herbivore *Plutella xylostella*. Interestingly, some *A. lyrata* ssp. *petraea* lines showed induced emission of *(E)*-□-caryophyllene, a major sesquiterpene emitted from *A. thaliana* flowers but not from the foliage of this species under stress conditions. Following transcriptional analysis, we have identified and characterized a gene encoding *(E)*-□-caryophyllene from *A. lyrata* ssp. *petraea*.
Promoter analyses of this and other TPS genes from *A. lyrata* ssp. *petraea* and further characterization of terpene volatile biosynthesis in other close relatives of *A. thaliana* will provide more insights into the differential genetic regulation and evolution of terpene biosynthesis in these species.

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P2

In vitro inhibition of GABA-transaminase by anxiolytic plant extracts and compounds

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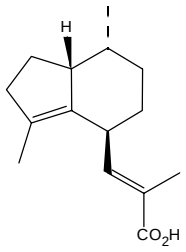
Botanicals used to treat anxiety and related nervous disorders are among the most popular medicinal plants on the market today. For many of these plants, little is known about their safety, efficacy and mode of action. □-amino butyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system (CNS). It has been proposed that anxiety may result from and imbalance in GABAergic transmission. Previous evidence has shown that inhibiting the metabolic enzyme GABA-transaminase (GABA-T) may relieve nervous tension by increasing GABA levels in the brain. A high-throughput spectrophoto-metric method has been developed in our laboratory to rapidly assess the effects of commercial plant extracts and pure compounds on *in vitro* GABA-T activity from rat brain.
Results to date show that aqueous extracts of valerian (*Valeriana officinalis*) and gotukola (*Centella asiatica*) inhibit 50% of enzyme activity at 2.28 mg/mL, and 2.82 mg/mL respectively. Of the three valerian standards tested, only valerenic acid (100 □M) inihibited GABA-T by 34%. Since this is a high concentration it is possible that other compounds are responsible for the activity observed in the extract.



Valerenic acid

P3

Characterization and involvement of lipophorins in cuticular hydrocarbon transport in termites.

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Lipophorin (plasma lipoprotein) is the major hemolymph protein responsible for transport of lipids in insects. In social insects lipophorin ensures transport of cuticular hydrocarbons that play important roles in nestmate and species recognition. We have performed various experiments using high-density lipophorin (HDLp) isolated from *Reticulitermes flavipes* plasma. Hydrocarbons accounted for 20.4% of the 33.8% total lipid component of the lipophorin (and 66.2% of protein). Western blotting showed strict association of Lp with an antiserum generated in rabbit. Immunoprecipitation confirmed antibody specificity in two different *Reticulitermes* species (*R. flavipes* from USA *and R. lucifugus* from Europe) and one cockroach (*Supella longipalpa*). A double immunodiffusion technique was used to investigate cross-reactivity of the antiserum with lipophorin from ten termite species in the genera *Reticulitermes*, *Coptotermes*, *Zootermopsis*, and *Kalotermes*, and five cockroach species. A sensitive ELISA test developed to quantify lipophorin titers in different castes of *R. flavipes* revealed a gradual increase in successively larger workers and differences among castes. Experiments based on injection of anti-HDLp serum into *Zootermopsis nevadensis* and monitoring the *de novo* biosynthesis of hydrocarbons and their transport to the cuticular surface further supported involvement of lipophorin in hydrocarbon transport. Antiserum injection significantly disrupted hydrocarbon transport.

P4

Foliar phenols in trees as prooxidants in caterpillars

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This study tested the hypotheses that (1) tree leaves with lower ascorbate:phenol ratios cause greater levels of oxidation in the guts of caterpillars, and (2) phenol-tolerant caterpillars have lower levels of phenol oxidation in their guts than do phenol-sensitive species. Foliar ascorbate and phenol concentrations were compared in red oak and sugar maple in the spring and summer, and the effects of ingesting these leaves were compared in *Orgyia leucostigma* (phenol-tolerant) and *Malacosoma disstria* (phenol-sensitive). Maple leaves had significantly lower ratios of ascorbate:phenol than oak in the spring and summer. Semiquinone (SQ) radicals, produced by phenol oxidation, were measured in the gut fluids of the caterpillars with electron paramagnetic resonance spectrometry. Much higher levels of SQ radicals were formed in caterpillars when they fed on maple than oak in both seasons, supporting the first hypothesis. Lower levels of SQ radicals were formed in the guts of *O. leucostigma* than in *M. disstria*, supporting the second hypothesis. Higher levels of SQ radicals were associated with higher levels of protein carbonyls and peroxides in caterpillar gut fluid, demonstrating that the ingested phenols had a net prooxidant effect, and that maple has particularly active oxidative defenses.

P5

Oviposition deterrents from bitter gourd leaves, *Momordica charantia* to a leaf mining fly, *Liriomyza trifolii*

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The American serpentine leaf mining fly, *Liriomyza trifolii* (Burgess) is well characterized by its high degree of polyphagy whose larva feeds on many economically important plants including cucurbitaceous, solanceous and leguminoceous plants. Development of pest control based on understanding of the ecological aspect of insect-plant interactions is thus desired.
Bitter gourd, *Momordica charantia* L. (Cucurbitaceae) is widely cultivated in East and South East Asia countries. This plant, however, shows highly resistance to *L. trifolii*. The methanol extract of *M. charantia* leaves strongly deterred females of *L. trifolii* from laying egg on kidney bean, *Phaseolus vulgaris*, leaves dipped into concentration of 1g leaf equivalent extract/ ml. Bioassay was conducted using leaf-dipping method. The active methanol extract was then dissolved in water and partitioned with hexane, diethyl ether and butanol successively. Repeated column chromatography of the active BuOH fr. over silica gel followed by HPLC led to the isolation of four active triterpenoids, momordicine I (**1**), 7,23-dihydroxy-3-*O*-malonylcucurbita-5,24-dien-19-al (**2**), momordicine II (**3**) and 7-*O*- -D-glucopyranosyl-3,23-dihydroxycucurbita-5,24-dien-19-al (**4**). The ovipositing of *L. trifolii* was totally deterred when bioassays were carried out using kidney bean leaves treated with compounds **1**, **2**, **3** and **4** at concentration of 150, 180, 80 and 40 □g/cm^2, respectively

P6

Variations in elaiosome lipid composition reveal a geographic mosaic of interactions between ants and the myrmecochore *Helleborus foetidus*

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Helleborus foetidus (Ranunculaceae) is a perennial herb which seeds wear a white food body (elaiosome) composed of large quantities of palmitic, linoleic, oleic and stearic acids, mostly in the form of triglycerides (TG). Diglycerides (DG) and free fatty acids (FFA) are also found though in much smaller amounts. Many omnivorous ant species collect the whole diaspore, feed on the elaiosome and discard the intact seed in or outside their nest, therefore providing dispersal in exchange of food. A phenotypic selection analysis carried out in 5 populations from 2 regions of the Iberian Peninsula (Sierra do Caurel and Sierra de Cazorla) distant of about 800 km revealed a contrasting effect of the lipid composition on the rate of dispersal. Dispersal rate was positively associated to the total quantity of lipids (TG + DG + FFA) in Sierra do Caurel but not in Sierra de Cazorla. In general, elaiosomes from Sierra do Caurel showed a greater amount of all types of lipids than those from Sierra de Cazorla. A bioassay conducted in the laboratory further indicated that ants, irrespective of their species and geographic origin, almost systematically preferred to remove diaspores from Sierra do Caurel than from Sierra de Cazorla. Overall, the results suggest a geographic mosaic of interactions in which elaiosome chemical traits better match ant preferences in Sierra do Caurel than in Sierra de Cazorla.

P7

Attractants to capture the white pine cone beetle (*Conophthorus coniperda*) (Schwarz) (Coleoptera: Scolytidae)

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The white pine cone beetle, *Conophthorus coniperda* (Schwarz), is one of the most destructive pests of the eastern white pine (*Pinus strobus* L.), cones in natural stands and seed orchards. Males are attracted to pityol, a female-produced sex pheromone, which can be used to capture them in traps or to disrupt mating. Females are not attracted to the pheromone, but may use the host volatiles to find cones. Experiments were conducted in 2003 to determine the attractiveness of various beetle-produced compounds (myrtenol, *trans*-pinocarveol and *trans*-verbenol) and *P. strobus* host cone volatiles ((-)-□-pinene, (-)-□-pinene and S-(-)-limonene). The compounds were used in binary and tertiary combinations as well as in blends, with and without pityol, in attempt to catch both males and females. Trap catch was greatest in those baited with the combination of (+)-pityol, (-)-□-pinene and S-(-)-limonene. Experiments in 2004 will involve further testing with pityol, (-)-□-pinene and S-(-)-limonene, as well as looking at the effect of R-(+)-limonene.

P8

Nitrile-specifier proteins in plants and insect herbivores – functionally related proteins with different functions

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Glucosinolates, a group of naturally-occurring thioglycosides, and myrosinases, their hydrolytic enzymes, constitute an activated chemical defense system in the Capparales. Its defensive function results from the toxic isothiocyanates, the major products released when glucosinolates are hydrolyzed by myrosinases upon tissue disruption. In plants, nitriles are often formed instead due to the presence of epithiospecifier proteins (ESPs), protein factors which alter the outcome of the hydrolysis reaction. Larvae of *Pieris rapae* are specialist herbivores on glucosinolate-containing plants. These larvae circumvent the formation of isothiocyanates by redirecting glucosinolate hydrolysis towards the formation of nitriles. We have identified a larval gut protein, designated nitrile-specifier protein (NSP), that facilitates nitrile formation without having any hydrolytic activity on glucosinolates (1). We are currently investigating the biochemical properties of the *Arabidopsis thaliana* ESP and the *P. rapae* NSP. A comparison of the two nitrile-specifier proteins and their assumed interaction with plant myrosinases will shed light on the catalytic mechanism and the role of these proteins in plant-insect interactions.

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P9

QTL mapping of phytochemical resistance factors to maize weevil in tropical maize

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The maize weevil (MW), *Sitophilus zeamais* (Motsch.) is a cosmopolitan pest that causes severe losses in stored maize (*Zea mays*, L). In this study, we analyzed QTL of phytochemical factors associated with MW resistance in tropical maize. A mapping population was derived from the cross CML290 (susceptible) x Muneng-8128C0HC1-18-2-1-1 (moderately resistant). The QTL analysis was performed using 151 RFLP and SSR markers. Phytochemicals such as ferulic acid, (FA) *p*-coumaric acid (CA), diferulic acids (DiFA: 5,5'-DiFA, 8,5'-DiFA, 8-O-4'-DiFA, 8,5'-DiFAb), and putrescines (DiFP, diferuloyl and CFP, coumaroyl-feruloyl) were evaluated on grain from F_3 lines from two seasons. An average of ten QTL were found for *trans* and *cis* FA, 8,5'-DiFA, 8-O-4'-DiFA, 8,5'-DiFAb, DiFP, and CFP distributed within the maize genome, and account for 32% to 47% of the phenotypic variance (σ^2_p). Seven QTL dispersed across the genome were associated with *p*-CA, 5,5'-DiFA, total FA, total DiFAs, and total phenolic acids, these explain between 25.88% and 43.84% of σ^2_p. Alleles associated with phytochemical composition were derived from both parents. QTL showed dominance, and additive gene action. Nine QTL regions were common between MW susceptibility and phytochemical components. Further fine mapping of these areas will assist in the development of molecular tools to accelerate the developments of MW resistant varieties.

P10

Effects on insect moulting of selected sterol mixtures and peniocerol isolated from *Myrtillocactus geometrizans*

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We are interested in the secondary metabolites isolated from Cactaceae Family. Since K. Djerassi and collaborators published their results of systematic studies of cacti, few autors had been working to isolate and elucidate chemical structures of those plants. Our results are focused in the relationship between the secondary metabolites of *Myrtillocactus geometrizans* and *Moneilema variolare*, a root-feeding insect that recently was discovered using this plant as host (Salazar et al., 2004. In press). In this occasion, the extracts, isolated sterol mixtures and peniocerol were tested against two generalist insects, *Spodoptera frugiperda* and *Tenebrio molitor*. We obtained the methanol (**EM**) and dicloromethane (**ED**) extracts from the roots. **EM** was fractionated by column chromatography (CC) to give a mixture of sterols (**CCM1**). From **CCM1** was isolated peniocerol (Cholest-8-en-3β,6α-diol) by recrystalization. The chemical elucidation of peniocerol was carried out by ^{1}H y ^{13}C-NMR one and two dimentional. Confirmation was made by X-ray diffraction experiment. Both extracts EM and ED, the sterol mixture and peniocerol shows inhibitory activities to the moulting process in *S. frugiperda*, and caused deformed pupas. While with *Tenebrio molitor*, peniocerol caused an accelerated moulting process compareted with solvent control. Those results suggest that sterols presents in roots, have an effect on moulting process in generalist insects and can be acting as protective metabolites. The role of sterols in roots of *M. geometrizans* in the relationship with *M. variolare* will be discussed. This work was supported in part by DGAPA-UNAM grant IN243802. Corresponding author: ccespede@servidor.unam.mx, http://www.iquimica.unam.mx/cespedes.html

P11

The chemical variation of the host *Pittocaulon (Senecio) praecox* (Asteraceae) and its relationship with the wax scale *Ceroplastes albolineatus (Homoptera: Coccidae)*

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Pittocaulon is a genus of five species of strange shrubs and small trees with broomstick-like branches from dry parts of central and southern Mexico. Along with the genera *Telanthophora* and *Villaseñoria* , *Pittocaulon* was segregated from the enormous genus *Senecio* of the Sunflower Family (Asteraceae). In addition to the strange appearance of these plants, *Pittocaulon* is of interest because of the remarkable range of habitats in which the species occur, from dry highland scrub well above 3000 meters, to tropical dry forest in hot country as low as 300 meters. On the stems of *P. praecox* growth and live *C. albolineatus*, our question is ¿Which is the final fate that give *C. albolineatus* to secondary metabolites obtained for feeding from the stems of this plant?. The wax scale is formed by a cover of sestertepenes and diterpenes and all aerial parts of *P. praecox* contain furanermophilane and terpenoids and the roots pyrrolizidine alkaloids, such as some diterpenes, which may function as chemical defenses against herbivores. Evolutionary hypotheses predict that levels of chemical defenses will vary in function of herbivory pressure. At present, *C. albolineatus* occur mainly in several population of *P. praecox* from "Valle de Mexico", resulting at times in complete defoliation, rotting and death of the plant. Here, we study the variation in the production of alkaloids and furaneermophilane from root and aerial parts of *P. praecox,* respectively, in populations under a variety of infested and uninfested regimes. This work was supported in part by DGAPA-UNAM grant IN243802. Corresponding author: ccespede@servidor.unam.mx, http://www.iquimica.unam.mx/cespedes.html

P12

Antioxidant and anti-inflammatory activities of *Penstemon gentianoides* (Scrophulariaceae) and *Barkleyanthus salicifolius* (Asteraceae).

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Pentstemon gentianoides (Scrophulariaceae) and *Barkleyanthus salicifolius* (Asteraceae), are popular medicinal species employed by different indigenous people from Mexico, for the treatments of diverse ailments and other uses as insect repellent. Previous works report the presence of iridoids, terpenes and flavonoids; however there are not report about anti-inflammatory and antioxidant activities. Extracts and fractions were assayed against in vitro experiments, with the purpose of corroborate the anti-inflammatory activity, in the induce rat paw-edema by carrageenan and TPA (12-O-tetradecaoylphorbol acetate) induced mouse ear edema. In addition, is known that the antioxidant effects of some secondary metabolites is strongly relationed with anti-inflammatory activities, by this was carried out a screening of the extracts and fractions obtained from these plants, on the diverse antioxidant measurements for to relate both activities.
This work was supported in part by DGAPA-UNAM grant IN243802 and USAID-TIES-ENLACES-PROGRAM. Corresponding author: ccespede@servidor.unam.mx, http://www.iquimica.unam.mx/cespedes.html

P13

Insecticidal and growth regulatory activities of essential oil from Chilean natives Magnoliidae.

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Was studied the secondary metabolites that occur in essential oils of five species of Magnoliidae: *Drimys winteri* J. R. Et G. Forster (canelo), *Laurelia sempervirens* (R. et P.) Tul. (laurel), *Laureliopsis philippiana* Looser (tepa), *Persea lingue* (R. et P.) Nees ex Kopp (lingue), y *Gomortega keule* (Mol.) Baillon (queule)- that belongs to the most primitive families of angiospermae that are growing in the rain-forest slope of Southern Chile and Argentina: Winteraceae, Lauraceae, Monimiaceae and Gomortegaceae. It was evaluated the insecticidal activity of these compounds on Fall Army worm (*Spodoptera frugiperda*) a common insect pest in corn crops of South America and North America. Almost all essential oils were insecticidal with lethal doses between 20-50 ppm. In addition, these essential oils also demonstrated scavenging properties toward 2,2-diphenyl-1- picrylhydrazyl (DPPH) in TLC autographic and spectrophotometric assay. Our results indicate that essential oils from *D. winteri* and *G. keule* showed the stronger insecticidal effect than oils from *Laurelia sempervirens*, *Laureliopsis philippiana*, *P. lingue*, these essential oils appear to have selective effects on the pre-emergence metabolism of the insect. The results were fully comparable to known natural insect growth inhibitors such as toosendanin, gedunin and Cedrela extracts and have had a possible role as natural insecticidal agents. This work was supported in part by DGAPA-UNAM grant IN243802. Corresponding author: ccespede@servidor.unam.mx, http://www.iquimica.unam.mx/cespedes.html

P14

Antioxidant, insecticidal and insect growth regulatory activities of *Roldana barba-johannis* against fall army worm *Spodoptera frugiperda.*

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The methanol extract from aerial parts of *Roldana barba johannis* afforded Sargachromenol 1, sargachydorquinoic acid 4, and Sargaquinoic acid 7. These natural products and their acetylated derivatives showed insecticidal and insect growth regulatory activity against Fall armyworm (*Spodoptera frugiperda*) an insect pest of corn. The most active compounds were 1, 3, 4, 6 and a mixture of 1+4+7 and its acetylated form, which had significant effects between 5.0 and 20.0 ppm in diets. Almost all compounds were insecticidal with lethal doses between 20-35 ppm. In addition, these compounds also demonstrated scavenging properties toward 2,2-diphenyl-1- picrylhydrazyl (DPPH) in TLC autographic abd spectrophotometric assay. Our results indicate that these compounds appear to have selective effects on the pre-emergence metabolism of the insect. The results were fully comparable to known natural insect growth inhibitors such as gedunin and Cedrela extracts and have had a possible role as natural insecticidal agents.
This work was supported in part by DGAPA-UNAM grant IN243802. Corresponding author: ccespede@servidor.unam.mx, http://www.iquimica.unam.mx/cespedes.html

P15

Identification of components in casein hydrolysate that activate biosynthesis of phenolic compounds and induces cell death in *Vitis labrusca* cell cultures.

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Concord grapes (*Vitis labrusca* L.) are native to northeastern North America and unlike wine grapes (*Vitis vinifera* L.), they are cultivated mainly for production of grape juices. As an alternative to using whole plants for our research, we have initiate plant cell suspension cultures since they can be grown in desired quantities throughout the year and are excellent sources of characteristic secondary metabolites and their related biochemical pathways. Casein hydrolysate (CH) contains a mixture of essential amino acids and is an excellent source of reduced nitrogen for many tissue culture systems. Although media containing CH is required for growing *Vitis vinifera* cell suspension cultures, initial trials showed that CH completely inhibited the growth of *Vitis labrusca* cell suspension cultures and caused cell death within a few days of subculture.
The present report describes the establishment of rapidly growing *Vitis labrusca* cell suspension cultures, and identifies an individual component of CH responsible for activating phenylpropanoid metabolism and that may also trigger cell death.

P16

Molecular genetics of saponin biosynthesis in *Saponaria vaccaria.*

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Cowcockle or *Saponaria vaccaria* produces a variety of saponins of the quillaja type, as well as sterol glycosides, especially spinasterol glucoside. These saponins have a variety of commercial uses including a role as adjuvants in vaccines. As part of a larger program to investigate and identify the genes involved in plant natural product biosynthesis using expressed sequence-tag-based approaches, we have initiated a study of saponin biosynthesis in S. vaccaria. A cDNA library and corresponding expressed sequence tag collection from S. vaccaria has been generated. Two cDNA clones have been isolated which show sequence similarity to sterol glucosyltransferases. Full length clones have been isolated and expressed in E. coli. Characterization of the enzyme activities, especially substrate specificities of these putative glucosyltransferase will be reported.

P17

Extracts of Canadian botanicals inhibit the growth of *Neisseria gonorrhoeae*

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Ethanol extracts of 22 Canadian plants, commonly used in traditional and alternative medicine, were tested for their antimicrobial activity against *Neisseria gonorrhoeae*. Initially, disc diffusion assays were conducted, using *N. gonorrhoeae* strain ATCC 49226, to qualitatively screen the plants for their antimicrobial properties. Eighteen Canadian plant species demonstrated antimicrobial activity, with *Arctostaphylos uva ursi, Hydrastis canadensis* and *Oenothera biennis* being most active. Subsequently, nine active plant extracts were investigated by determining their Minimum Inhibitory Concentrations (MICs) against 31 phenotypically diverse *N. gonorrhoeae* strains. Extracts of *Sambucus nigra, Oenothera biennis, Equisetum arvense, Berberris vulgaris, Cimicifuga racemosa,* and *Ledum groenlandicum* showed weak antimicrobial activity with MICs $\geq$ 128 mg/L. Strong antimicrobial activity was seen with extracts of *Arctostaphylos uva ursi*, *Hydrastis canadensis* and *Rhodiola rosea*, demonstrating MICs ranging from of 32 mg/L, 4-32 mg/L and 64 mg/L, respectively. Consequently, Canadian botanicals represent a potential source of novel antimicrobial agents for the treatment of *N. gonorrhoeae* infections.

P18

Interactions of host specificity and hunger status on the sensitivity of sensilla styloconica in larvae of *Manduca sexta* to host plant compounds

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Larvae of the moth *Manduca sexta* are facultative specialists on plants in the family Solanaceae. The induction of host specificity in *M. sexta* is at least in part a result of a chemosensory tuning to indioside D, in which taste receptor neurons reduce their responses to a number of plant compounds while retaining sensitivity to indioside D. This effect is found in 50% of the taste receptor cells of the lateral and medial sensilla styloconica, which are both sufficient and necessary for the feeding preferences of host-restricted *M. sexta* larvae. In the present study, we compared the role of hunger status on the sensitivity of the lateral and medial sensilla styloconica of specialist feeders and polyphagous feeders. Electrophysiological recordings of the responses of sensilla styloconica to indioside D, aqueous potato extract and KCl, all at natural concentrations, were compared between larvae which had either just finished a feeding bout (satiated) or had been starved for 2 to 4 hours (hungry). We found that in the polyphagous larvae, hunger status has a significant effect on the sensitivity of the taste receptor cells of the sensilla styloconica, while in the specialist feeders, hunger status had no significant effect. We propose a model to explain at least in part how the sensory information transduced by the taste receptor cells of the sensilla styloconica may modulate the motor feeding centers in the central nervous system of *M. sexta* larvae of different dietary preferences and hunger states.

P19

Genetic control of geographic variation in pheromone blend of the pine engraver beetle, *Ips pini* (Coleoptera: Scolytidae).

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North American pine engraver beetles, *Ips pini* (Say) (Coleoptera: Scolytidae), exhibit two distinct pheromonal types. Western populations utilize *R*-(-)-ipsdienol, while eastern populations utilize higher proportions of *S*-(+)-ipsdienol, with a cline between types in British Columbia. We performed line crosses between different populations to assess geographic variation in pheromone blend genetic architecture. By employing line means analysis, we estimated the number of effective factors. Line cross experiments consistently revealed few effective factors separating any two given populations. In line means analysis of crosses between opposing variants from the hybrid zone, and between CA and NY populations, autosomal additive and dominance effects, X-linked effects, and epistasis are needed to explain the results. These results suggest that at least two autosomal loci and one X-linked locus contribute to pheromone blend differences. However, segregation is visibly strong and one autosomal locus may explain most of the variation. Compared to crosses between variants from within the hybrid zone, those between the more distant populations (CA x NY) yielded a more complicated genetic architecture, including the possibility of a lethal factor. Complementation crosses between Washington and California, and Alberta and New York populations reveal no transgressive segregation. However there are significant autosomal additive and dominance, and x-linked factors that separate the latter populations. The results suggest localized evolution of genetic architecture for pheromone blend.

P20

Bruchin responsive genes of pea

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Bruchins are insect elicitors that cause the formation of localized neoplasms when applied to pods of pea and certain other legumes. Differential display, when used to screen for changes in gene expression brought about by application of bruchin B to pea pods, revealed several mRNA's whose level increased upon bruchin application, namely: (1) An mRNA coding for isoflavone synthase, an enzyme involved in the biosynthesis of pisatin, a phytoalexin from pea, (2) Three mRNA's coding for 12-oxophytodienoic acid-10,11-reductase (OPDAR), and (3) An mRNA similar in sequence to the *MtN19* gene, which is expressed in root nodules of *Medicago truncatula,* and is, in turn, similar to genes from several other leguminous and nonleguminous plants. Real time quantitative RT-PCR showed that the level of each of these mRNA's increased by one or more orders of magnitude subsequent to bruchin treatment. Given its general toxicity one can hypothesize a role for the phytoalexin pisatin (which increases in concentration upon bruchin treatment) in plant defense against both microorganisms and herbivores. Whether or not the increase in levels of the OPDAR and *MtN19*-like transcripts are related to plant defense is unclear, although it may be significant that specific OPDAR's are involved in biosynthesis of jasmonic acid, a plant regulator involved in mediating systemic resistance to herbivore attack.

P21

Surface hydrocarbons of queen eggs regulate worker reproduction in large colonies with highly fertile queens in the ant *Camponotus floridanus* (Formicidae, Hymenoptera)

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A hitherto unresolved problem is how workers are prevented from reproducing in large insect societies. The queen informs about her fertility and health which ensures sufficient indirect fitness benefits for workers. In the ant *Camponotus floridanus*, we found such a signal located on eggs of highly fertile queens. Groups of workers were regularly provided with different sets of brood. Only in groups with queen eggs workers refrain from reproducing. Thus, the eggs seem to inform the workers about queen presence. The signal on queen eggs is presumably the same that enables workers to distinguish between queen and worker-laid eggs. Workers from queenright colonies destroy worker-laid eggs whereas eggs from their own or a foreign highly fertile queen are accepted. Queen and worker-laid eggs differ in their surface hydrocarbons in a similar way as highly fertile queens differ from workers in the composition of their cuticular hydrocarbons. When we transferred hydrocarbons from the queen cuticle to worker eggs the eggs were no longer destroyed, indicating that they now carry the signal. There is no effect of sex of the egg or of caste specific origin. Eggs from queens with low egg-laying rates in small colonies show a similarly high destruction rate as worker-laid eggs when presented to workers from large colonies. Queens from such small colonies and such little egg-laying rates do not show the characteristic cuticular hydrocarbon profile and the respective egg profile that highly fertile queens show. The typical queen profile develops along with increasing fertility accompanied by an increasing colony size. In colony foundations chemical communication of queen status may not be necessary to prevent workers from reproducing, since workers may benefit more from investing in colony growth and increased productivity of large colonies rather than from producing male eggs in incipient colonies.

P22

Searching for pine weevil antifeedants: finding an active compound in bark from linden, *Tilia cordata* Mill.

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The pine weevil, *Hylobius abietis*, is a major pest on newly planted spruce and pine saplings in Sweden. To protect the plants they are treated with the pyrethroid Cypermethrin. We are searching for alternative protection methods and concentrate on antifeedants, compounds that either through taste or smell or both, protect the trees from weevil feeding. Earlier we have found that the weevils are feeding significantly less on linden branches than on pine branches. Our hypothesis was that the linden bark contains one or several compounds that act as antifeedants against the weevil. To identify these compounds, the bark was extracted and the resulting extract was tested for antifeedant activity. An active extract was found, which was fractionated by column chromatography and the resulting fractions were tested for activity. Two fractions were found to be active and the chemical content of these was determined.

P23

Physical and chemical defense in facultative and obligate ant mutualists in the genus *Piper*

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The tropical rainforest shrub *Piper cenocladum*, which is normally defended against herbivores by an obligately mutualistic ant, contains three amides that have various defensive functions. A closely related shrub, *Piper imperiale*, is facultatively inhabited by ant colonies. We describe amides isolated from the leaves of *Piper imperiale*, which appear to be present at lower concentrations in the plant than *P. cenocladum* amides. In order to compare the chemical and physical defenses of these two *Piper* species, we performed bioassays, toughness measurements, and herbivory surveys. Diets spiked with amides were offered to leaf-cutting ants (*Atta cephalotes*). Leaf toughness and herbivory by different classes of herbivores was measured for a random sample of *Piper* leaves. *P. cenocladum* and *P. imperiale* amides were strongly deterrent to *A. cephalotes*. Total herbivory and beetle herbivory were higher on *P. imperiale*, while *Eois* herbivory was more frequent on *P. cenocladum*, which was the tougher of the two species. Lacking obligate ant mutualists and possesing lower concentrations of amides and less tough leaves, *P. imperiale* appears to be significantly less well defended than *P. cenocladum*. Accordingly, *P. imperiale* tends to sustain herbivory by generalist herbivores more often and lose more leaf area than *P. cenocladum*.

P24

Bio-assay guided fractionation demonstrates that the sea lamprey migratory pheromone is a mixture of at least three sulfated steroids

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The sea lamprey (*Petromyzon marinus*) starts its life in freshwater streams which it then leaves to parasitize lake/oceanic fishes before eventually re-entering streams to spawn. Laboratory and field studies have shown that adult lampreys locate spawning streams using a pheromone released by stream-resident larval lampreys. Initial biochemical characterization of this cue found it to contain the sulfated bile acid petromyzonol sulfate (PS) which adult lampreys detect at 10^{-12} Molar (M). Using a combination of HPLC fractionation, olfactory recording, behavioral assays, and mass spectrometry we have recently isolated two additional compounds from larval holding water that have pheromonal activity. The most important has a molecular weight of 704 daltons, is sulfated, and is behaviorally attractive at concentrations below 10^{-14} M, a record for fish. The second has a molecular weight of 590, is also sulfated, and is less potent. Both appear to be novel compounds. In a two-choice preference maze, adult lampreys did not distinguish between larval water and a mixture comprised of PS and these two compounds, demonstrating that this mixture constitutes the majority of the pheromone. It seems likely that the lamprey has evolved to respond to multiple compounds released by larvae either to increase their sensitivity to larval odor or to discern it more specifically. Efforts are presently underway to elucidate the structures of the unknown sulfated compounds using various forms of NMR. Funded by the Great Lakes Fishery Commission.

P25

Glycerolipid biosynthesis in the sex pheromone gland of the moth *Heliothis virescens*.

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Most moths biosynthesize their sex pheromone components *de novo* through synthesis of the saturated fatty acids (FAs), palmitic or stearic, and limited metabolism of these acids to form unsaturated alcohols, aldehydes, or acetates. In addition to the sex pheromone components, the pheromone gland of most moths typically contains large quantities of glandular lipids (GLs), predominantly triacylglycerols. In addition to common saturated and unsaturated FAs, these GLs also contain significant quantities of FAs of the pheromone components and related intermediates. We have hypothesized that the GLs serve as a governor for FA concentrations in the gland, 'soaking up' FAs when concentrations are high (i.e., during pheromone biosynthesis) and releasing them when FA concentrations are low. In this way, GLs allow pheromone biosynthesis to proceed smoothly, rather than be punctuated by high or low FA concentrations, which might inhibit or limit enzyme function. Because studies on GL biosynthesis *in vivo* are complicated by the multiple fates of applied FAs (e.g., to GLs or to pheromone components), we have studied this process using a fatty acid (heptadecanoic) that has no pheromone fate. We report the results of this work here.

P26

Infochemical releases from aphids: kairomonal role of (E)-β-farnesene toward syrphid and ladybird predators

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The volatiles released from several aphid species were studied for their infochemical role in prey location. Using a four-arm olfactometer, the attraction of four aphid species, namely *Myzus persicae, Acyrthosiphon pisum, Megoura viciae* and *Brevicoryne brassicae* toward two aphidophagous predators at larval stages, the *Adalia bipunctata* ladybird and the *Episyrphus balteatus* hoverfly was observed. Whether the predatory beetle larvae were only attracted by *A. pisum* and *M. persicae* when they were crushed, the hoverfly positively responded to all aphid species as odour sources, either crushed or as non stressed whole aphids. Identification and quantification of the volatile releases from each aphid species were performed using SPME and GC-MS methods. Whether the (E)-β-farnesene (EBF), the common aphid alarm pheromone, was found alone in *A. pisum* and *M. persicae* volatile pattern, other molecules were associated with EBF from *M. viciae* and *B. brassicae* releases. As the presence of the (E)- β-farnesene seemed to be an efficient kairomone from aphid preys toward both predatory ladybird and hoverfly, pure EBF was tested in olfactometry assays on *A. bipunctata* and *E. balteatus* larvae. The latter molecule was shown to display kairomonal role toward both predatory species. In conclusion, even if both tested predators were considered to present polyphagous feeding behaviour, preferences according to the aphid species in relation with their volatile releases were shown for *A. bipunctata*.

P27

Comparative study of pupae cuticular hydrocarbons of flour beetles related to their cannibalism and predation vulnerability (Coleopterae: Tenebrionidae).

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There is evidence of cuticular hydrocarbons involvement in species recognition. Thus, cuticular hydrocarbons were extracted from 3-days old emerged *Tribolium* pupae with n-hexane at room temperature and GC-MS (gas chromatograpy-mass spectometry) investigations were performed to analyse profiles patterns. Components were quantified by n-eicosane as internal standart. The major cuticular hydrocarbons identified were n-C25; n-C26; n-C27; 3-Me-C27; n-C28; n-C29. Qualitative and quantitative significant differences were observed in cuticular hydrocarbon found between species. Cuticular hydrocarbon patterns are closely related to cannibalism and predation vulnerability patterns. The most intriguing case was remarkably expressed by *T. brevicornis* specie which pupae had the most of chemical compound found with the highest rate but were unable to be consumed by different predators. Impending investigations on chemical signals perception could probably explain this particularity.

P28

Preliminary investigations into olfactory cues involved in host ant recognition for an obligately myrmecophilous butterfly, *Jalmenus evagoras* (Lepidoptera: Lycaenidae).

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Within the Australian butterfly genus *Jalmenus* (Lycaenidae), most species share the same host plant (*Acacia*) but differ with respect to their mutualistic ant partner. Thus, a shift in host ant, rather than host plant, may facilitate genetic divergence and speciation in this group. In general, ant association (termed myrmecophily), is thought to be an important factor contributing to diversification within the Lycaenidae, particularly among obligately ant-associated taxa such as *Jalmenus*, whose larvae have species-specific associations with ants, and whose adults use host ants as cues when selecting egg-laying sites. Olfactory cues appear to play a role in host ant recognition by *J. evagoras* adults. To elucidate these cues, we used GC-EAD to record electroantennographic responses of butterfly antennae to headspace volatiles of an attendant ant species (*Iridomyrmex anceps*) and to a closely related, non-attendant ant species (*I. purpureus*). Up to eight response peaks were detected in each EAD recording; female and male antennae responded to the same array of chromatogram peaks. Volatiles from the two *Iridomyrmex* species elicited EAD responses in the same areas of their respective chromatograms. Identifications of electrophysiologically-active compounds are in progress using GC-MS. Preliminary work indicates that iridodial or related monoterpenoid compounds stimulate olfactory receptors in the butterfly antenna.

P29

Endocrine regulation of pheromone production in the pinyon *Ips*

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Bark beetles are among the most economically important forest pests in the northern hemisphere and are responsible for the loss of millions of cubic meters of coniferous standing timber each year. In fact, it is estimated that over 8 million hectares of forest in the western U.S. are at high risk of death by bark beetle attack, and forest health is declining at a rapid rate. Many bark beetles produce monoterpenoid pheromone components that coordinate host colonization and mating, and populations may be managed, at least at a local level, by disrupting pheromone biosynthesis. To fully exploit the pheromone-mediated behavior of these beetles, a thorough understanding of identity, expression, and activity of these chemical signals is paramount. *Ips confusus*, the pinyon Ips, has become a major pest in the southwestern United States, destroying hundreds of thousands of hectares of pinyon pines. In this study, we investigate the interplay between feeding on host phloem and the induction of *de novo* pheromone biosynthesis in *I. confusus*. We have found that feeding on host phloem strongly induces pheromone production in male *I. confusus*. Moreover, feeding, but not juvenile hormone (JH) III treatment, stimulates the activity of a key mevalonate pathway enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-R), only in males. However, feeding and JH III both significantly up-regulate mRNA levels of HMG-R and other mevalonate pathway genes. Our data suggest that JH III alone cannot stimulate pheromone biosynthesis in male *I. confusus,* and some other regulatory factor, perhaps a brain hormone, is required for pheromone production.

P30

Expression of conifer sesquiterpene synthases in *Arabidopsis* and tobacco.

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Terpenoids are a diverse group of natural products with a wide array of biological functions. Common examples include but are not limited to toxins, floral scent and parasitoid recruiting info-chemicals. Conifer trees contain a vast variety of terpenoids, making them a great pool for terpene synthase genes. Presently we commonly study these genes using *E. coli* and *S. cerevisiae in-vitro* expression assays. In an attempt to study terpenoid synthases and their functions more comprehensively, we have tested if model plant systems such as *Arabidopsis* and tobacco could express conifer sesquiterpene synthases. In this work, we show that it is possible to express (E)-α-bisabolene synthase, a sesquiterpene synthase, under the control of a wound and jasmonate inducible promoter (PINII) in both tobacco and *Arabidopsis*. These transgenic plants will allow us, in this case, to study (E)-α-bisabolene's effect on insects.

P31

The chemical ecology of the northern spruce engraver, *Ips perturbatus* (Coleoptera: Scolytidae), and associated insects in Alaska

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The attractant aggregation pheromone of the northern spruce engraver, *Ips perturbatus* (Eichhoff), consists of the three monoterpene alcohols, ipsenol, ipsdienol, and *cis*-verbenol. These pheromone components were identified by GC-FID and GC-MS from Porapak Q and abdominal tissue extracts, elicited responses in GC-EAD assays, and were attractive to flying beetles at two locations in Alaska in Lindgren funnel trap assays. Various combinations of verbenone and conophthorin, potential interruptive semiochemicals of *I. perturbatus*, were also bioassayed at south-central (Lutz spruce, *Picea xlutzii*) and interior (white spruce, *P. glauca*) Alaskan forest sites. The best interruptant was the synergistic combination of commercially available 84%-(−)-verbenone and racemic *trans*-conophthorin, which yielded reductions in trap catch ranging from approx. 30- to 150-fold, relative to attractant-baited control traps. The interruption by verbenone was enantiospecific; (−)-verbenone was more effective than (+)-verbenone. Three associated subcortical insects, *Pityophthorus nitidulus* (Mannerheim), *P. recens* Bright (both Scolytidae), and *Lasconotus borealis* Horn (Colydiidae) responded significantly to traps containing conophthorin. A fourth associated subcortical insect, the predator *Rhizophagus dimidiatus* Mannerheim (Rhizophagidae), responded significantly to all treatments containing the *I. perturbatus* attractant. A marked biodiversity of Alaskan subcortical insects was recorded in this study, including 14 species of bark and ambrosia beetles (Scolytidae), 17 species of longhorned beetles (Cerambycidae), 3 species of metallic woodboring beetles (Buprestidae), and 1 species of woodwasp (Siricidae). The collections of *P. recens* and two cerambycid species [*Pygoleptura brevicornis* (LeConte) and *Phymatodes maculicollis* LeConte] are new state records for Alaska.

P32

Cytotoxicity of leaf extracts and flavonoids against an insect cell line, CF-1

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Secondary compounds in the leaves of plants can act as a natural defence against defoliating insects. We used an agarose lawn assay to investigate the cytotoxicity of crude leaf extracts and purified flavonoid compounds in vitro. Freeze-dried ethanolic extracts of leaves from 10 species of trees and 15 purified compounds from various classes of flavonoids were assayed in this system against the lepidopteran cell line, IPRI-CF-1, and their cytotoxic activities compared. Samples were prepared in 50% dimethyl sulfoxide and applied directly against the cells without encountering any solvent toxicity. Threshold doses of flavonoids that were toxic to CF-1 ranged from 0.25 μg (quercetin and morin) to 16 μg ((+) catechin). The range for leaf extracts was 0.13 μg (mulberry, *Morus alba* L.) to 2 μg (scotch pine, *Pinus sylvestris* L.). The activities of several leaf extracts were markedly enhanced when their pH was increased to 10.5, similar to that of the larval lepidopteran midgut. Trembling aspen (*Populus tremuloides* Michx.) toxicity increased 16-fold at the higher pH. The pH effect was largely abolished in the presence of dilute larval gut juice.

P33

Identification and characterization of *O*-glucosyltransferases from Vitis sp. Involved in anthocyanin biosynthesis

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The terminal steps of anthocyanin biosynthesis involve different hydroxylation reactions that may be substituted with sugars, phenolics and/or methyl groups. Since each of these substitutions modify the color and/or stability of anthocyanins, they are important for breeding and biotechnological production of novel flower colors in horticultural crop species. In grapes the amount of anthocyanin pigments and the particular pigmentation that they contribute to red wine are very important for its visual attractiveness and antioxidant properties. As part of our program to identify and functionally characterize new genes in anthocyanin biosynthesis in grape, the UDP-glucose: anthocyanin 5-O glucosyltransferase (5gt) responsible for producing the dark blue 5-O-glucosylated anthocyanins found in many North American grape and some hybrid wine grape species has been studied. Molecular cloning of the 5gt from *Vitis labrusca* yielded a putative 448 amino acid protein exhibiting 30-50% identity to previously characterized 5gts from several other horticultural species. Developmental studies indicate that this gene is expressed in grape peels shortly before veraison (8 weeks after flowering) and it increases to a maximum at 16 weeks just before the grapes are harvested. The properties of this gene in relation to the biochemical production and accumulation of 5-O-glucosylated anthocyanins will be described.

P34

The role of odor receptors in odor coding

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We are undertaking a large-scale analysis of odor receptors in *Drosophila melanogaster*. The *Or* family of receptor genes contains ~60 members, which are expressed in at least 35 functional classes of olfactory receptor neurons (ORNs). We are examining the functions of odor receptors using a mutant fly that contains an "empty" antennal ORN (Δab3A) that lacks its endogenous odor receptors. Individual receptors are expressed in the empty ORN (Δab3A:OrX) and odor response is assayed electrophysiologically using a panel of diverse odorants. We are establishing a receptor-to-neuron map by matching the odor response spectra of Δab3A:OrX ORNs to the odor response spectra of wild-type ORNs. We have found that the odor receptor dictates not only the odor response spectrum, but also the signaling mode (excitation v. inhibition) and the response dynamics of the ORN in which it is expressed. Different receptors, when expressed in the same ORN and given the same odorant stimulus, can confer responses that differ in signaling mode. Moreover, an individual receptor can confer responses of different modes to different odorants in the same ORN. The results thus show that odor receptors contribute to multiple aspects of odor coding in *Drosophila* ORNs, and they suggest a model for odor receptor transduction. Finally, we express two odor receptors from the malaria vector mosquito *Anopheles gambiae* in *Drosophila* and find that the female-specific receptor AgOr1 responds to 4-methylphenol, a component of human sweat.

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P35

Neuroprotective activities of plant-derived phenolic compounds.

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Because secondary metabolites from plants possess biological activity of interest to human health, plants and plant-derived compounds are used throughout the world as medicines and dietary supplements. Naturally occurring phenolics, which are produced in abundance by a wide variety of plants, display several biological activities and often act as anti-oxidants. Considering the evidence implicating reactive oxygen species (ROS) in the development of differing neurodenegerative diseases such as Alzheimer's disease and diabetic neuropathy, plant-derived phenolics may be of therapeutic benefit. To test this hypothesis, a library of phenolic compounds (flavonoids, benzoates, and cinnamic acids) was screened for protection against various insults (hyperglycemia, PAF, etoposide, ceramide) where ROS is believed to contribute to neurotoxicity. Undifferentiated PC12 cells were grown and treated in 96-well plates and cell viability was determined colorimetrically by mitochondrial reduction of WST. By calculating the lowest bond dissociation enthalpy for the hydroxl groups of the tested compounds, theoretical anti-oxidant capacities were predicted and plotted against the compound's neuroprotectivity. Three compounds demonstrating robust protection in PC12 cells, quercetin, hesperitin and orsellinic acid, were selected for advanced assessment in dorsal root ganglion neurons.

P36

Pheromones use in smart-autoinoculation systems using insects as vectors of plant pathogens in weed biocontrol.

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Biological control of weeds has been traditionally centred on the use of insects and microbial pathogens as biocontrol agents. While each approach has brought some success in weed control, there is a potential to combine the use of these two groups of biocontrol agents to achieve a synergistic effect in further improving control. In New Zealand, there are currently over 250 weed species of economic importance with gorse (*Ulex europaeus*), broom (*Cytisus scoparius*) and thistle (*Circium* spp.) topping the list. While a number of insect biocontrol agents have been introduced, monitoring their establishment is difficult. However, with the success in identifying the sex attractants of two introduced insects- gorse soft shoot moth (*Agonopterix ulicetella*) and gorse pod moth (*Cydia succedana*), these tools have began to demonstrate value for comparing the seasonable pattern of insect and plant phenology, population size, and distribution. Current research is also being conducted to identify pheromones of other insects present in New Zealand for the purpose of biological control. Further, we now propose the development of a smart-autoinoculation system based on a tripartite model, using insects as deliberate and enhanced vectors of plant pathogens as a novel weed control strategy. This novel approach proposes using attractant-based systems to bring insects to the pathogen inoculum and thereafter infecting the weed ("lure-load-infect"). However, the potential for deliberately using insects to deliver plant pathogens to weed hosts can only be realized if a wide range of hypothetical tripartite system conditions is met. Each of the tripartite components (insects, pathogens and plants) brings ecological and biological complexity, including the need for practical functionality that constrains the possible options and risk of non-target impacts. The functionality of this system will require an evaluation of the life cycle compatibility of each component whilst management of non-target risks can be achieved by the choice of general or specialised characteristics of insects and pathogens used. Therefore, a feasibility analysis is required to evaluate the various options of the system i.e. full benefits and risks of each component. Hence, a strategic approach is to examine the specificity of interactions between the insect, pathogen and weed.

P37

Host selection by pine processionary moth *Thaumetopoea pityocampa* females

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Thaumetopoea pityocampa (Den. & Schiff.) (Lep.: Thaumetopoeidae) is one of the most destructive defoliators of natural and artificially established *Pinus* spp in the Mediterranean region. Infestation rates differ dramatically among species and tree individuals, suggesting female choice of hosts during egg-laying. We have identified two main factors affecting the host selection process:
- Tree silhouette, suggested as a dominant factor in the litterature. In a rearing room we offered to moths several *Pinus nigra* trees, a preferred species, of different sizes. A significant positive correlation was found between the number of egg masses laid on each tree and both tree height and silhouette.
- Volatiles of host plant may play an important role in habitat selection. We investigated the olfactory host recognition mechanisms of females by the gas chromatographic-electroantennographic technique (GC-EAD). We tested natural volatile blends and a synthetic mixture of monoterpenes present in pine needles. Repeatable responses were among others to minor compounds like *E* -ocimene, □-terpinol and neodihydrocarveol. In addition, the identification of suitable needles through contact sensilla on tarsi is evaluated as an additional input for the host choice by females.

P38

Major components in the hairpencil secretion of the danaid butterfly, *Euploea mulciber*, and evidence for their origin

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Male danaid butterflies secrete a diversity of characteristic compounds from the abdominal hairpencils which are thought to function as an aphrodisiac during the sequence of courtship. We examined the secretion components of field-caught males of *Euploea mulciber* by GC and GC-MS. Two major compounds were detected from a CH_2Cl_2 extract of the hairpencil: One was determined to be 9,10-epoxytetrahydroedulan (ETE), a compound previously identified from the butterfly, and the other, viridifloric–ß-lactone (VL), which is known to be one of the male sex pheromones of another danaid butterfly, *Idea leuconoe*. Various feeding experiments with larvae and male adults suggested that ETE is biosynthesized *de novo* probably from nutrients ingested by larvae. In contrast, laboratory-reared males never produced VL at all. Male adults of *E. mulciber* as well as other danaids are attracted to PA-containing plants in the field. When males were orally administered any one of three PAs, intermedine, heliotrine, and monocrotaline-HCl, only those fed with intermedine produced a significant quantity of VL. This clearly indicates that the production of VL exclusively depends on the acquisition of PA(s) of particular chemical structure(s) which contain trachelanthic or viridifloric acid as a necic acid moiety. Strong and selective attraction of males to intermedine observed in laboratory bioassays seems to further lend support to their limited ability of PA utilization.

P39

Apigenin-7-*O*-*p*-hydroxybenzoate, a new flavone ester from the fern *Pteris vittata*

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A new flavone ester has been found in *Pteris vittata* L.,a fern that iperaccumulates arsenic[1] and is reccomended for use in the remedation of arsenic-contaminated soils. This product has been isolated from an ethanolic extract of aerial parts of this plant by preparative paper chromatography followed by Sephadex LH-20 column chromatography . Electrospray mass spectrum, ultraviolet spectral analysis in the presence of usual shift reagents, acid hydrolysis and alkaline hydrolysis have shown that the isolated compound is apigenin-7-*O*-*p*-hydroxybenzoate, a new natural product. This is the first report of the occurrence of a flavone aglycone acylated with an aromatic acid in ferns; however some flavone and flavonol aglycones acylated with aliphatic acids (acetic and butyric) have previously been found[2] in ferns belonging to the genus *Notholaena* (Pteridaceae).

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P40

Gene expression profiles of *Drosophila melanogaster* adults exposed to the botanical insecticide *Piper nigrum*: identification of novel cytochrome P450 genes associated with insect defense

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The synergistic activity of *Piper nigrum* extracts in combination with pyrethrum has been investigated in toxicity assays with *Musca domestica* and *Drosophila melanogaster*. Tropical plants of the *Piper* genus (Piperaceae) are characterized by a high level of insecticidal activity, which has been attributed to the presence of secondary metabolites, particularly isobutylamides (piperamides) and lignans. *P. nigrum* or black pepper is a ubiquitous table spice and as such is readily available and is considered to be of low risk for humans (possesses GRAS status). The ethylacetate fraction of a *P. nigrum* extract was evaluated for use as a synergist with the classical botanical insecticide pyrethrum. When *Musca domestica* was employed as the test insect, LC_{50} values for contact toxicity were determined using a Potter's tower and a synergist ratio of 13 was obtained. This indicates a high level of synergy, comparable in efficacy to synthetic synergists such as piperonyl butoxide. A similarly high ratio was found to occur when *Drosophila melanogaster* was substituted as the test insect. Further investigation of this synergy has been pursued by a study of the gene expression profiles induced in *D. melanogaster* in response to treatment with *P. nigrum* used alone or in combination with pyrethrum. cDNA microarrays containing 7000 *D. melanogaster* genes were used for this purpose. This microarray study demonstrates changes in the regulation of three cytochrome P450 genes: *Cyp6a8*, *Cyp9b2* and *Cyp12d1*.

Laboratory and field evaluation of the insecticidal activity of *Piper* species (Piperaceae) employed alone or in combination with pyrethrum for the control of Dipteran species

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This study investigates the use of active botanical extracts as effective, low risk substitutes for synthetic insecticides and synergists. Tropical plants of the *Piper* genus (Piperaceae) are characterized by a high level of insecticidal activity which has been attributed to the presence of secondary metabolites, particularly isobutylamides (piperamides) and lignans. *P. nigrum* and *P. guineense* were evaluated as agents for the control of mosquito larvae which has been a growing concern since the advent of the West Nile virus in North America. Water pools containing $3^{rd}/4^{th}$ instar larvae of *Aedes atropalpus* were treated and control was achieved after 24 hours with a concentration of 6 ppm for both *P. nigrum* and *P. guineense*. A *P. nigrum* extract was evaluated for use as a synergist with the classical botanical insecticide pyrethrum. When *Musca domestica* was employed as the test insect, LC_{50} values for contact toxicity were determined using a Potter's tower and a synergist ratio of 13 was obtained. This indicates a high level of synergy, comparable in efficacy to synthetic synergists such as piperonyl butoxide. A similarly high ratio was found to occur when *Drosophila melanogaster* was substituted as the test insect. Further investigation of this synergy has been pursued by a study of the gene expression profiles induced in *D. melanogaster* in response to treatment with *P. nigrum* used alone or in combination with pyrethrum. cDNA microarrays containing 7000 *D. melanogaster* genes were used for this purpose.

P41

Isolation and identification of the ovipositional deterrent in the sweet pepper, *Capsicum annuum* against *Liriomyza trifolii*.

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Liriomyza trifolii (Burgess), which has a broad host range and a high pesticide resistance, is well known as a pest to many vegetables and crops in the world.
This fly species fiercely attacks a sweet pepper (Solanaceae) on young stage as well as other solaneceae plants. We, however, found that *L. trifoii* seldom attacked and laid an egg on the sweet pepper on mature stage. It is, therefore, tried to elucidate the factor of this resistance of *Capsicum annuum* on mature stage against *L. trifoii*.
Giving the sweet pepper leaves on the four-leaves stage, this fly could leave a lot of the ovipositional marks (3.51 marks/cm^2 ± 1.65) on them. On the contrary, giving the leaves on mature stage, the fly could not leave the marks (0.76 marks/cm^2 ± 0.26) on the leaves at all. Even when *L. trifoii* was allowed to feed on the kidney bean's primary leaves which were treated with the MeOH extract (1 g leaf equivalent/ml) of the top leaves of sweet pepper in fruiting period, the ovipositional marks could not observed on it at all. These bioassay results suggested that the resistance of *C. annuum* on mature stage to *L. trifoii* was based on the ovipositional deterrent in the leaf.
According to the bioassay guidance, the ovipositional deterrent was isolated and identified as luteolin 7-*O*-□-D-apiofuranosy-l→2-□-D-glucopyranoside through the spectroscopic analysis.

P42

Gene expression in the pheromone producing midgut of the pine engraver beetle (*Ips pini* (Say))

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The pine engraver beetle is a significant pest of North American coniferous forests. The male is the pioneering sex and attracts mates to its nuptial chamber in the phloem of a host tree with a pheromone. Upon feeding, and regulated by juvenile hormone III (JH), the pheromone component ipsdienol is biosynthesized *de novo* via the mevalonate pathway in the male midgut. Identifying and characterizing genes in the midgut that respond to feeding or JH-treatment will provide information on the regulation of pheromone biosynthesis and might yield new targets for novel pest management strategies as well as the mode of JH action. We used cDNA microarrays and quantitative real-time PCR to examine gene expression in midgut tissue of fed, JH-treated, and control beetles of both sexes. Several genes were significantly up or down regulated by JH-treatment or feeding, including known mevalonate pathway genes, other known genes, and unknown genes. Transcript levels in the midgut of both sexes respond to JH and feeding although there was a sex-specificity consistent with pheromone biosynthesis.

P43

Efficient synthesis of the main pheromone component of the pink gypsy moth, *Lymantria mathura*, (2*S*,3*R*)-2-(2*Z*,5*Z*-octadienyl)-3-nonyloxirane

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The pink gypsy moth, *Lymantria mathura* Moore, is a widespread defoliator of hardwood forest and orchard trees in Asia. It could represent a potential threat to North American forest ecosystems and forest industries, if introduced. For detection and monitoring of this invasive species we have developed two new syntheses of its main sex pheromone component, (2*S*,3*R*)-2-(2*Z*,5*Z*-octadienyl)-3-nonyloxirane. The principal steps in the first route were the construction of (*Z*,*Z*)-1-bromo-1,4-heptadiene and the coupling with 2-iodomethyl-3-nonyloxirane via a Grignard reaction. The second method benefited from a straightforward alkylation of 1,4-heptadiynyl lithium with epoxy triflates in ether/hexane. The ~4:1 ratio of pheromone enantiomers, the most attractive to pink gypsy moth males, can be directly crafted using the Sharpless asymmetric epoxidation. Field trapping of the male *L. mathura* with the synthetic pheromone mixtures will also be discussed.

P44

Relationships between terpene deployment and leaf structure in *Eucalyptus polybractea*

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Eucalyptus polybractea (R.T. Baker) is an Australian terpene-accumulating species, which stores terpenes in specialized oil glands embedded within the leaf lamina. Using regression analysis, we showed that leaf thickness, measured as leaf mass per area (LMA), influenced terpene content, apparently through regulation of gland dimensions, and thus, gland volume. Given that LMA is known to be governed in part by prevailing environmental conditions, terpene deployment may therefore be theoretically regulated by environmental factors such as nutrient supply or drought, either directly through regulation of supply side processes, or indirectly by regulation of LMA. However, neither water stress, measured using carbon isotope ratios as an indicator, nor nutrient stress, measured as foliar nitrogen and phosphorus content, accounted for observed variation in either terpene content or LMA in field grown trees. Phenolic content, measured as a possible competing carbon sink, did also not account for variation in terpene content and variation in environmental stresses could not account for differences in growth rate.

P45

Chemical defenses in freshwater plants: activated or constitutive

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Although macrophyte-herbivore interactions in freshwater systems were generally disregarded for many years, recent data suggest that herbivory can be intense and important in structuring freshwater communities. This has led to the hypothesis that chemical defenses should be common among freshwater plants, but few studies have reported such chemical defenses, and no previous studies have assessed the frequency of chemical defenses among a substantial number of freshwater plant species. In a study of *21* macrophyte species co-occurring with the omnivorous crayfish *Procambarus vernicosus in* a Southeastern USA wetland environment, we found that extracts of I I species (52 %) deterred feeding by P. verrucosus when tested in artificial foods at natural concentrations. Of these I I chemically defended species, one species, Eupatorium capilfifolium, consistently had a more unpalatable extract following mechanical damage to plant tissue, indicative of an activated chemical defense. Because herbivores are commonly nitrogen-limited and select food based on several plant traits, including plant nutritional value, it might be expected tkat chemical. defenses would be especially important for protein-rich plants. However, we found no relationship between soluble protein concentration and deterrence of plant extracts. Overall, chemical defenses are common among freshwater plants, but activated defenses appear to be rare.

P46

Male-produced aggregation pheromone of the cerambycid beetle *Neoclytus acuminatus acuminatus*

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Field studies with male and female *Neoclytus acuminatus acuminatus* (F.) (Coleoptera: Cerambycidae) caged separately on host logs revealed that adult males produce a compound that attracted beetles of both sexes. This response was confirmed in outdoor olfactometer studies. Extracts of odors from caged males contained a single major male-specific compound, identified as (2*S*,3*S*)-hexanediol. In field trials, both sexes of *N. a. acuminatus* were attracted by the racemic blend of (2*S*,3*S*)- and (2*R*,3*R*)-hexanediols. Subsequent field bioassays determined that the racemic blend was comparable in activity to enantiomerically enriched (2*S*,3*S*)-hexanediol (e.e. 80.2%), but a blend of all four 2,3-hexanediol stereoisomers attracted few beetles, indicating inhibition by one or both of the (2*R**,3*S**)-stereoisomers. This is the first fully verified report of an aggregation pheromone produced by a cerambycid beetle species. Fourteen of 15 females of a second cerambycid species, *Curius dentatus* Newman, were attracted to traps baited with the four component blend. This suggests that it is likely other species in this large subfamily will be found to use variations of this structural motif as attractant pheromones.

P47

Antioxidant activity of wheat pearling fractions and their by-products

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Pearling is a process by which layers of seeds are removed to different depths. Wheat pearling fractions (10, 20, 30, 40 and 50% pearling from outside inwards) and their by-products from the wheat cultivar CWAD, grown in Western Canada, were examined for their total phenolic content and *in-vitro* antioxidant activity. Crude phenolics were extracted into 80% ethanol. Total phenolic content (TPC) was determined using Folin-Ciocalteu reagent and expressed as mg ferulic acid equivalents per g extract (FAE/g). Total antioxidant activity (TAA) was determined by Trolox equivalent antioxidant capacity (TEAC) assay and expressed as μM Trolox equivalents (TE). The antioxidant activity was evaluated in terms of the ability of wheat extracts to scavenge a variety of free radicals {1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion, hydroxyl} and inhibit copper-induced oxidation of human low density lipoprotein (hLDL). In addition, the reducing power of wheat extracts was determined and expressed as μM ascorbic acid equivalents (AE). TPC of wheat pearlings and their by-products was 5.7-13.1 and 6.1-26.5 FAE/g, respectively. The corresponding TAA was 10.4-18.8 and 21.7-35.2 TE. DPPH, superoxide anion and hydroxyl radical scavenging activities of wheat pearling fractions were 46.7-62.6, 36.6-43.3 and 65.9-77.9%, respectively. The corresponding data for their by-products were 57.2-91.9, 39.2-57.1 and 69.7-83.7%. Wheat pearling fractions and their by-products inhibited hLDL oxidation by 50.6-68.9 and 56.5-94.8%, respectively. The reducing power of pearled wheat was 44.1-104.3 AE while that of by-products was 74.1-218.8 AE. The antioxidant activity decreased toward the interior of the seed, corresponding to a decrease in the content of phenolics towards the interior of the seed and in agreement with results of a previous study on wheat bran and its milling fractions.

P48

Bumblebee males of *B. lucorum*: Connection between lipid metabolism and biosynthesis of the male marking pheromone.

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The equimolar blend of deuterium labeled fatty acids was applied to the head or to the abdomen of a bumblebee body. The metabolites formed were identified and quantified in the different parts of body by a two-dimensional GC with MS TOF detection. Labeled analogs of pheromone components such as saturated and unsaturated esters and alcohols were found in the labial gland. Labeled fatty acids were also built into lipids of the fat body; incorporated precursors have been identified in the fraction of triacylglyceroles, diacylglyceroles and free fatty acid in the fat body of the male. The haemolymph and the rest of body were searched for labeled metabolites as well. Metabolites originated from the labeled fatty acids in the different tissues were compared and balanced.
If the precursors were applied into the abdomen, we found more unsaturated esters than in case of application into the head. This confirms our previous assumption that desaturases are not localized directly in the labial gland.
Generally, the highest amount of hexadecanoic acid was metabolized in the labial glands and incorporated into lipids and other tissues. The least incorporated acid was dodecanoic acid. Such high level of this acid is not physiological for a bumblebee organism and most of it was probably catabolized and eliminated. Higher level of deuterated metabolites was found in the head-application then in the abdomen-application. We assume that a massive enzyme system for modification of free fatty acid to the pheromonal components is localized in the labial gland.

P49

Is the post-mating resumption of sexual receptivity in *Choristoneura rosaceana* females associated with eupyrene sperm depletion in the spermatheca?

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In *Choristoneura rosaceana*, it has been previously demonstrated that females receiving a small spermatophore from a previously-mated males were significantly less fecund and fertile than those receiving a large spermatophore from a virgin male. Subsequent studies revealed that the decline observed in the size of the spermatophore with successive matings was associated with a concomitant decline in the number of both apyrene and eupyrene transferred at the time of mating. In Lepidoptera, it is well known that sperm present in the spermatophore, located in the bursa copulatrix, must migrate to a storage organ, called spermatheca in order to fertilise the eggs and stimulate oviposition. Furthermore, in C. rosaceana the presence of sperm within the spermatheca is also involved in the post mating inhibition of pheromone synthesis, a phenomenon called pheromonostasis. However, multiply-mated females are frequently observed in natural populations of C. rosaceana suggesting that pheromonostasis is a transitory rather than permanent process. Consequently, we hypothesised that the ability of mated *C. rosaceana* females to resume sexual receptivity is probably associated with the progressive loss of sperm within the spermatheca. We, therefore, predict that (i) females mated with previously-mated males would be more likely to resume pheromone production and remate sooner than those mated with virgin ones, and (ii) the level of sperm in the spermatheca would be similar at the time females resumed calling, irrespective of whether they were inseminated by previously-mated or virgin males. In this paper, we present results that partially supported these predictions.

P50

Biosynthesis of 2-alkanones and of mandibular acids in honey bees and bumble bees.

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In the course of our study on semiochemicals in social bees, worker mandibular glands components of honey bees and bumble bees were analyzed by GC/MS. 2-Alkanones (C7 and C9) and 3-hydroxyalkanoic acids (C8 and C10) were identified in three *Bombus* species. In *Apis* species, the Japanese honey bee, *Apis cerana japonica*, gave 3-hydroxyotanoic acid as a forager-specific major compound with a small amount of 2-heptanone, whereas *Apis mellifera* gave about 1 microgram of 2-heptanone and a trace of 3-hydroxyoctanoic acid. These results implicate that 2-alkanones in bee mandibular glands are biosynthesized from corresponding 3-hydroxyalkanoic acids by oxidative decarboxylation.
In order to demonstrate this hypothesis, deuterated 3-hydroxy alkanoic acids were prepared and applied onto worker mandibular glands of *Apis* and *Bombus* species. After incubation, the mandibular glands were extracted with ether and analyzed by GC/MS.
In *Apis mellifera*, deuterated 3-hydroxyoctanoic acid was smoothly transformed into deuterated 2-heptanone. Results from *Apis cerana japonica* and other *Bombus* species will be presented.

P51

Heterologous expression of two *Fusarium* trichothecene P450 genes

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Fusarium graminearum and *F. sporotrichioides* produce the trichothecene mycotoxins 15-acetyldeoxynivalenol and T-2 toxin, respectively. In both species, disruption of the P450 monooxygenase-encoding gene *Tri4* blocks production of the mycotoxins and leads to the accumulation of the trichothecene precursor trichodiene. To further characterize its function, the *F. graminearum Tri4* (*FgTri4*) was heterologously expressed in the trichothecene-nonproducing species *F. verticillioides*. Transgenic *F. verticillioides* carrying the *FgTri4* converted exogenous trichodiene to the trichothecene biosynthetic intermediate isotrichodermin. Conversion of trichodiene to isotrichodermin requires seven steps, two of which can occur non-enzymatically. Precursor feeding studies done in the current study indicate that wild-type *F. verticillioides* has the enzymatic activity necessary to carry out the seventh step, the C-3-acetylation of isotrichodermol to form isotrichodermin. Together, the results of this study suggest that the *Tri4* protein catalyzes the remaining four steps and is therefore a multifunctional monooxygenase. We also used this approach to look at oxygenation steps near the end of the biosynthetic pathway controlled by *Tri1*.

P52

Molecular analysis of wound-inducible condensed tannin accumulation in trembling aspen, *Populus tremuloides* Michx.

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Many plant stress responses involve rapid changes in gene expression resulting in an accumulation of protective metabolites. In trembling aspen (*Populus tremuloides* Michx.), mechanical wounding of leaves has been found to induce an accumulation of condensed tannins (CTs), antinutritive compounds that may deter feeding by some insects. This induction is preceded by an upregulation of genes encoding flavonoid biosynthetic enzymes. Our research aims to characterize inducible CT accumulation in trembling aspen at the molecular level and to identify key regulatory genes controlling this response. Several biosynthetic genes thought to be key in channeling flavonoid metabolites into CT production have been isolated and their functions and expression are being studied. Additionally, a number of genes encoding Myb transcription factor proteins sharing high sequence similarities to known regulators of flavonoid biosynthesis have been isolated. Several of these exhibit stress-inducible upregulation expression patterns that correlate with the activation of flavonoid biosynthetic genes. The functions of these regulators are being studied further. This research will contribute to our understanding of what may be an important inducible defense mechanism, with potential applications in tree biotechnology.

P53

Correlation between antioxidant and hypoglycemic activities in eight *Bauhinia* species.

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Bauhinia plants belong to the family Leguminosae and are very know in Brazil as possessing hypoglycemic effects. In Rio de Janeiro State, 8 species belonging to this genus were collected in order to ascertain which of them has in fact that activity. In a complete study in animals fed and not fed we could conclude that only half of them possess that activity. For all the aqueous extracts tested, an HPLC was made in order to achieve their chemical (chromatographic) profiles. It was observed that the four species with some hypoglycemic activity had in their chromatogram one signal in the same retention time. This compound was analyzed by NMR techniques and its structure could be determined as a flavonoid glucoside. The other species with no detectable activity showed in the HPLC profile a great number of signals in higher retention times, probably other flavonoids. Since flavonoids are molecules with very know antioxidant activity, an evaluation was made for each *Bauhinia* ethanolic extract in order to compare with the hypoglycemic activity. A good correlation could be observed when this analyses were made. Acknowledgements: FUJB, FAPERJ, PRONEX, CNPq, CAPES.

P54

You are what you eat: the role of diet on glucose oxidase activity.

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Plant responses to insect herbivory have traditionally been viewed as an extension of the wound response; however, it is now clear that although there are shared components with mechanical damage, insect herbivores elicit distinct responses in plants. In fact, plants are able to differentiate between herbivorous Lepidopteran species and elicit specific defences against distinct pest species. Elicitors present in the oral secretions of caterpillars are believed to be a key mechanism used by the plant to identify the herbivorous species. These elicitors not only induce plant responses but recent evidence suggests that they may also suppress defense responses. Hydrogen peroxide produced by the salivary enzyme glucose oxidase (GOX) suppresses herbivore-induced nicotine production in tobacco plants (Nature (2002) 419:599). Throughout larval development, GOX activity fluctuates in caterpillars of the beet armyworm, *Spodoptera exigua*, with the highest levels associated with 4^{th} instar salivary glands (0.161 U/mg protein, pH 7.0). However, salivary gland GOX activity was 3.4 times higher in caterpillars reared on artificial diet compared with those fed plants. In gel enzyme assays verify that as quickly as an hour after transfer from the plant to artificial diet, an increase in GOX activity is observed. This implies that a factor in the diet is involved in the regulation of salivary enzyme activity, perhaps, at the transcriptional level.

P55

Cyanogenesis in Australian tropical rainforests: resource allocation to a nitrogen-based defence

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A quantitative study for cyanogenesis was conducted in upland and lowland tropical rainforest in far north Queensland, Australia. There have been few community-based studies on the frequency of cyanogenesis, no work on cyanogenesis in Australian rainforest communities, and no quantitative studies in relation to resource availability at the community level. As a constitutive, resource-demanding defence, effective against generalist herbivores, cyanogenesis provides an ideal system to test the effect of nutrient availability on deployment of a nitrogen-based defence in plant communities. Specifically, this survey aimed to investigate the hypothesis that relatively resource-rich environments will support a greater abundance of cyanogenic plants as measured by the number of cyanogenic species, and the contribution of these species to total biomass. All species occurring in 30 plots (200 m^2) at five sites were screened for cyanogenesis. Two pairs of sites on the Atherton Tableland, matched for rainfall and altitude, but differing in soil nutrient status were surveyed, as well as one site in lowland rainforest. Over 400 species from 85 families were screened. Within upland rainforest, substrate nutrient status significantly affected foliar nutrients; there was greater foliar nitrogen, a greater number of cyanogenic species, and greater contribution of cyanogenic species to total biomass on higher nutrient sites. The majority of cyanogenic species in the survey are endemic to Queensland. Cyanogenesis has not previously been reported for 17 of the 18 cyanogenic species. In several cases, the species belong to plant families or orders in which cyanogenesis has been little reported, if at all. The cyanogenic glycosides were identified for several species from families in which cyanogens are little known. Findings include novel distribution of known cyanogenic glycosides, and one potentially novel cyanogenic glycoside.

P56

Isolation of terpenoids from sunflowers as oviposition stimulants for the banded sunflower moth, *Cochylic hospes*.

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The banded sunflower moth (*Cochylis hospes*) is endemic to North America and restricted to the genus *Helianthus*. It is a major pest of sunflower crops in the northern Great Plains. Females lay the majority of eggs on bracts of pre-bloom sunflower heads, and larvae feed on bracts and developing seeds. Extracts made by dipping sunflower heads in 3:1 methanol/dichloromethane for 60 seconds stimulated oviposition by *C. hospes* when applied to filter paper disks in a laboratory bioassay. Bioassay directed chromatographic fractionations of these extracts resulted in the isolation of several terpenoids, which were identified by NMR spectroscopy and mass spectrometry. Testing the purified compounds in the bioassay allowed relative activities and dose responses to be determined, and suggested structure-activity relationships.

P57

Inter- and intra-specific activity of compounds identified from sex pheromone glands of currant borer, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae)

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Gas chromatographic and mass spectrometric analyses of crude sex pheromone gland extracts revealed that virgin *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae) females produced 6 compounds structurally related to sex pheromone components of clearwing moths. Comparing retention times and mass spectra of synthetic standards these compounds were identified as *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OAc, *Z*13-18:OAc, *E*2,*Z*13-18:OH, *Z*13-18:OH and 18:OH in the ratio 100:0.7: 2.7:3.2:traces:traces. The first 3 compounds have been previously known to occur in the sex pheromone gland extracts of currant borer, while the last 3 chemicals are reported for the first time. Trapping tests carried out in the black currant field revealed that *E*2,*Z*13:OAc, tested separately, attracted *S. tipuliformis* males, while addition of *E*3,*Z*13-18:OAc to the main component increased effectiveness of *E*2,*Z*13:OAc over seven times. Attractiveness of 6 component lures did not differ significantly from the one of the binary mixture confirming that, *E*2,*Z*13-18:OAc and *E*3,*Z*13-18:OAc in the ratio100:0.7 are essential sex pheromone components of *S. tipuliformis*. Trapping tests carried out at the edge of a deciduous forest with old birch-trees dominant which is typical dwelling place of *Synanthedon scoliaeformis* (Borkhausen) (Lepidoptera: Sesiidae) revealed, that in addition to synergistic effect, *E*3,*Z*13-18:OAc increased specificity of pheromone signal for *S. tipuliformis* acting as attraction antagonist to *S. scoliaeformis* males. Other compounds identified from sex pheromone gland of *S. tipuliformis* did not have significant effect on attraction of *S. scoliaeformis* males.

P58

Chemical and ecological relationship between the olive (*Olea europaea*) and the olive weevil (*Dyscerus perforatus*) (Part II)

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The olive weevil, *Dyscerus perforatus*; Coleopetera; Curculionidae, is a native species in Japan and now the most serious pest of the olive tree. Originally, this weevil seemed to colonize on *Ligustrum japonicum* Thunb and other trees, which belong to the oleacea family, like the olive. However, when olive trees were introduced to Japan and planted on large scale, the weevils immediately attacked the plants and soon preferred them to the former hosts. Unlike in the former hosts where the weevils live in low population densities, it is extraordinary high in the case of the olive tree and the assault thereby becomes seriously damaged for the host plant. During the course of our study on the relationship between the olive tree and the olive weevil, we have been interested in the possible chemical constituents of this plant, that are responsible for host selection and attraction of the olive weevil. Previously, we reported that a secoiridoid glucoside, some lignans andβ-sitosteryl-D-glucoside from the olive tree stimulated feeding habit of the weevil.

In this study, we found that the olive trees and the olive weevils release some volatile compounds as attractants to the weevils, since the preliminary bioassay by using the olfactometer showed that the weevils were attracted by these volatiles. Therefore, we are trying to isolate and identify the attractive components. Here, the characterization and the activity of such attractants will be discussed.

P59

GC/MS analysis of frass volatiles of *Platypus quercivorus* (Coleoptera: Platypodidae)

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The mass attack of oak platypodid, *Platypus quercivorus* (Coleoptera: Platypodidae), to *Quercus crispula* resulted in the heavy mortality of oak in Japan. The fungus, vectored by the beetle and designated as *Raffaelea quercivora*, disordered the water flow in the trunk of attacked oak trees. It is the first case that the ambrosia beetle was demonstrated to kill the healthy host tree with the associated pathogen.

The morphology of frass dumped from the gallery of oak platypodid changed according to the reproductive status of it. The GC-MS analysis of the head space volatiles of the frass revealed that the frass of different shape emitted different volatiles. At the beginning the reproductive sequence of the ambrosia beetle, unmated pioneer males tunnelled galleries, produced monoterpenes and impregnated them into the fibrous frass. While mated females engraved galleries, only sesquiterpenes were detected as the volatiles of the round shaped fibrous frass. Although hatched larvae helped their parents to tunnel galleries and produced a large quantity of granular frass, the amount of volatiles from the frass was completely reduced.

P60

Host range expansion and host shifts inferred from oviposition stimulants in papilionid butterflies.

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Papilionid butterflies are thought to have evolutionarily changed their hosts, probably from Aristolochiaceae, via Magnoliaceae, Lauraceae, Annonaceae, and Rutaceae, eventually to Apiaceae. Although many investigations have demonstrated that phytochemicals played a significant role in their host selection, very little is known about host-plant chemicals that can reasonably explain how they have accomplished drastic shifts in host affiliation.
Two compounds (*trans*-4-hydroxy-*N*-methyl-L-proline and 2-*C*-methyl-D-erythronic acid) identified from *Toddalia asiatica* and *Glycosmis citrifolia*, and five compounds (stachydrine, proline, quinic acid, synephrine, and choline) from *Citrus depressa,* were found to synergistically stimulate oviposition by *Papilio polytes* (Rutaceae feeder). On the other hand, pinitol, identified from *Michelia compressa* and *Magnolia grandiflora* stimulated oviposition by *Graphium doson* (Magnoliaceae feeder). It is intriguing that certain cyclitols and related compounds are also involved in stimulation of oviposition by some Aristolochiaceae and Rutaceae feeders. Based on the present findings and previous data, we discuss possible phytochemical mechanisms involved in host range expansion and host shifts in papilionids in relation to the evolution of biosynthesis of cyclitols in plants.

P61

Behavioural differences in host selection between the generalist and tobacco-specialized subspecies of *Myzus persicae*

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Host specialization in aphids may involve different strategies for host selection and acceptance. *Myzus persicae* is a generalist aphid that uses more than 400 plant species as hosts; however, a subspecies highly specialized on tobacco has been recognized. These characteristics make these aphids particularly suitable for the study of the effect of host range on host selection behaviors. We studied the first two steps of the host selection process by alate virginoparae of the generalist and tobacco-specialist aphids on host and non-host plants using three kinds of behavioral assays: wind tunnel, olfactometry, and video-recording. We demonstrated that alate virginoparae of the subspecies of *M. persicae* specialized on tobacco recognized and chose their host plant more efficiently than generalist *M. persicae*, on the basis of olfactory and visual cues, and factors residing at cuticular and subcuticular levels. Host recognition was evident before phloem tissues were contacted. Olfactory cues were not apparently involved in host selection by generalist *M. persicae*. Host specialization is concomitant to behavioral adaptations to the physical and chemical defense mechanisms of tobacco.

P62

Identification, synthesis and activity of sex pheromone components of the autumn gum moth, *Mnesampela privata,* (Lepidoptera:Geometridae), a defoliator of *Eucalyptus*

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The autumn gum moth, *Mnesampela privata* (Lepidoptera:Geometridae) is an endemic Australian moth whose larvae feed upon species of *Eucalyptus.* The moth´s favoured host plants are *E. globulus* and *E. nitens* which are the most important species used in commercial plantations of the Australian pulpwood industry. The autumn gum moth represents a threat to this industry and has become one of the most significant outbreak insects of eucalypts plantations throughout Australia. The purpose of this study is to identify the sex pheromone of the autumn gum moth and then use the sex pheromone to take control of the moth without using any synthetic pyrethroids.
Using gas chromatography-mass spectrometry (GC-MS) and gas chromatography analysis with electroantennographic detection (GC-EAD) we are at the moment working with the identification of the found compounds in the autumn gum moth. So far we have identified three of these compounds.

P63

Feeding behaviour of forest tent caterpillars on nutrient supplemented leaves

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Levels of primary nutrients and secondary metabolites vary both within and between trembling aspen trees (*Populus tremuloides*). Herbivores, including the forest tent caterpillar (*Malacosoma disstria*), exhibit differences in feeding behaviour and in performance on aspen foliage from different sources. Our study was designed to assess the effects of the ratio of the primary nutrients, protein and sugar, in foliage on the performance and choice behaviour of the forest tent caterpilllar, an oligophagous forest folivore whose primary host is aspen. Fourth-stadium larvae were fed aspen leaves supplemented with 5% (wet weight) either casein or sucrose. Sugar supplementation increased survivorship relative to control leaves, but decreased development and growth rates; protein supplementation had no significant effects. Behavioural observations showed that the insects did not differentiate between supplemented leaves over the short-term, but, over the longer term, they fed slightly less on the protein supplemented leaves than on control or sugar-supplemented leaves. Decreasing the protein:carbohydrate ratio via sugar supplementation does not appear to influence feeding behaviour and has ambiguous consequences on fitness. Increasing the protein:carbohydrate ratio via protein supplementation slightly decreases preference for that food but has no effect on performance.

P64

Anthocyanins of *Cleome hassleriana*, Capparidaceae.

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Cleome hassleriana, commonly known as spider flower because of its long thin stamens, is widely grown as a garden annual. The plants may grow as tall as 180 cm and have large terminal racemes of white, rose or mauve flowers that bloom continuously. Five variations of petal colour were selected for the present study: white (#1), pale rose (#2), deep rose (#3), pale mauve (#4) and deep mauve (#5). The buds of #s 2, 3, 4 and 5 gradually develop pigmentation as they expand, requiring about 18 days at 20C to reach maturity. The paler colours of #2 and #4 reach maximum concentration by about 10 days whereas the deeper colours of #3 and #5 continue to be produced until maturity. Pigmentation is localized in both the lower and the upper epidermal cells. The pigmentation of #3 and #5, however, is accompanied by the appearance of pigment in the mesophyll cells and of intensely coloured spherical structures, one per cell, but not necessarily in every cell and not necessarily always accompanied by soluble pigmentation. These structures are variously called anthocyanoplasts, cyanoplasts, or anthocyanic vacuolar inclusions (AVIs) and are known to occur in a wide range of plants. The intensity of pigmentation in petals of all shades declines markedly the day after full opening and the AVIs disappear. Extraction of the pigments and their separation by TLC and HPLC show that several acylated anthocyanins are present. Hydrolysis of the pigments shows that those of #2 and #3 are pelargonidin glycosides whereas those of #4 and #5 are cyanidin glycosides. No pigment band could be specifically linked to the presence, or absence, of AVIs but the deeper shades had at least one more such band than did the paler shades. Kaempferol glycosides are the dominant flavonols. Further analysis of the pigments is in progress.

P65

Electrophysiological and behavioural activity of chocolate volatiles: attractants for the pyralid moths *Ephestia cautella* and *Plodia interpunctella*

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The use of food odours to monitor females in pest populations would be a valuable complement to pheromone-based methods. In a chocolate factory stored product moths were observed flying in the vicinity of containers with chocolate-based products. We tested three chocolate-based products in flight tunnel experiments and found that they all attracted males and mated females of the pyralids *Ephestia cautella* and *Plodia interpunctella*. The substances in chocolate-based products were isolated by two different extraction methods; steam distillation and head space collection. GC-EAD experiments revealed several electrophysiologically active substances in the different products. The extracts and headspace samples were analysed with GC-MS and the substances identified. The electrophysiological activity of the corresponding synthetic samples was investigated by EAG, and three components with a positive dose-response relationship were detected for both species and sexes, ethyl vanillin, phenylacetaldehyde and nonanal. These three compounds were also behaviourally active when tested individually in a flight tunnel. A blend of the three components induced landing rates corresponding to the levels found when testing chocolate-based products, suggesting that we had found the chemicals mediating the attraction to chocolate. Field tests with the blend are now running to evaluate the potential use in monitoring traps.

P66

Origin of a species: potential impacts of peripheral chemoreception on host fidelity in the rhagoletis species complex

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The recent shift of *Rhagoletis pomonella* (a true fruit fly) from its native host hawthorn to introduced, domestic apple has been implicated as an example of sympatric speciation. Studies indicate that differences in host plant preference are important in reproductively isolating *pomonella* flies via premating barriers to gene flow. Single cell electrophysiology was used to determine if differences in peripheral chemoreception could contribute to host preference and fidelity in the *pomonella* group. Individuals from apple, hawthorn, and flowering dogwood origin populations were analyzed as well as *R. mendax*, the most closely related confirmed species. Host volatiles were selected as stimuli from electroantennographic and/or behavioral studies of host fruit. Analysis of olfactory receptor neuron (ORN) responses revealed similar chemical selectivity and topographical receptor location in all populations. However, population comparisons revealed differences in ORN sensitivity and adaptation rate to various host volatiles. It is concluded that these differences in peripheral sensitivity and adaptation rate may contribute to host fidelity in the *pomonella* group, which plays a key role in the speciation of this complex.

P67

Priority of color to scent: chromatic and olfactory responsiveness in flower visiting by the Indian red admiral, *Vanessa indica*

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Adult butterflies conduct pre-alighting flower selection using several sensory cues such as color and scent. However, the relative significance among different cues is likely variable with butterfly and flower species. In this study, we attempted to reveal the chromatic and olfactory cues that stimulate flower visiting and the preference hierarchy between them using naïve adults of the nymphalid butterfly *Vanessa indica*. Among 12 different-colored models, *V. indica* frequently visited yellow and blue, indicating the color preference in flower visiting. On the basis of the proboscis extension reflex (PER), we also examined the olfactory preference among 16 single compounds identified in the floral scents of *Taraxacum officinale* and *Cirsium japonicum*. The six compounds, including benzaldehyde and nerolidol, had relatively high PER-eliciting activities. In two-choice bioassays with purple flower models, the color of which was relatively unattractive to the butterfly, the scented models with these active compounds were significantly more attractive than the odorless controls. However, the effect of odorizing was not conspicuous with the yellow models, and the butterfly significantly preferred the odorless-yellow models to the scented-purple ones. These results demonstrate that flower visiting by *V. indica* is more dependent on color than scent.

P68

Major components in the hairpencil secretion of the danaid butterfly, *Euploea mulciber*, and evidence for their origin

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Male danaid butterflies secrete a diversity of characteristic compounds from the abdominal hairpencils which are thought to function as an aphrodisiac during the sequence of courtship. We examined the secretion components of field-caught males of *Euploea mulciber* by GC and GC-MS. Two major compounds were detected from a CH2Cl2 extract of the hairpencil: One was determined to be 9,10-epoxytetrahydroedulan (ETE), a compound previously identified from the butterfly, and the other, viridifloric-ß-lactone (VL), which is known to be one of the male sex pheromones of another danaid butterfly, *Idea leuconoe*. Various feeding experiments with larvae and male adults suggested that ETE is biosynthesized *de novo* probably from nutrients ingested by larvae. In contrast, laboratory-reared males never produced VL at all. Male adults of *E. mulciber* as well as other danaids are attracted to PA-containing plants in the field. When males were orally administered any one of three PAs, intermedine, heliotrine, and monocrotaline-HCl, only those fed with intermedine produced a significant quantity of VL. This clearly indicates that the production of VL exclusively depends on the acquisition of PA(s) of particular chemical structure(s) which contain trachelanthic or viridifloric acid as a necic acid moiety. Strong and selective attraction of males to intermedine observed in laboratory bioassays seems to further lend support to their limited ability of PA utilization.

P69

Biological activity of 4-hydroxybenzoic and chlorogenic acid on the root rot pathogen *Pythium aphanidermatum* and its biological control agent *Pseudomonas chlororaphis in vitro*

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The purpose of this study was to determine the biological activity of phenolic acids found in diseased and healthy roots of hydroponic bell pepper (*Capsicum annuum* L.) on the root pathogen *Pythium aphanidermatum* and its biological control agent, *Pseudomonas chlororaphis*. Roots infected with *P. aphanidermatum* have elevated levels of 4-hydroxybenzoic acid (4-HBA), whereas healthy roots contain chlorogenic acid (CA). It is unknown how *Ps. chlororaphis* responds to changes in concentrations of phenolics present in *Pythium*-infected roots. To test the effects of 4-HBA and CA against each microorganism, pure compounds were incorporated at concentrations of 0, 0.2, 2 and 20 $\mu M \cdot L^{-1}$ into water agar (for *P. aphanidermatum*) and tryptic soy broth (for *Ps. chlororaphis*) buffered at pH 5.8. Additionally, challenge assays on buffered potato dextrose agar, amended with the phenolics, tested the competitive ability of the microorganisms against each other. *Pythium aphanidermatum* colony diameters in 4-HBA were not significantly different from controls, whereas CA-treated colonies were smaller at concentrations of 0.2 and 2 $\mu M \cdot L^{-1}$. However, 12 h after treatment with 4-HBA ($R^2 = 0.72$, $P = 0.0027$) and CA ($R^2 = 0.82$, $P = <.0001$) counts of reproductive sporangia of *P. aphanidermatum* were similar to controls or elevated. Regardless of the concentrations of 4-HBA and CA, the optical densities of *Ps. chlororaphis* were not different from controls ($R^2 = 0.75$, $P = 0.8869$), although treatments containing phenolics were visibly more orange-brown. In the challenge assay, inhibition zones between *P. aphanidermatum* and *Ps. chlororaphis* were significantly smaller in control treatments (7.3 mm) than in treatments containing 4-HBA (9.5, 9.2 and 10.7 mm, for 0.2, 2 and 20 $\mu M \cdot L^{-1}$) and CA (10.2 and 10.6 mm for 0.2 and 2 $\mu M \cdot L^{-1}$) ($se = 0.46$, $R^2 = 0.76$, $P = 0.0033$). The results suggest that phenolic compounds in diseased and healthy pepper roots are important in the epidemiology of *P. aphanidermatum* and the effectiveness of *Ps. chlororaphis* as a biological control agent.

P70

Insecticidal activity of the essential oil of *Ligusticum mutellina* roots

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Continuing our work on the insecticidal activities of essential oils we have now tested the root oil of *Ligusticum mutellina* (L.) Crantz. (Apiaceae), an aromatic medicinal plant, which grows in the alpine and subalpine regions of southern Germany (1). Topical administration to 3rd instar armyworms (*Pseudaletia unipuncta*, Noctuidae) produced a 24 hr LD_{50} of 25.1 □g/larva. There are different reports in the literature as to the major constituents of *L. mutellina*. Using GC/MS with plants exclusively from the Black Forest, Brandt and Schulze (2) identified myristicin as the main compound, with lesser amounts of ligustilide and dillapiol. Spitaler et al. (3) isolated sarisan, an isomer of myristicin, and two other phenylpropanoids from Tyrolean plants. The predominant compound in the root oil from our plants cultivated in the region of the Bavarian forest was dillapiol. Ligustilide and myristicin were also present, but we did not find sarisan.

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2 Brandt, J., Schulze, W. (1995) J. Essent. Oil. Res. **7**, 231-235.
3 Spitaler, R., Ellmerer-Müller, E., Zidorn, C, Stuppner, H. (2002) Sci. Pharm **70**, 101-109.

P71

Hydroxymethylbutenyl diphosphate reductase has a limiting role in plant carotenoid biosynthesis

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The biosynthesis of the universal isoprenoid precursors IPP and DMAPP is accomplished in parallel via two compartmentally separated pathways in plant cells responsible for the formation of different products. However, under certain conditions, common intermediates are exchanged between the plastidially localized methylerythritol phosphate (MEP) pathway and the cytosolic mevalonate pathway. We used transgenic overexpression and regulatory studies to define limiting steps in the relatively recently discovered MEP pathway of plastids. This included a regulatory study of (*E*)-4-hydroxy-3-methyl-but-2-enyl diphosphate reductase (HDR), which synthesizes plastidial IPP and DMAPP, in ripening tomato fruit and deetiolating Arabidopsis seedlings, and transformation of Arabidopsis with the corresponding gene from tomato under the control of the 35S promoter. We found that HDR is upregulated during tomato fruit ripening in a process that parallels both the accumulation of carotenoids (C40 plastidial isoprenoids) and the catabolism of chlorophyll. In dark grown Arabidopsis seedlings, HDR is sharply induced upon exposure to light and is accompanied by an accumulation of chlorophyll and carotenoids, especially □-carotene. Transgenic Arabidopsis plants overexpressing tomato HDR accumulated approx. 50% more carotenoids than the wild type when grown in light. Prolonged seed dormancy was also observed in the HDR transgenic line, suggestive of higher abscisic acid levels.
However, there was no change in carotenoid content in etiolated (dark grown) HDR overexpressing plants, indicating that a light activated signaling pathway is required to activate downstream genes involved in carotenoid biosynthesis. To test the effect of altering isoprenoid pathway flux independently of this light activated signaling transduction, we engineered Arabidopsis to produce the non-native diterpene taxadiene by transforming it with taxadiene synthase (TXS) from *Taxus baccata*. While single transgenic plants overexpressing TXS accumulated taxadiene at 20-25 ng/g tissue (fresh weight), double transgenics co-expressing either 1-deoxy-D-xylulose 5-phosphate synthase (DXS) or HDR in addition to TXS accumulated approx. 7 and 13 times as much taxadiene, respectively, as the single TXS transgenic plants. These results suggest a limiting role of HDR in plastidial isoprenoid production. Other notable features in TXS expressing plants were a gibberellin deficient phenotype (reduced hypocotyl length in dark grown seedlings and retarded growth and flowering) and pale appearance, possibly related to depleted GGPP pools.

P72

Chemical basis for mate location and recognition in the longhorned beetle *Megacyllene caryae* (Gahan) (Coleoptera: Cerambycidae)

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Male *Megacyllene caryae* (Gahan) respond to females only after touching them with their antennae, indicating that mate recognition is cued by a contact sex pheromone. GC-MS analyses of whole-body solvent extracts of male and female *M. caryae* revealed substantial differences in hydrocarbon profiles, with nearly half of the hydrocarbons in the cuticular wax layer of females being absent in extracts of males. In arena behavioral bioassays, we assessed the activity of reconstructed blends of the most abundant straight-chain compounds (nC27, nC28, nC29) and unsaturated compounds (Z9:C_{29}, Z13:C_{29}, Z14:C_{29}, Z13:C_{31}, Z14:C_{31}, Z15:C_{31}) in extracts of females, and a fraction of crude extract containing methyl-branched alkanes. Males showed limited response to dead females treated with blends of straight-chain and methyl-branched alkanes, but responded strongly to the blend of alkenes. Bioassays indicated that the complete sequence of mating behaviors, up to and including coupling the genitalia, was elicited by Z9:C_{29} alone.

P73

Chemotypes of the tropical tree *Calophyllum brasiliense* in Mexico, and differences in the anti-fungal activity of their main constituents

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Taxonomically genus *Calophyllum* (Clusiaceae) is represented in Mexico by one species: *C. brasiliense* Cambess. However, we have detected two populations that although bearing the same triterpenoids, differ in other main constituents of the leaves. Specimens belonging to chemotype 1 contain 4-alkyl- or 4-phenyl- coumarins, such as mammea A/BA, while those of chemotype 2 contain cromanone carboxylic acids (apetalic acid). In order to investigate the effects of both types of compounds on organisms that interact with this tree, we isolated and identify five fungi that colonize *C. brasiliense* leaves (chemotype 2). Compounds or extracts were tested *in vitro* using fungal growth as an estimate of toxicity. Only two species of fungi *Rhizoctonia repens* & *Curvularia lunata* var. *aeria*, were susceptible to the extracts of both chemotypes. Apetalic acid was more potent than mammea A/BA. TLC analysis of a number of specimens showed that chemotype 2 has a wider range of distribution in Mexico than chemotype 1. The above results may allow to hypothesize an adaptative role for chromanone carboxylic acids in *C. brasiliense* interactions with fungi. Previously it has been reported two chemically distinct groups in *Calophyllum* species of Sri Lanka. Occurrence of 4-phenyl-coumarins, or alternatively cromanone carboxylic acids, could be tracked to a common biosynthetic route as suggested by Gautier *et al* (1979). Acknowledgments: Project UNAM/DGAPA IN207301.

P74

Xanthones from the heartwood of *Calophyllum brasiliense* inhibit the mycelial growth of the wood rotting fungus *Lenzites trabea*.

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Calophyllum brasiliense ("Bari") -Clusiaceae- is a big tree of the tropical rain forests from Brazil to México. The timber has commercial importance, and is known for its durability and high resistance to termites and, wood destroying fungi. We have previously reported that *C. brasiliense* heartwood contains xanthones able to inhibit the growth of the fungus *Postia placenta.* In this occasion we are reporting the effects of these compounds against a second brown rot fungus *Lenzites trabea*. We also investigated their toxicity on mammal tissues, as well as blockade of K^+ channels, using a rat aorta smooth muscle model. The main component, 1,3,5,6-(tetrahydroxy-2-(3,3-dimethyl allyl)-xanthone (**V**) was the most active compound (75% inhibition), while other xanthones showed moderate or none inhibition. In the rat aorta model, all the natural xanthones were toxic at concentrations higher than 10^6 M, but did not block K^+ channels or show any effect on contractility of smooth muscle. Since the minoritary xanthone **I,** as well as the triacetyl (**Va**) and the mixture of trimethyl (**Vb**) and tetramethyl ether (**Vc**) derivatives were inactive, it is possible to conclude that hydroxyls on xanthone moiety are necessary in order to inhibit *Lenzites trabea* mycelial growth. Acknowledgments: Project UNAM/DGAPA IN207301

P75

The effect of fungal infection on the induced indirect defence of maize

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Plants attacked by insects can release volatile compounds that attract the noxious herbivores' natural enemies. This so-called indirect defence is plastic and may be affected by an array of biotic and abiotic factors. We investigated the effect of fungal infection as a biotic stress agent on the emission of herbivore-induced volatiles and the possible consequences for the attraction of two parasitoid species. Maize seedlings that were simultaneously attacked by the fungus *Setosphaeria turcica* and larvae of *Spodoptera littoralis* emitted a blend of volatiles that was qualitatively similar to the blend emitted by maize that was damaged by only the herbivore, but there was a clear quantitative difference. When challenged simultaneously by fungus and herbivore the maize plants emitted in total 47% less of the volatiles. Emissions of the green leaf volatiles (*Z*)-3-hexenal and (*Z*)-3-hexen-1-yl acetate were unaffected. In a six-arm-olfactometer naïve females of the parasitoids *Cotesia marginiventris* and *Microplitis rufiventris* responded equally well to odours of herbivore-damaged and fungus- and herbivore-damaged maize plants. Healthy and fungus-infected plants were not attractive. An additional experiment showed that the performance of *S. littoralis* caterpillars was not affected by the presence of the pathogen, nor was there an effect on larvae of *M. rufiventris* developing inside the caterpillars. Our results confirm previous indications that naïve wasps may respond primarily to the green leaf volatiles and/or other related compounds that may have been released in minor amounts. The observed non-discriminatory responses are adaptive as the wasps develop equally well in hosts on healthy and on fungus-infected plants. Based on measurements of the expression of the representative genes *MPI* and *MPR1* for plants that underwent the above treatments we conclude that indirect defence signalling is not compromised by cross-talk between the salicylic acid and jasmonic acid signalling pathways, but that the overall reduction in doubly infested plants was likely due to other physiological processes in the plant.

P76

Cuticular hydrocarbon profiles and aggregation in four *Periplaneta* species (Insecta: Dictyoptera)

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Cuticular hydrocarbons (HC) are involved in the aggregation behaviour of some cockroach species [1]. Our aim was to compare the HC profiles of four *Periplaneta* species (*P. americana*, *P. brunnea*, *P. fuliginosa* and *P. australasiae)* and to evaluate their role in aggregation and in interspecific recognition. HC were extracted in dichloromethane from first instar larvae and identified with gas chromatography and mass spectrometry. The HC profiles are specific and include from 19 to 25 hydrocarbons. The HC profiles of *P. brunnea*, *P. fuliginosa* and *P. australasiae* had more molecules in common than with *P. americana*. The non-polar chemical profile of *P. americana* includes hydrocarbons with 24 to 43 carbon atoms per molecule and it varies from 21 to 41 atoms in the three other species. The major component of the HC profile is 6,9 heptacosadiene in *P. americana* but it is absent in the three other species. The major component in the three other HC profiles is 13 methyl pentacosane that is absent in *P. americana*. The role of the HC in inducing aggregation in the four species was evaluated by binary choice tests. First instar larvae were given the choice between a paper impregnated by their specific HC extract and a control paper. The minimum response thresholds varied with species. *P. fuliginosa* aggregated on 20 equivalent larvae extracts, whereas the three other species react only to more concentrated specific extracts. These results suggest that HC are involved in aggregation and in interspecific recognition.
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P77

Social behavior and recognition system against parasitic *Varroa* mite in honey bees.

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Social behaviors in honey bees can serve as models for investigating recognition and communications among intra- and interspecies as well as those towards environmental conditions.
Studies on honey bees' response and recognition against parasitic *Varroa* mite (*Varroa destructor*) were conducted to investigate how honey bees develop social behaviors. Observations and experimental results show that (1) *Apis mellifera* performs allo-grooming between workers but easily suffer *Varroa* parasitism, (2) *Apis cerana japonica* performs hygienic allo-grooming behavior in which workers grab *Varroa* mites on other workers by their mandibles and eventually kill the mites, (3) the older workers of *Apis cerana japonica* respond more quickly and persistently against mites than the younger ones, suggesting that the recognition against mites develops along with aging. Investigations on chemical cues for hygienic allo-grooming behavior and responses of worker bees towards some fatty acids were underway.

P78

Testing the sensory exploitation hypothesis: the evolution of the male sex pheromone in the European beewolf *Philanthus triangulum* **(Hymenoptera: Sphecidae)**

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Pheromone communication plays a crucial role for social interactions and mate finding as well as courtship. However, how these signals have been shaped by natural and/or sexual selection is not well understood. The sensory exploitation hypothesis predicts that male sexual signals evolve according to sensory abilities of the females that have been evolved in a non sexual context. Here we present the first results testing the sensory exploitation hypothesis in a solitary wasp, the European beewolf, *Philanthus triangulum* (Hymenoptera: Sphecidae). Beewolf females hunt exclusively honeybees as provisions for their progeny. Males mark territories with a pheromone to attract females. The co-occurrence of (Z)- 11 -eicosen- I -ol in the male pheromone and in the alarm pheromone of honeybees suggests that males might exploit the sensory ability of females. Our results support a three step scenario for the evolution of the male pheromone. First, (Z)- 11 -eicosen- 1 -ol can be found on the cuticles of honeybees and in the air surrounding foraging honeybees. Thus, foraging honeybees smell of (Z)- 11 -eicosen- 1 -ol and beewolf females could use this characteristic odour as a kairomone to locate and identify their prey. Second, bioassays show that olfactory cues are responsible for eliciting attacks on honeybee prey, Most interestingly, (Z)- 11 -eicosen- 1 -ol plays a crucial role in the identification of the honeybees. Third, a reanalysis of the beewolf sex pheromone revealed an extensive congruence between the marking secretion of male beewolves and the cuticular hydrocarbons of honeybees. This congruence strongly supports our hypothesis that beewolf males evolved a pheromone that exploits the females' pre-existing sensory sensitivity.

P79

Floral nectar composition of some monocotyledons – a NMR study

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Floral nectar samples of several members of the monocotyledonous plant families Musaceae, Haemodoraceae and Zingiberaceae have been analyzed by nuclear magnetic resonance (NMR) spectroscopy in order to determine sugar composition, amino acids, and trace components. Composition of floral nectar usually has been analysed by TLC, HPLC or GC-MS, in part requiring derivatization. In this study, we demonstrate that NMR spectroscopy is a useful tool for nectar analysis without or with minimum sample preparation and without derivatization.

Nectar samples were collected from greenhouse-grown plants. A three-step analytical procedure, employing various NMR techniques, was used according to the largely different concentration of the different classes of compounds. The ratio of sucrose and hexoses was determined by ^{13}C NMR of the diluted or undiluted nectar sample without further sample preparation. Low abundant compounds such as amino acids were identified by ^{1}H- and homo- and heteronuclear correlated 2D-NMR techniques using a cryogenic NMR probe. Non-polar trace component were enriched by solid phase extraction prior to NMR analysis. Identification was based on comparison with reference spectroscopic data and *de novo* structure elucidation using cryogenic NMR spectroscopy. The results will be discussed in view of their significance for plant-pollinator interactions.

P80

Biosynthesis of anacardic acids in glandular trichomes of *Pelargonium xhortorum* and bioactivity assessment of anacardic acids against Coleoptera pests.

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Anacardic acids are a group of 2-hydroxy-6-alkylbenzoic acids likely produced by a combination of fatty acid synthesis and polyketide synthesis pathways. In *P.* □*hortorum*, the secretion of anacardic acids from glandular trichomes presents an effective resistance system against small pests such as spider mites, aphids, and whitefly. To better define the biosynthetic pathway(s) involved in anacardic acid production in glandular trichomes we conducted [1-^{14}C]acetate time-course labeling studies and compared labeling patterns of glycerolipid and anacardic acid fractions. Initially, the polar lipid fraction was most heavily labeled. However, later time points indicate a reduction in incorporation into polar lipids with a corresponding increase in incorporation into anacardic acids. We are currently analyzing the acyl composition of polar lipids to determine if these serve as a pool of fatty acid substrates for anacardic acid synthesis. In *P.* □*hortorum*, anacardic acids provide resistance to small pests. To determine effectiveness against larger pests, we analyzed the effect of anacardic acids on development of Colorado potato beetle larva. When supplied with food treated with anacardic acids (1 mg □ ml$^{-1)}$, we found a significant decrease (-15%) in larval weight through 8 days of development. This indicates anacardic acids may be effective against a broader range of pests than previously known.

P81

Studies on the aggregation pheromone of the pinyon ips, *Ips confusus* (Coleoptera: Scolytidae) in the southwestern United States

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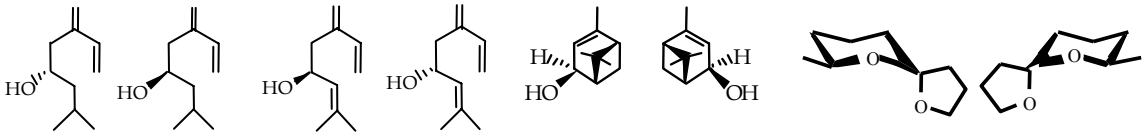
The pinyon ips, *Ips confusus* (LeConte), is a pine bark beetle that is the main insect mortality agent of two species of pinyon pines (*Pinus monophylla* and *P. edulis*) at intermediate elevations in the desert southwest of the USA and Mexico. In 2003, a USDA Forest Service survey revealed that between 15 and 30% of pinyon pines had been killed recently over nearly 4 million acres in the southwestern US. Land managers in these areas are interested in monitoring the flight of and reducing tree mortality caused by *I. confusus*. The attractant aggregation pheromone of *I. confusus* consists of the three monoterpene alcohols, ipsenol, ipsdienol, and *cis*-verbenol. These pheromone components were isolated, identified, and reported in the 1970's by M.C. Birch, R.M. Silverstein, and colleagues. Using insect and plant material collected from the Pinenut Mountains in Nevada, we have used GC-MS and GC-EAD to analyze Porapak-Q-trapped volatiles from male- and female-infested *Pinus monophylla* logs and from logs of *P. monophylla* and the non-host, *Juniperus osteospermae*. Our analyses confirmed the earlier work and revealed that males produced (4*S*)-(–)-ipsenol and (4*S*)-(+)-ipsdienol. *cis*- and *trans*-Verbenol and verbenone were present not only in male samples, but also in female and host alone samples. Of the two enantiomers, only (–)-ipsenol was EAD-active on female and male antennae, whereas both enantiomers of ipsdienol were EAD-active. Field studies of the flight behavior of *I. confusus* with the commercially available enantiomers of ipsdienol in Nevada, Utah, and Colorado revealed a distinct preference by both sexes for (+)-ipsdienol. The spiroacetal conophthorin, which interrupts aggregation in other *Ips* spp., did not interrupt the flight behavior of *I. confusus*.



S *R* *S* *R* *S* *R* 5*S*,7*S* 5*R*,7*R*

Ipsenol Ipsdienol *cis*-verbenol *trans*-conophthorin

P82

Quantification of anti-microbial activity of (+)-(R)-α-pinene and (-)-(S)- α-pinene

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There are many published studies on anti-microbial activity of various phytochemicals, but only a few reports on the effect of chemical structure on anti-microbial activity. Previous results on this project indicated that molecular rigidity and introduction of oxygen or nitrogen containing functional groups alters anti-microbial activity of α-pinene. The purpose of this project is to quantify anti-microbial activity and compare the enantiomers of α-pinene. The minimal inhibitory concentration (MIC) of (+)-(R)-α-pinene and (-)-(S)- α-pinene on *Escherichia coli, Micrococcus luteus, Staphylococcus aureus* and *Candida albicans* was determined by comparing results from broth dilution and agar well diffusion methods. Results of the study will be presented.

P83

The use of plastic bags in chemical ecology: encapsulation of plant parts for headspace analysis.

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Methods for capturing and analyzing leaf volatiles have steadily improved with the development of guillotine entrainment vessels, inlet-outlet push-pull systems and thermal desorption. However, the continued use of bulky glass vessels for encapsulation has made it difficult to take samples from plants growing naturally in the field. Plastic bags offer an easily transportable and disposable alternative that would allow many samples to be taken in a field locality. As for any encapsulation material, chemical contamination, chemical absorption and light wavelength absorption are issues to consider. Presented are results from experiments using a glass chamber, nylon bags and polyester bags that test headspace recoveries and wavelength absorption properties. Using ten compounds of known importance in chemical ecology, recoveries of headspace injected into static air sealed in the vessels are assessed. Recoveries are also quantified when headspace chemicals are added to air being drawn through the different vessels, which perhaps better replicates a real entrainment. Overall, recoveries were very good except for alpha-pinene whose recovery was poor from all materials. Recovery of cis-jasmone was also poor but only from the glass vessel and it is suggested that these low recoveries are caused by absorption onto the materials. After a spectrophotometric study, the glass chamber and the polyester bag were tested in field entrainments, sampling volatiles from an aphid infested apple tree growing in a commercial orchard. Entrainment chromatograms using the two materials were consistent suggesting validity and such a bag-based system will facilitate future field sampling as well as replication

P84

Characterization of an Arabidopsis mutant defective in C4H

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In plants, the "general" phenylpropanoid pathway consists of three enzymatic reactions, catalyzed by phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-(hydroxy)cinnamoyl CoA ligase (4CL). These reactions are common in the biosynthesis of flavonoids, monolignols, and an array of other secondary metabolites. As far as we are aware, no mutants defective in these genes have been described, although the impact of their down-regulation has been studied using transcriptional suppression technologies. Here we describe the identification and characterization of an Arabidopsis mutant defective in the gene encoding C4H. The three alleles of *reduced epidermal fluorescence 3* (*ref3*) comprise an allelic series of moderate to severely dwarfed plants. The mutant *C4H* genes were heterologously expressed in *E. coli* and the encoded proteins were observed to be compromised in either protein stability or substrate binding. This reduction in C4H function in *ref3* results in reduced levels of sinapoylmalate and the accumulation of a novel soluble phenylpropanoid, cinnamoylmalate, decreased levels of wall bound phenylpropanoid esters, and a decreased deposition of lignin. Furthermore, an increased incidence of lateral branching and the presence of swellings at the nodes suggest that the mutant is inhibited in auxin transport. Finally, the lignin content of the strongest allele, as measured by pyrolysis-GC-MS, was found to consist of *p*-hydroxy-, guaiacyl-, and syringyl-derived subunits, similar to that of the wild type. This finding is discussed in the context of possible metabolic channeling in the phenylpropanoid pathway.

P85

Spatial and temporal aspects of the flight response of the pine engraver beetle, Ips pini (Coleoptera: Scolytidae), to its aggregation pheromone in a red pine forest

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Several studies of the flight behavior of the pine engraver, Ips pini, were conducted in northern Michigan (USA) during the summers of 2002 and 2003 in a healthy forest stand consisting primarily of red pine (Pinus resinosa) and big tooth aspen (Populus grandidentata). The flight response of the beetle was measured using multiple funnel traps baited with the aggregation pheromone racemic ipsdienol and lanierone. The temporal flight pattern of I. pini was examined during both years, and peak flight occurred in early to mid-July. Additionally, the vertical flight response was assessed throughout each summer using a series of 4 or 5 traps suspended in tandem from the forest canopy. Each trap in the column was baited with the aggregation pheromone. The lowest trap caught significantly fewer beetles than any of the upper traps, indicating that I. pini flies in greater densities 1 m above the forest floor. Lastly, the flight response of I. pini at a distance from a point source of pheromone was evaluated and correlated with a tracer gas (sulfurhexafluoride, SF6) at two sites during the summer of 2003. In these arrays, of twenty-one traps arranged radially and placed zero to 30m from the point source, I. pini was trapped up to 5 m from the source in concordance with a decline in SF6 concentrations after 5 m.

P86

Cloning , expression and characterization of putative flavonoid glucosyltransferases from grapefruit (*Citrus paradisi*) Leaves

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Flavonoids, plant secondary metabolites, are an integral part of our lives. They are chemically modified by glycosylation, hydroxylation, methylation, etc. During glycosylation the sugar moiety from UDP-sugar is transferred to aglycone flavonoid substrates by specific enzymes and those catalyzing the transfer of glucose are known as glucosyltransferases (GTs). Flavanone-specific 7-O-glucosyltransferase (7GT) catalyzes the first glycosylation reaction leading to production of bitter naringin in grapefruit. Primary structure of 7GT couldn't be determined due to low yield, N-terminal blockage, etc. This research was designed to obtain full-length grapefruit GT cDNA clones, express them and characterize them. Specific primers were designed from the Plant Secondary Product Glucosyltransferase (PSPG) box to amplify grapefruit GTs. RNA was extracted from young grapefruit leaves. SMART RACE RT-PCR was used to obtain 5' clones. Clone specific primers were used to obtain the 3' ends. Compiled sequences were obtained by matching overlaps of partial clones. Primers were designed from compiled sequences to obtain full-length cDNA clones. To date one full length clone was inserted into expression vector (pET32A) and transformed into the expression host BL21 (DE3) RIL. The 34kDa protein was tested for GT activity using 6 different flavonoid aglycones: naringenin, naringenin chalcone, apigenin, kaempferol, quercetin, and hesperetin using UDP-^{14}C glucose as glucose donor. Young grapefruit leaf extract was tested with naringenin as control for 7GT assay. Results indicated that the expressed protein was probably not a flavonoid GT. An EST library has been constructed and is being evaluated. To date, there are 3-4 more additional unique clones being analyzed.

P87

Pheromonal and other factors influencing speciation of some Crambid moths

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In Crambid moths (Lepidoptera: Crambidae) some agricultural crop pests are members of "species groups" or "intra-specific strains" and thus often cause confusion for species identification that is often critical for IPM. We have conducted biosystematic studies on such moths by combined techniques of morphology, physiology, molecular biology, and chemical ecology.

An example of species group in the genus *Ostrinia* is of *O. latipennis* and *O. ovalipennis*. These two non-pest species feeding on knotweeds are sympatric as well as synchronous. Although only 5% difference in COII gene sequence was found, reproductive isolation between them may be complete by different sex pheromone blends, resulting in finding the latter as a new species.

Two other studies, on the differentiation of two strains in *Chilo suppressalis*, based on the difference in host plants, rice and water-oats, and on the geographic variation of female sex pheromone blends in *Cnaphalocrocis medinalis*, in Asia will also be introduced.

P88

The resistance factor against *Liriomyza trifolii* in sweet pepper induced by jasmonic acid

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Lyriomyza torifolii (Burgess) (Diptera, Agromyzidae) is a serious pest of horticultural and vegetable crops. The leafminer fly has both habitat of a short life cycle and a highly resistance against chemicals, and it is difficult to control by using pesticides. Recently, induced resistances against pest in plant have been investigated and its application in the crop protection has also been undertaken. Here we investigated that the effect of jasmonic acid (JA) on the resistance in cotyledon of sweet pepper seedling against leafminer. After the cotyledons were floated on JA aqueous solution for 48 hours, they were exposed to oviposition of leafminer. The cotyledons treated with JA at the concentration over 50 mM showed resistance against leafminer. Comparing the secondary metabolites in the treated cotyledon with that in the untreated cotyledon by HPLC, an enhanced peak was observed. This peak was purified with octadesyl silica gel column and reversed phase HPLC, and identified as caffeoylputrescine by using LC-MS and NMR.

P89

Effects of isoquinoline alkaloids from hydrastis canadensis on growth and viability of *Fusarium* spp.

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ABSTRACT

Goldenseal (*Hydrastis canadensis* L.) is a popular medicinal plant, whose rhizome and rootlets have been traditionally used as an anti-microbial treatment, for mucosal infections, urinary tract infections, as a treatment for cholelithiasis and other liver disorders. Goldenseal contains the isoquinoline alkaloids berberine, canadine and hydrastine. These compounds have shown activity against a large number of microorganisms, including fungi. A bioassay was developed to determine the effect of goldenseal isoquinoline alkaloids on growth of selected toxigenic Fusarium isolates, both native to the goldenseal root and isolates from unrelated plant species. We have studied the effects of whole plant isoquinoline alkaloid extracts versus individual alkaloids on both growth of *Fusarium* spp. as well as their survivability when exposed to goldenseal alkaloids. Phylogenetic relationships between fungi, patterns of mycotoxin production, conidial formation and germination rates have been correlated with host adaptation by Fusarium to the isoquinoline alkaloids found in goldenseal.

P90

Aggregation pheromone of the birch bark beetle, *Scolytus ratzeburgi* Janson(Coleoptera: Scolytidae)

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Scolytus ratzeburgi, the birch bark beetle, is the largest species of its genus in Europe and the only one developing in birch (*Betula* spp.). Performing one single generation per year, female beetles create characteristic rows of gallery ventilation holes in the bark, making infested trees easily recognizable. Aggregation usually takes place over the whole tree (from the trunk to branches of 5 cm Ø). Infested trees normally do not survive the attack. In order to identify the semiochemicals involved in the aggregation process, both frass and head space extracts of beetles colonizing fresh birch logs were examined using GC/MS and GC-EAD.
A total of six EAD active compounds were identified, eliciting clear responses with both male and female antennae. Three of the active compounds are beetle-derived and already known as semiochemicals from other *Scolytus* species, while the remaining three substances clearly originate from the infested host tree.
In preliminary field bioassays, the effect of the EAD active compounds on *Scolytus ratzeburgi* was investigated during the flight period in June.

P91

Formation of male marking pheromones in bumblebees

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Marking pheromone of the bumblebee males is produced by the cephalic part of the male's labial gland. Labial glands of *Bombus lucorum* males were incubated *in vitro* with deuterium-labeled fatty acids, d_5-ethanol and d_4-methanol. In all cases, esters were formed. Ethyl esters and to a small extent also methyl esters were formed from deuterium-labeled fatty acids. A preference for ethyl esters formation from shorter-chain-acids (12, 14) was observed when an equimolar mixture of acids C_{12}–C_{18} was incubated. No products of desaturation were found *in vitro* despite the main pheromonal component is ethyl tetradec-9-enoate. This was a significant difference between *in vitro* and *in vivo* experiments.
The tissue of the dissected labial gland contained high amounts of free fatty acids the proportions of which were the same as ethyl esters in the pheromonal blend. We assume that fatty acids are stored in the labial gland and transformed into pheromonal components by an esterase. It seems that the proportions of ethyl esters in the pheromonal blend are not given by the selectivity of the esterase but by the availability of substrates.

P92

Identification of volatile emissions from the Ambrosia beetle *Platypus sulcatus (=mutatus*) (Coleoptera: Playtpodidae) and their pheromonal activity

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Platypus mutatus (Chapuis,1865) is a Coleoptera (Platypodidae) ambrosia beetle native to South America. *Platypus mutatus* is a primary pest because they affect only living standing trees. This insect drills the trunks boring large internal tunnels in the xylem. *P mutatus* is a serious problem in commercial plantations of poplars (*Populus deltoids*)
Concerning the genus Platypus, pheromone production has been demonstrated for *P. apicalis*, *P. gracilis* and *P. caviceps* and its chemical identity has been studied for *P. flavicornis*.
The aims of this study were to determine the behavioural responses of males and females of *P. sulcatus* to odors emitted by boring males in tunnels and the identification of components of volatile emissions. Headspace analysis using SPME techniques showed the presence of 6-methyl-5-hepten-2-ol (sulcatol) and 6-methyl-5-hepten-2-one (sulcatone) in the volatiles emitted by the boring male. Both sulcatol and sulcatone showed electroantennographical activity on females. Behavioral assays showed that females are more attracted than males to galleries with boring males inside. The fact that females and not males are attracted to galleries containing males suggests the existence of a pheromone that could be a sexual rather than an aggregation pheromone.

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Biosynthesis of volicitin-related compounds in noctuid caterpillars

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Volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] was first identified as an elicitor of plant volatiles from the regurgitant of *Spodoptera exigua*. In recent studies, we identified volicitin-related compounds from three noctuid species, *Spodoptera litura*, *Helicoverpa armigera* and *Mythimna separata*, and one sphingid species, *Agrius convolvuli*. All the conjugates comprise only L-glutamine as the amino acid moiety [1]. Interestingly, amino acid analyses of regurgitants showed that L-glutamine was a minor amino acid component in the regurgitants of noctuid caterpillars. Nevertheless, L-glutamine was exclusively conjugated with fatty acids *in vivo* experiments [2] and most of L-glutamine in the gut was found as the conjugated forms. Considering the fact that degrading enzyme(s) for volicitin-related compounds was also found in the gut lumen [3], the fatty acid amides might be crucial physiological components in caterpillar metabolism.

[1] Mori et al., Biosci. Biothechnol. Biochem., 67, 1168-1171, 2003
[2] Yoshinaga et al., Biosci. Biothechnol. Biochem., 67, 2655-2657, 2003
[3] Mori et al., J. Insect Physiol., 47, 749-757, 2001

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Treatment of mature Norway spruce (*Picea abies*) with methyl jasmonate triggers multiple defense responses and protects trees against *Ceratocystis polonica* infestation.

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When conifers such as *Picea abies* (Norway spruce) are attacked by insects or pathogens, they often produce increased quantities of terpenoid oleoresin. This response can be mimicked in young *P. abies* seedlings by treatment with methyl jasmonate. In this study, we determined the effect of methyl jasmonate on the terpenoids and other chemical defenses of mature *P. abies*, and investigated if this treatment protected trees against attack by the blue-stain fungus *Ceratocystis polonica*, the most important fungal associate of the bark beetle *Ips typographus*. Methyl jasmonate treatment induced the formation of traumatic resin ducts in the developing xylem, enhanced resin flow, and stimulated increased accumulation of monoterpenes, sesquiterpenes, and diterpene resin acids. However, almost no significant changes in terpene composition were detected. In addition, no changes in soluble phenolic content were observed. There was a very high variability both among and within clones in the timing and degree of response to methyl jasmonate. These chemical and anatomical changes were correlated with increased resistance to *C. polonica*, suggesting that terpenoid oleoresin may function in defense against this pathogen.

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Development of semiochemical-based biological control systems for suppressing soybean aphid populations

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Since its first appearance in North America, infestations of the newly invasive soybean aphid, *Aphis glycines* Matsumura, have continued to spread causing a significant soybean yield loss due to either direct feeding damage or the vectoring of plant viruses by the aphid. The present study reports our recent findings on using semiochemical-based suppression strategies (kairomones-enhanced biological control, pheromone mass trapping and mating disruption) to reduce populations of this pest, as well as their effects on soybean yields.

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Induction by AM colonization of phytomedicinal isoflavonoids in red clover

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This study postulated that the symbiosis between red clover, *Trifolium pratense* L., and the arbuscular mycorrhizal (AM) fungus, *Glomus intraradices* Schenck & Smith, enhances secondary phenolic metabolism and nitrogen assimilation. To test this hypothesis, the four objectives set forth were to determine the impact of AM colonization and/or rhizobial inoculation in red clover on the: 1) growth parameters; 2) nitrogen assimilation, via the activity of glutamine synthetase (GS, E.C. 6.3.1.2), total N content and soluble protein concentrations; 3) contents of isoflavonoids (e.g. genistein, daidzein, biochanin A, and formononetin); and 4) contents of these four isoflavonoids over time and with a hormonal factor, jasmonic acid (JA). To accomplish these objectives, four greenhouse experiments were performed. The overall results indicate that the colonized red clover plants, with 22% to 37% of AM root colonization, had enhanced shoot height and shoot and root dry masses. Co-inoculation with *Rhizobium* enhanced the growth parameters more than inoculation with either microsymbiont alone. The concentrations of micronutrients significantly increased in red clover with AM colonization and/or rhizobial inoculation, while the macronutrients did not vary to any great degree. The GS activity in the shoots and roots of red clover increased with AM colonization, indicating that AM fungi are able to take up and/or assimilate N forms. Growing red clover with AM fungi and/or *Rhizobium* significantly enhanced the isoflavonoid contents in both the shoots and roots. In addition, the JA treatment approximately doubled the concentrations of formononetin, biochanin A and genistein in the shoots. Over time, the isoflavonoid concentrations increased in the shoots and decreased in the roots, suggesting their translocation. Overall, these results support the hypothesis that AM colonization enhances GS activity and the isoflavonoid contents. Our findings support the idea of using AM symbioses to promote a natural method of harnessing phytomedicinal compounds such as the isoflavonoids