

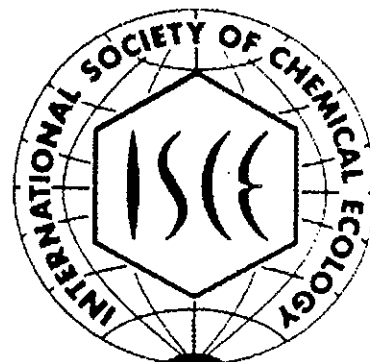
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# INTERNATIONAL SOCIETY OF CHEMICAL ECOLOGY

**11<sup>TH</sup> ANNUAL MEETING**

**JUNE 4 – 8, 1994**

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***Program and Abstracts***

State University of New York  
College of  
Environmental Science and Forestry



Syracuse, New York, USA

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## ACKNOWLEDGMENTS

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# PROGRAM SCHEDULE

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## *Saturday, June 4*

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2:00 – 6:00 PM	REGISTRATION	MARSHALL HALL LOBBY, SUNY ESF
3:00 – 5:00 PM	ISCE EXECUTIVE COUNCIL MEETING	229 BRAY HALL
6:00 PM	WELCOME BARBECUE	ESF QUAD

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## *Sunday, June 5*

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8:00 AM	REGISTRATION OPENS	MARSHALL HALL LOBBY, SUNY ESF
9:00 AM	WELCOME	MARSHALL HALL AUDITORIUM
	<i>William P. Tully</i> , Vice President and Provost SUNY College of Environmental Science and Forestry	
9:15	PLENARY SPEAKER	MARSHALL HALL AUDITORIUM
	Sustainable Development, Biodiversity and Chemical Ecology: A Costa Rican View.	
	<i>Rodrigo Gamez</i> Instituto Nacional de Biodeversidad, Heredia, Costa Rica	
10:25	Break	Nifkin Lounge

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10:45

**PLENARY SPEAKER**

Turnabout is Fair Play:  
Secondary Functions for Primary Compounds

*May Berenbum*  
University of Illinois  
Urbana, Illinois

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12 Noon

*Lunch*

*Schine Student Center or On You Own*

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1:30 PM

**SYMPOSIUM I:**

*MARSHALL HALL AUDITORIUM*

**CHEMICAL PROSPECTING**

*Organizers: Thomas Eisner and Jerrold Meinwald*

---

Natural Products in Drug Discovery.

*Paul S. Anderson*, Dupont MerckPharmaceutical Co.,  
Wilmington, Delaware

Prospecting in the Oceans.

*Janice E. Thompson*, CalBioMarine Technologies, Inc.,  
Carlsbad, California

Revival of Screening: Targeted Screening  
Using Molecular Biology Techniques.

*Prabhavathi B. Fernandes*, Bristol-Myers Squibb,  
Princeton, New Jersey

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4:00—4:30 PM *Break*

*Nifkin Lounge*

Chemical Prospecting: An Overview of the International  
Cooperative Biodiversity Groups Program.

*Francesca T. Grifo*, National Institutes of Health,  
Bethesda, Maryland

Biorationality: Improving the "Hit" Rate.

*Thomas Eisner*, Cornell University, Ithaca, New York

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5:30 PM

*Dinner*

*On your own*

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7:30—9:30 PM SYMPOSIUM II:

MARSHALL HALL AUDITORIUM

**PLANT-HERBIVORE INTERACTIONS: *QUO VADIMUS?***

*Organizers:* Dietland Müller-Schwarze and Jack Schultz

---

You Can't Tell the Players Without a Program:  
Plant-Herbivore Interactions Aren't Just Interactions Between  
Plants and Herbivores.

*Jack C. Schultz*, Pennsylvania State University,  
University Park, Pennsylvania

Resource Allocation as a Constraint in Plant Chemical Defense.

*Ian T. Baldwin*, State University of New York, Buffalo, New York

Tracking Variable Environments:

There is More Than One Kind of Memory.

*Frederick D. Provenza*, Utah State University, Logan, Utah

Significance of Early Ontogenetic Variation in Chemical Defense of  
Woody Plants Against Browsing by Mammals.

*John P. Bryant*, University of Alaska, Fairbanks, Alaska

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**Monday, June 6**

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8:00 AM

SYMPOSIUM III:

MARSHALL HALL AUDITORIUM

**INTERSPECIFIC CHEMICAL RECOGNITION**

*Organizer:* Alan Renwick

---

Molecular Determinants of the Plant/Fungus Interaction.

*Olen C. Yoder*, Cornell University, Ithaca, New York

Communication/Recognition between the Parasitic Weed, *Striga*,  
and its Hosts.

*Larry G. Butler*, Purdue University, West Lafayette, Indiana

Interspecific Chemical Recognition: Insect Plant Relationships.

*Erich Städler*, Swiss Federal Research Station,  
Wädenswil, Switzerland

How Parasitoids Use Chemical Cues to Track Host and Food  
Resources in a Tritrophic System.

*W. Joe Lewis*, US Department of Agriculture, Tifton, Georgia

Olfactory Temporal Analysis of Spatial Gradients in Turbulent  
Odor Dispersal.

*Jelle Atema*, Boston University Marine Program,  
Woods Hole, Massachusetts

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10:30 AM      *Break*      *Nifkin Lounge*

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11:00 AM      **CONTRIBUTED PAPERS, SIMULTANEOUS SESSION 1A**      *MARSHALL HALL  
AUDITORIUM*

---

Inter and Intra-Species Chemical Recognition in Social Insects:  
Efficiency and Plasticity of the Chemical Signature,  
*Jean-Luc Clement.*

Host Recognition by Monarch Butterflies, *Danaus plexippus*,  
*Meena Haribal.*

Do *Brassica*-Phytoalexins have an effect on Host-Plant Selection in  
the Cabbage Root Fly, *Robert Baur.*

Tannin Action is a Real Phenomenon: Protein Precipitation in the  
Midgut Fluid of Gypsy Moth Caterpillars, *Heidi M. Appel.*

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11:00 AM      **CONTRIBUTED PAPERS, SIMULTANEOUS SESSION 1B**      *5 ILLICK HALL*

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The Influence of Tansy Odour on European Grapevine Moth  
Behavior, *Denis Thiery.*

Ovipositional Responses of the Polyphagous Herbivore, European  
Corn Borer (*Ostrinia Nubilalis* (Hubner)), to Chemical Constituents  
of Host Plants, *Sujaya Udayagiri.*

Extreme Differences in Alkaloid Concentrations Within Rosette  
Plants of *Cynoglossum Officinale*: Are Plants Smart Investors?  
*Nicole M. Van Dam.*

Ultraviolet Radiation Influences Protective Shield on the Plant  
Surface, *Alicja M. Zobel.*

---

12:00 PM      *LUNCH*      *ON YOUR OWN OR SCHINE STUDENT CENTER*



1:30 PM	<b>SYMPOSIUM IV:</b>	<i>MARSHALL HALL AUDITORIUM</i>
	<b>CHEMICAL ECOLOGY OF FOREST INSECTS</b> <i>Organizers: Stephen Teale and David Wood</i>	
	Genetic Correlation of Signal Production and Response: A Quantitative Genetics Approach to Pheromone-Based Assortative Mating in <i>Ips pini</i> . <i>Stephen A. Teale</i> , State University of New York College of Environmental Science and Forestry, Syracuse, New York	
	Chemotaxonomic Studies of the Pine Engraver Beetle, <i>Ips pini</i> (SAY) (Coleoptera: Scolytidae). <i>Steven J. Seybold</i> , University of Nevada, Reno, Nevada	
	Biosynthesis of Monoterpenoid Pheromones of Bark Beetles. <i>Desiree Vanderwel</i> , University of Winnipeg, Winnipeg, Manitoba.	
3:00 PM	<i>BREAK</i>	<i>NIFKIN LOUNGE</i>
3:30 PM	<b>SYMPOSIUM IV (CONTINUED):</b>	<i>MARSHALL HALL AUDITORIUM</i>
	New Semiochemicals from Bark Beetles. <i>Wittko Francke</i> , University of Hamburg, Hamburg, Germany	
	Chemical Identification and Biological Activity of Volatiles Produced by the White Pine Cone Beetle. <i>Göran Birgersson</i> , University of Goteburg, Goteburg, Sweden	
4:30 PM	<b>CONTRIBUTED PAPERS, SESSION 2</b>	<i>MARSHALL HALL AUDITORIUM</i>
	Factors Affecting Production of Pheromone and Defensive Secretions in the Red Flour Beetle, <i>Tribolium Castaneum</i> , (Coleoptera: Tenebrionidae), <i>Thomas W. Phillips</i> .	
	Chemical Defenses of the Thysanoptera, <i>Murray S. Blum</i> .	
	Diet-Dependent Sensitivity of Larvae to a Host Plant Feeding Deterrent, <i>J. A. A. Renwick</i> .	
	Fast Growth Versus Superior Defense: Evidence for a Trade-off in the Leaf Beetle <i>Oreina elongata</i> , <i>Susanne Dobler</i> .	
5:30 PM	<i>DINNER</i>	<i>ON YOUR OWN</i>
8:00 PM	<b>POSTER SESSION</b>	<i>MARSHALL HALL—NIFKIN LOUNGE AND ROOM 103</i>

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## Tuesday, June 7

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8:00 AM

CONTRIBUTED PAPERS, **SIMULTANEOUS SESSION 3A**

MARSHALL HALL  
AUDITORIUM

---

Induced Defence in Cotton Plants as a Response to Damage by *Spodoptera littoralis* larvae. *Hans T. Alborn.*

Tannin Sensitivity and Tolerance in Larval Lepidoptera, *Raymond V. Barbehenn.*

Pharmacophagous Relation Between *Zonacerus* Grasshoppers and the Introduced Weed *Chromolaena odorata* in Africa, *Michael Boppré.*

Comparative Antifeedancy of Gaba/Glycinergic Antagonists to Diabroticite Beetles, *Herbert Eichenseer.*

Attractiveness of Plant Pyrrolizidine Alkaloids (PAs) as Defensive Compounds for Insects: Biochemical Answers, *Thomas Hartmann.*

Chemical Camouflage in an Aphid-Ant-Parasitoid System, *Caroline Liepert.*

Phagostimulants Isolated from Fungi Associated with the Western Subterranean Termite, *Reticulitermes hesperus* Banks L., *Brice A. McPherson.*

Feeding Strategies of the Aphid Species, *Rhopalosiphum Padi* and *R. Maidis* (Homoptera: Aphididae), In Wheat, *Hermann M. Niemeyer.*

---

8:00 AM

CONTRIBUTED PAPERS, **SIMULTANEOUS SESSION 3B**

5 ILLICK HALL

---

Structure-Activity Relationships (SAR) of Avian Chemoirritants: Implications for Plant Animal Evolution and Conservation Biology, *Larry Clark.*

The Influence of Phenolic Defences of Eucalypts on Habitat Use by Arboreal Folivorous Marsupials, *Steven J. Cork.*

The Paradox of Polymorphic Plant Defence and the Low Costs of Defence, *David A. Jones.*

Effects of Plant Cardenolides on Generalist Herbivores, *Stephen Malcolm.*

---

Capsaicin Detection by Trained Starlings: The Importance of Olfaction and Trigeminal Chemoreception, *J. Russell Mason*.

Why do Courting Male Garter Snakes Chin-Rub Backwards? Evidence for a Copulatory Pheromone, *Robert T. Mason*.

Ecological Theory: A Useful Guide to the Presence / Absence of Tannins and Other Secondary Metabolites? *Simon Mole*.

Musth Chemosignals Among Asian Elephants, *L. E. L. Rasmussen*.

---

10:00 AM

BREAK

NIFKIN LOUNGE

---

10:30 AM

CONTRIBUTED PAPERS, **SIMULTANEOUS SESSION 4A**

MARSHALL HALL  
AUDITORIUM

---

Trends in the Evolution of Contact Oviposition Stimulants in Swallowtail Butterflies, *Paul Feeny*.

The Postpharyngeal Gland as a "Gestalt" Organ for Nestmate Recognition in the Ant *Cataglyphis Niger*, *Abraham Hefetz*.

Phomodiol, A Novel Compound Produced by *Phomopsis*, *Wendy S. Horn*.

Ontogeny of *Drosophila* Cuticular Signature and Courtship Behavior, *Jean-Marc Jallon*.

Synthetic Attractants for Western Corn Rootworm Larvae in Laboratory Bioassays, *Darryl K. Jewett*.

Hairpencil Volatiles of a Giant Danaine Butterfly, *Idea leuconoe*, *Ritsuo Nishida*.

Aphid-Repellent Effects of Polygodial, A Plant Sesquiterpenoid, *Glen Powell*.

---

10:30 AM

CONTRIBUTED PAPERS, **SIMULTANEOUS SESSION 4B**

5 ILICK HALL

---

Can Small Rare Prey be Chemically Defended? The Case for Marine Larvae, *Mark E. Hay*.

Information in Scent Counter-Marks: A Striking New Phenomenon in Hamsters and Voles, *Robert E. Johnston*.

Arrestment by Ixodid Tick Species On Contact Chemostimuli from Steer, *Thomas H. Krober*.

Long-Range Female Sex Pheromone of the Longhorn Beetle *Migdolus fryanus*, *Walter S. Leal*.

Tobacco is Unable to Recover Nitrogen Investment in Defense, *Thomas E. Ohnmeiss*.

Bioactivity of Major Phenol and Neutral Compounds from Beaver (*Castor Canadensis*) Castoreum, *Bruce A. Schulte*.

The Adaptation of the Defensive Secretion of *Bledius* (Col., Staph., Oxy.) To Natural Predators, *Johannes L. M. Steidle*.

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12:30 PM

Lunch

Schine Student Center or On Your Own

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1:30 PM

SYMPOSIUM V:

MARSHALL HALL AUDITORIUM

**CHEMICAL ECOLOGY AND CHEMICAL ANALYSIS:  
ADVANCE IN DETECTION AND IDENTIFICATION**

*Organizers: Wendell Roelofs and Fran Webster*

---

Measurement of Pheromone Density in Disruptant-Treated Plots with an Electroantennogram System,  
*Ring T. Cardé*, University of Massachusetts, Amherst, Massachusetts

Gas Chromatography - Olfactometry in Flavor Analysis,  
*Terry E. Acree*, Cornell University, Geneva, New York

Isolation, Separation and Identification of Chiral Pheromones from SawFlies and Antlions, *L. Gunnar Bergström*,  
University of Göteborg, Göteborg, Sweden

---

3:00 — 3:30 PM BREAK

NIFKIN LOUNGE

Development of Liposome-Based Immunoanalytical Methods for Toxic Chemicals.

*Richard A. Durst*, Cornell University, Geneva, New York

ICP Spectrometry: A Means of Studying Sodium Uptake and Nuptial Transfer in a Puddling Moth,

*Scott R. Smedley*, Cornell University, Ithaca, New York

---

Microscale Activity Bioassays for the Isolation of Arthropod Neurotoxins, *Pierre Escoubas*.

Quantification of Cantharidin in Canthariphilous Ceratopogonidae (Diptera), Anthomyiidae (Diptera) and Cantharidin-Producing Oedemeridae (Coleoptera), *Mark Frenzel*.

Chiral Purity of Disparlure Samples, *James E. Oliver*.

Some Thermodynamic Considerations for the Anaerobic Degradation of Hydrocarbons, *John M. Brand*.

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6:30 PM

RECEPTION AND BANQUET

SKYDECK, CAROUSEL MALL

---

## Wednesday, June 8

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8:00 AM

SYMPOSIUM VI:

MARSHALL HALL AUDITORIUM

### SEMIOCHEMICALS OF BENEFICIAL ARTHROPODS

Organizer: Jocelyn Millar

---

Harvesting Predators and Parasitoids with Semiochemicals: Testing the "New Associations" Biocontrol Concept,  
*Jeffrey R. Aldrich*, USDA, Beltsville, Maryland

Why Not Be A Queen? Regioselectivity in the Mandibular Secretions of Honey Bee Castes,  
*Keith N. Slessor*,  
Simon Fraser University, Burnaby, British Columbia

Multifunctional Communication in the Bean Bug, *Riptortus clavatus* (Heteroptera: Alydidae), *Walter S. Leal*,  
Cornell University, Ithaca, NY

Aggressive Chemical Mimicry by Bolas Spiders,  
*Kenneth V. Yeargan*, University of Kentucky, Lexington, Kentucky

Evidence of a Female Sex Pheromone in the Aphid Parasitoid,  
*Aphidius nigripes*, *Jeremy McNeil*,  
Université Laval, Quebec City, Quebec

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10:30 – 11:00 AM BREAK

Nifkin Lounge

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11:00 AM

CONTRIBUTED PAPERS, SESSION 6

MARSHALL HALL AUDITORIUM

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Pheromonal Mediation of Nest Location in Honey Bees,  
*Justin O. Schmidt.*

Do Specialist Bees Orient to Key Volatiles in Pollen?  
*Heidi E. M. Dobson.*

Effect of *Aphanamixis polystachya* on Insects and Fungal Pests of  
Stored-Products, *Farid A. Talukder.*

Egg Parasitoids of Galercuinae (Coleoptera; Chrysomelidae): Role  
of Chemical Cues for Host Finding and Host Acceptance,  
*Monika Hilker.*

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12 NOON – 12:30 PM

ISCE BUSINESS MEETING

MARSHALL HALL AUDITORIUM

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Thursday, June 9

Finger Lakes Wine Country Tour

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# ABSTRACTS — INVITED PLENARY AND SYMPOSIUM SPEAKERS

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## GAS CHROMATOGRAPHY - OLFACTOMETRY IN FLAVOR ANALYSIS.

*Terry E. Acree*, Cornell University, NYSAES, Geneva, NY 14456.

Sensory tests designed to measure odor-active chemicals most commonly measure one or more of three properties: dose, character and potency. Gas chromatography - olfactometry (GCO) or "GC - Sniffing" as it is often called, not only produces measures of dose, potency and character of odorants but also yields chromatographic properties of odorants that can be used to identify the chemical causing the odor. For example, the characteristic odor and gas chromatographic retention properties of compounds like  $\beta$ -damascenone and geosmin make their detection in natural products at the femtogram level routine. This presentation will discuss some of the applications of GCO and its implications for other GC based bioassays.

## HARVESTING PREDATORS AND PARASITOIDS WITH SEMIOCHEMICALS: TESTING THE "NEW ASSOCIATIONS" BIOCONTROL CONCEPT.

*Jeffrey R. Aldrich*, USDA Insect Chemical Ecology Laboratory, Beltsville, MD 20705 USA.

Males of the spined soldier bug (SSB), *Podisus maculiventris* (Heteroptera: Pentatomidae), produce a powerful attractant pheromone that is exploited as a host-finding kairomone by a complex of parasitic species. The availability of synthetic SSB pheromone is enabling the "new associations" concept (Hokkanen and Pimentel, 1989) to be tested in two ways. The idea that evolutionarily new exploiter-victim associations are optimal for effective biocontrol is being tested from a practical standpoint by using the SSB to control the Colorado potato beetle, a pest which has recently invaded the SSB's range. A USDA Pilot Test is underway to use pheromone to harvest wild SSB adults early in the spring to mass-produce young predators for "Suppression of Colorado Potato Beetle Infestation by Predator Augmentation." Secondly, the theoretical premise of the new associations concept is being tested by giving parasitoids a choice between native and exotic hosts. Wild adult females of a generalist tachinid fly parasitoid, *Euclytia flava*, which are captured in pheromone-baited traps, more often than not prefer to oviposit on exotic pentatomid species in the laboratory even when their choice includes a native host species. These results suggest that exotic species are actually maladapted and vulnerable to native parasitoids, although native beneficials do not recognize the pheromones of foreign potential hosts.

## NATURAL PRODUCTS IN DRUG DISCOVERY.

*Paul S. Anderson*, The DuPont Merck Pharmaceutical Company, Experimental Station, P.O. Box 80353, Wilmington, DE 19880-0353, USA.

Receptor-based screening has become a valuable method for discovery of novel natural products which can serve as leads for development of useful drugs. This approach to drug discovery produced a novel CCK antagonist, asperlicin, from *Aspergillus alliaceus* and a structurally unique cyclic hexapeptide with oxytocin antagonist activity which was isolated from *Streptomyces silvensis*. Using the structural information encoded in these natural products, subsequent medicinal chemistry efforts succeeded in elaboration of antagonists suitable for clinical study. Advances in molecular biology and X-ray crystallography have made it possible for the target molecule to be the template which drives drug discovery rather than the ligand. In the case of HIV protease, the crystal structure of this enzyme has played a major role in the design of potent, selective enzyme inhibitors. The modified natural product acetyl pepstatin also played a key role in the evolution of this science as it was the first inhibitor of HIV-1 protease to be studied while bound to the enzyme active site.

## OLFACTORY TEMPORAL ANALYSIS OF SPATIAL GRADIENTS IN TURBULENT ODOR DISPERSAL.

*Jelle Atema*, Jennifer Basil, George Gomez, Paul Moore and Rainer Voigt. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543 USA.

Turbulent odor dispersal is characteristic of most macroscopic environments. It results in patchy, filamentous odor concentration patterns. High resolution analysis of the spatial distribution of odor patches shows the existence of a spatial gradient of concentration parameters that points to the odor source. Lobsters do locate odor sources and use antennular information in the process. We describe dynamic tuning properties of lobster antennular chemoreceptor cells that may be used to extract pertinent odor pulse features for chemotactic behavior. These temporal properties include flicker fusion frequency, stimulus integration time, time course of background adaptation and cumulative adaptation, and response reliability (i.e. receptor noise). We will also consider aspects of stimulus intensity coding and central processing of temporal features. Overall, the results indicate that the lobster olfactory system is designed to selectively amplify a variety of odor pulse onset slopes above local background concentrations at repetition rates not exceeding 4 Hz. This frequency corresponds to the animal's maximum flick (i.e. sniff) rate, as well as to the 200 ms sampling bins of its receptor cells. The instantaneous distribution of pulse onset slopes provides one of the best spatial gradients to follow to the odor source.

## RESOURCE ALLOCATION AS A CONSTRAINT IN PLANT CHEMICAL DEFENSE.

*Ian T. Baldwin*, Department of Biological Sciences, SUNY at Buffalo, Buffalo, NY 14260, USA.

The hegemony of the second law of thermodynamics has dominated the development of plant chemical defense theory; the fact that resources can not be simultaneously allocated to secondary metabolite production and growth represents a point of departure for theories addressing the patterns of defense metabolites in plants. Whether or not this "constraint" is the mechanism responsible for the patterns observed in multi-taxa comparative studies is unclear for the "opportunity" costs of allocating resources to defense may be



overshadowed by the opportunity costs of herbivory. Intraspecific experimental studies which manipulate resource allocation to growth and defense have underscored the importance of the ecological context of plant growth for the existence of tradeoffs. The controls over secondary metabolite production in some systems has advanced to the point where allocation patterns can be manipulated in the field and experimental tests of the cost-benefit paradigm of plant chemical defense are now possible.

#### TURNABOUT IS FAIR PLAY: SECONDARY FUNCTIONS FOR PRIMARY COMPOUNDS.

May Berenbaum, University of Illinois, Urbana, Illinois.

Chemically based resistance of plants to herbivorous insects is today essentially synonymous with allelochemically based resistance; the importance of plant secondary compounds in determining patterns of hostplant utilization has been established in a vast number of insect/plant interactions. In contrast, primary compounds, those involved in fundamental plant physiological processes, are rarely considered to be major determinants of hostplant resistance. The degree to which variation in plant primary metabolism results from the selective impact of herbivory, however, may be seriously underestimated in that the biosynthetic and structural diversity of primary metabolites and the consequences of that diversity on herbivore behavior and physiology are rarely taken into account in most studies of insect preference and performance. Qualitative variation in the production of primary metabolites can result from herbivore selection pressure if that variation is genetically based and if plant fitness in the presence of herbivores is associated in a predictable way with that genetically based primary metabolite variation. Variation in oligophagous herbivores with limited mobility, especially those confined to reproductive structures containing allelochemicals that could neutralized the benefits associated with compensatory feeding.

#### ISOLATION, SEPARATION AND IDENTIFICATION OF CHIRAL PHEROMONES FROM SAWFLIES AND ANTLIONS.

L. Gunnar Bergström and Anni-Brit. Wassgren, Department of Chemical Ecology, Göteborg University, Göteborg, Sweden.

Nanogram-amounts of pheromone precursors have been isolated from the sawflies *Neodiprion sertifer* and *Diprion pini*; they are enantiomers and homologs of diprionol (2S,3S,7S-3,7-dimethyl-2-pentadecanol); separation of the light enantiomers was achieved by combination of LC, GC, chiral derivatives, chiral columns and identification by GCMS (Ei), GCMS (Ci), GCEAD, GCMS (Sim). Potential sex pheromones of five species of antlions (Neuroptera) have been identified in the same way. Techniques for "cross-sectional" head-space analysis is discussed.

#### CHEMICAL IDENTIFICATION AND BIOLOGICAL ACTIVITY OF VOLATILES PRODUCED BY THE WHITE PINE CONE BEETLE.

Göran Birgersson, Chemical Ecology, Göteborg University, Göteborg, Sweden.

Females of white pine cone beetle, *Conophthorus coniperda* (Schwarz), attacking second-year cones of eastern white pine, *Pinus strobus* L., produce a sex-specific pheromone that attracted conspecific males in laboratory bioassays and to traps in the field. The female-produced compound was identified as (+)-*trans*-pityol (2R,5S)-2-(1-hydroxy-1-methylethyl)-

5-methyltetrahydrofuran. Beetle response was synergized by host monoterpenes. Both males and females produce and release a spiroacetal, E-(+)-(5S,7S)-7-methyl-1,6-dioxaspiro[4.5]-decane, which was not an attractant for either sex, but acted as an inhibitor for males. In addition, both sexes produce *trans*-pinocarveol, pinocarvone and myrtenol. The amounts of produced compounds were quantified and related to different treatments of the insects, and during different phases of the attack. Only virgin females produce *trans*-pityol, and cease if leaving host cone, even if not mated. Mated females and males produce the spiroacetal.

#### **SIGNIFICANCE OF EARLY ONTOGENETIC VARIATION IN CHEMICAL DEFENSE OF WOODY PLANTS AGAINST BROWSING BY MAMMALS.**

*John P. Bryant*<sup>1</sup>, Riitta Julkunen-Tiitto<sup>2</sup>, Rob Swihart<sup>3</sup>, and Paul Reichardt<sup>1</sup>. (1) University of Alaska, Fairbanks, Alaska, (2) University of Joensuu, Joensuu, Finland, and (3) Purdue University, West Lafayette, Indiana.

Browsing by mammals is an important factor in the ecology and evolution of woody plants. From the perspective of chemical ecology a very important, but poorly understood control over the relationship between woody plants and browsing mammals is allocation of resources by woody plants to chemical antiherbivore defense in the early stages of their ontogenetic development. We begin this paper by discussing how allocation of carbon to chemical defense by woody plants varies shortly after germination, and relating this ontogenetic variation in defense to the establishment of woody plants subject to browsing by mammals and competition. Then we describe a global biogeographic pattern in the chemical defense of northern woody plants, and suggest this pattern is the result of the effect browsing by mammals has had on woody plant establishment in boreal forests since the Ice Age. We conclude by discussing the implications this biogeographical pattern holds for further development of plant defense theory.

#### **COMMUNICATION/RECOGNITION BETWEEN THE PARASITIC WEED, *STRIGA*, AND ITS HOSTS.**

*Larry G. Butler* and Gebisa Ejeta. Purdue University, West Lafayette, IN 47907 USA.

*Striga* spp. (witchweeds) are obligate parasitic weeds of tropical cereals and legumes which are becoming a major constraint on grain production in Africa. *Striga* is exquisitely adapted to its host. Early phases of *Striga* development, even before attachment to host roots, are under control of host-produced chemical signals, which are being identified. Some non-hosts produce the same chemicals, so host recognition is not completely explained by the occurrence of these signals. An effective and durable mechanism of resistance is host failure to produce sufficient levels of more than one of these signals required for *Striga* development. Field screening for *Striga* resistance is very slow and difficult. Screening crop hosts for low signal production is a much more efficient method of breeding for *Striga* resistance.

#### **MEASUREMENT OF PHEROMONE DENSITY IN DISRUPTANT-TREATED PLOTS WITH AN ELECTROANTENNOGRAM SYSTEM.**

*Ring T. Carde*, Uwe Koch, and Robert Staten, Department of Entomology, University of Massachusetts, Amherst, USA.

A key to understanding and predicting efficacy of communication disruption is

measurement of airborne concentrations of disruptant. We have used a system based on electroantennograms (Sauer et al. 1982, *Chemical Senses*, 17:543-553) to measure over time intervals of a fraction of a second the distribution of formulated pheromone of the pink bollworm moth (*Pectinophora gossypiella*) in Arizona cotton fields with hand- and commercially-applied pheromone. The densities of pheromone are influenced by the type of formulation, and its rate of application, age and position with the canopy. This technique can be coupled with the use of large field wind tunnels and behavioral observations of released moths to establish how the distribution of formulated pheromone disrupts normal patterns of mate location.

#### **DEVELOPMENT OF LIPOSOME-BASED IMMUNOANALYTICAL METHODS FOR TOXIC CHEMICALS.**

*Richard A. Durst*, Analytical Chemistry Laboratories, Cornell University - NYSAES, Geneva, NY 14456 USA.

To address the need for more rapid, sensitive and specific analytical methods, a fully automated, flow-injection, liposome-based immunoanalysis (FILIA) system is being developed for the determination of toxic substances in environmental samples. Antibodies which bind an analyte of interest are immobilized in a FILIA reactor column as the first step in developing a reusable, automated immunoanalyzer. Detection and quantitation of the interaction between analyte in a sample and the immobilized antibodies on the immunoreactor column are achieved through the use of competitively bound analyte-tagged, marker-loaded lipid vesicles (liposomes). Using similar principles and methodologies, a simple, user-friendly, single-use immunobiosensing device for extra-laboratory screening applications is also being developed. In this approach, the analyte-labeled liposome competes with unlabeled sample analyte for the active antibody binding sites immobilized on the assay test strip and provides an analytical signal that can be directly related to the concentration of the toxic compound of interest.

#### **BIORATIONALITY: IMPROVING THE "HIT" RATE.**

*Thomas Eisner*, Cornell University, Ithaca, NY 14853 USA.

The first step in the search for drugs from nature is the selection of species for chemical screening. Species can be selected on a random basis, or on the basis of what may be termed *biorational leads*. Such leads have traditionally come from ethnobotany or indigenous medicine, but they may be increasingly forthcoming from currently unfolding scientific disciplines. Chemical ecology may itself become a significant source of leads for drug development.

#### **REVIVAL OF SCREENING: TARGETED SCREENING USING MOLECULAR BIOLOGY TECHNIQUES.**

*Prabhavathi B. Fernandes*, Bristol-Myers Squibb, Princeton, NJ 08543-4000, USA.

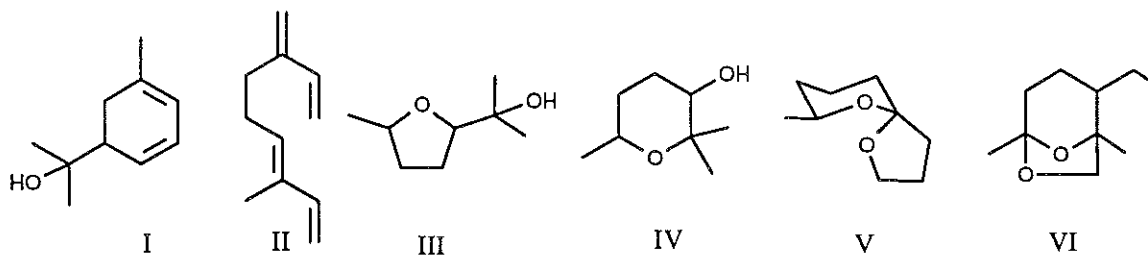
Molecular biology has introduced fundamental changes to screening, redirecting its main focus from that against whole cells and pharmacological targets into cloned targets of interest. The advantage to this is that the specific target selectivity of the leads isolated is known and, therefore, non-specific toxic side effects are avoided. New targets have been identified using molecular biology tools. Human enzymes and receptors are cloned and expressed in *E. coli*, *S. cerevisiae*, or mammalian cells for the purpose of developing high-

throughput screens. Expression of individual isoenzymes, such as the GMP phosphodiesterases, will allow isoenzyme specificity. Cloning of receptors has led to identification of receptor subtype selective antagonists and agonists such as endothelin receptors and adrenergic receptors. The cloning of human enzymes and receptors has allowed the identification of human target specific lead compound identification, while in the past, leads were identified using animal tissues or cells. In screening against cancer, the goal is no longer to identify cytotoxic agents. Rather, it is to reverse the cancer phenotype. In addition, molecular biology, electronic technology and robotics have taken away the boredom of repetitive motions involved in high-throughput screening. The expression systems are utilized keeping in mind the need for their compatibility with screening microbial and plant extracts, as well as synthetic compound collections.

### NEW SEMIOCHEMICALS FROM BARK BEETLES.

*Wittko Francke*, Institut für Organische Chemie, Martin-Luther-King-Platz 6, D-20146 Hamburg.

The *m*-mentha-4,6-dien-8-ol (I) is a strong repellent produced by males of *Ips typhographus*. It may be derived from 3-carene and appears to be wide spread among bark beetles. The homoterpene (II) is a repellent produced by *Ips typhographus* under stress. Reduction, epoxidation and ring closure of 6-methyl-5-hepten-2-one would yield pityol (III) and vittatol (IV), while epoxidation of 6-methyl-6-hepten-2-one, which is present in *Dendroctonus simplex*, would produce frontalin upon ring closure. Females of *Conophthorus* spp. produce pityol as an attractant, while males produce the spiroacetal (V) as a repellent, which was also found in other bark beetles. The identification of isomers and derivatives of brevicomin will be discussed. The new bicyclic acetal, 2-ethyl-1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane (VI), was identified from the beech bark beetle *Taphrorychus bicolor*.



### SUSTAINABLE DEVELOPMENT, BIODIVERSITY AND CHEMICAL ECOLOGY: A COSTA RICAN VIEW.

*Rodrigo Gamez*. Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, 3010 Costa Rica.

Chemical ecology offers significantly valuable opportunities to address the complex issues of linking biodiversity with sustainable development. Understanding the chemistry of ecosystems could help increase the value of biological resources and the conservation of tropical biodiversity through promoting the sustainable use of these resources. The implementation of this strategy presents complex problems. It requires national and international frameworks and mechanisms that can bring together bodies and agencies concerned with economic policy, environmental management and local government, labor and industry, facilitating also their interactions with the non-governmental sector.

Simultaneously, multisectoral and multidisciplinary activities are required, incorporating widely different sectors of society, from rural parataxonomists to scientists, lawyers, managers, economists and policy makers, from both developing and developed countries. The Costa Rican search for a new model of sustainable development based on the non-destructive use of its biodiversity will be discussed.

#### **CHEMICAL PROSPECTING: AN OVERVIEW OF THE INTERNATIONAL COOPERATIVE BIODIVERSITY GROUPS PROGRAM.**

*Francesca T. Grifo*, Fogarty International Center, National Institutes of Health, Bethesda, MD 20892, USA.

The International Cooperative Biodiversity Groups (ICBG) Program is an integrated conservation and development program which addresses the interdependent issues of biodiversity conservation, sustained economic growth, and human health in terms of drug discovery for diseases of concern to both developing and developed countries. The funding for this program is provided by the National Institutes of Health, National Science Foundation, and US Agency for International Development. Because biological resources which benefit local communities are among those most likely to be preserved, chemical prospecting or more specifically, development of pharmaceuticals from natural products can be used to promote biological conservation by providing an economic return from sustainable use of the resources. A critical component of the supported activities is to ensure that equitable economic benefits from these discoveries accrue to the country of origin, community, group, or organization which facilitated the discovery of the natural product. This is being achieved through the use of novel contractual mechanisms among the members of each group.

#### **MULTIFUNCTIONAL COMMUNICATION IN THE BEAN BUG, *RIPTORTUS CLAVATUS* (HETEROPTERA: ALYDIAE).**

*W.S. Leal*,<sup>1</sup> *H. Higuchi*,<sup>1</sup> *N. Mizutani*,<sup>1</sup> *H. Nakamori*,<sup>2</sup> *T. Kadosawa*,<sup>1</sup> and *M. Ono*.<sup>3</sup> <sup>1</sup>Ministry of Agriculture, Forestry, and Fisheries of Japan, <sup>2</sup>Okinawa Prefectural Agricultural Experiment Station, and <sup>3</sup>Fuji Flavor Co. Ltd.

Semiochemicals released by males of *R. clavatus*, (E)-2-hexenyl (E)-2-hexenoate, (E)-2-hexenyl (Z)-3-hexenoate, and myristyl isobutyrate, play the double role of aggregation pheromone for adults as well as "brood care pheromone", which enables the second-instar nymphs to find host plants. On the other hand, females of *Ooencyrtus nezarae*, the most effective parasitoid of the host in Kumamoto, Japan (where the field experiments were conducted), utilize these semiochemicals as kairomones in order to locate the potential host community.

#### **HOW PARASITOIDS USE CHEMICAL CUES TO TRACK HOST AND FOOD RESOURCES IN A TRITROPHIC SYSTEM.**

*W. Joe Lewis*, USDA/ARS, Tifton, Georgia 31793 USA.

Parasitic wasps of herbivores rely on plants as sources of insect hosts and for nectar and pollen food needs. The larval parasitoids, *Microplitis croceipes* and *Cotesia marginiventris*, and the egg parasitoid *Trichogramma* Spp. have served as model systems to elucidate how sophisticated use of chemical and visual cues enable the parasitoid to track these resources

in a complex environment where the host is playing hide-and-seek. The studies have revealed an array of precocious tracking methods used by the parasitoid. The egg parasitoid eavesdrops on the sex pheromone of the host moth as a source of volatile long range indicators of the vicinity of its egg stage host. Nonvolatile cues in moth scales and adhesives that attach the eggs to the plant are used as close range host location and recognition cues. The larval parasitoids rely on volatile "distress" signals emitted by the plant when fed on by the herbivore as long range indicators of the host's presence and vicinity. Nonvolatile, host specific chemicals in the host frass are used as close range host location and recognition cues. Visual cues associated with the host are also important and are used in combination with the chemical cues. Moreover, learning serves a crucial role in the ability of parasitoids to exploit these chemical and visual cues, especially the indirect long range cues. During visitations to host sites the parasitoids learn chemical and visual cues, including novel information, associated with host specific cues and subsequently use that information to improve host finding. Learned use of plant-related chemical and visual cues were found to be important also in the parasitoid's location of its adult food needs.

#### **EVIDENCE OF A FEMALE SEX PHEREMONE IN THE APHID PARASITOID, *APHIDIUS NIGRIPES*.**

*Jeremy McNeil*, Jacques Brodeur, Department of Biology, Laval University, STE FOY, P. Q., Canada 91K 7P4.

*Aphidius Nigripes* is the most abundant parasitoid attacking the potato aphid, *Macrosiphum euphorbiae* in Quebec. Recent experiments have demonstrated that the parasitoid actually passes the winter as a diapausing prepupa and is not, as previously assumed, a seasonal immigrant. A research program is now underway to develop methods to increase the parasitoid populations in the agroecosystems early in the season. One facet of the project addresses the reproductive biology of the parasitoid. Mating in the species involves a female sex pheromone. Data will be presented on (i) the effect of the age and mating status on both the production of, and receptivity to, the sex pheromone, (ii) the diel periodicity of pheromone and (iii) the use of the pheromone as a tool for monitoring parasitoid population density in the field.

#### **TRACKING VARIABLE ENVIRONMENTS: THERE IS MORE THAN ONE KIND OF MEMORY.**

*Frederick D. Provenza*, Utah State University, Logan, UT 84322-5230 USA.

Three kinds of memory help mammalian herbivores track changes in the environment. The first is the collective memory of the species with genetic instructions that have been shaped by the environment through the millennia. This includes skin- and gut- defense systems evolved under selective pressures of predation and toxicosis. Auditory and visual stimuli and sensations of pain impinge upon the skin-defense system that evolved in response to predation. The taste of food and the sensations of nausea and satiety caused by toxins and nutrients are an integral part of a gut-defense system that evolved in response to plant deterrents. Mother is the second kind of memory in social mammals, a source of transgenerational knowledge that increases efficiency and reduces risk of learning about foods and environments. The third kind of memory is acquired by experience. Postingestive feedback from nutrients and toxins enables animals to experience the consequences of food ingestion, and to adjust selection and intake commensurate with a food's utility. The three memories interact, each linking the past to the present, and in so

doing collectively shape the future of every individual. Thus, explaining the dynamics of foraging involves appreciating the uniqueness of individuals and subgroups of animals, each with its (their) own genetic and behavioral history, and recognizing that foraging behaviors may not be stable, optimal, or even predictable in the conventional sense.

#### **YOU CAN'T TELL THE PLAYERS WITHOUT A PROGRAM: PLANT-HERBIVORE INTERACTIONS AREN'T JUST INTERACTIONS BETWEEN PLANTS AND HERBIVORES.**

*Jack C. Schultz*, Penn State University, University Park, PA 16802 USA.

Progress in understanding the nature of plant-herbivore interactions (evolution of diet, structure of plant defenses, impact of participants on each other, etc.) has been slowed by restricting our view to the obvious participants: plants and herbivores. Unfortunately, it is clear that the characteristics of both are shaped strongly by interactions with other organisms and factors. I point out that plant responses to environmental stimuli as diverse as symbiotic microbes and wind are mediated by similar, broadly overlapping biochemical mechanisms that produce "crossed impacts" on other species, including herbivores. I review evidence that the outcome of plant-herbivore interactions frequently, if not always, is determined by "third parties" that may be neither plant nor herbivore. If our science is to be a predictive one, we must identify ALL of the influential participants in these interactions and identify the mechanisms involved, quantify their impacts, and integrate these interacting factors. This broader, but more specifically phenomenological view of plant-herbivore interactions is needed if we are to develop a lasting theoretical construct for our work. This broader approach is needed for the continued growth and increasing influence of chemical ecology in both basic and applied sciences.

#### **CHEMOTAXONOMIC STUDIES OF THE PINE ENGRAVER BEETLE, *IPS PINI* (SAY) (COLEOPTERA: SCOLYTIDAE).**

*Steven J. Seybold*<sup>1&3</sup>, *Toshikazu Ohtsuka*<sup>2</sup>, *David L. Wood*<sup>2</sup>, *Isao Kubo*<sup>2</sup>, *Jorge Santiago-Blay*<sup>2</sup>, *Lori J. Nelson*<sup>3</sup>, and *Michel J. Faucheux*<sup>4</sup>. <sup>1</sup>Department of Biochemistry, University of Nevada, Reno, NV 89557 USA, <sup>2</sup>Department of Environmental Science, Policy, and Management, University of California at Berkeley, Berkeley, CA 94720 USA, <sup>3</sup>USDA Forest Service, Pacific Southwest Research Station, Albany, CA 94710 USA, and <sup>4</sup>Laboratory of Endocrinology of Social Insects, University of Nantes, Nantes, FRANCE.

A geographic survey of the enantiomeric composition of the aggregation pheromone component ipsdienol produced by male *Ips pini* suggests that *I. pini* exists as at least three regional pheromone variants. A survey of cuticular hydrocarbon phenotypes from a subset of the populations surveyed for ipsdienol also indicates the presence of three regional variants. Limited comparative morphological analyses were conducted on populations from two of the three regions. The ultrastructure of the sound-producing organs (*pars stridens* and plectrum) from females originating from California and New York showed no differences in interstitial distances. Differences in these distances are key morphological discriminants for other closely related species of *Ips*. Preliminary morphological analyses of the ultrastructure of male and female antennal clubs from these two populations indicate differences in the distribution and abundance of chemosensory sensilla. Taken together, this morphological evidence supports olfactory, but not sonic, behavioral discrimination by these two interbreeding populations.

## BIOSYNTHESIS OF MONOTERPENOID PHEROMONES OF BARK BEETLES.

*Desiree Vanderwel*, Department of Chemistry, University of Winnipeg, Winnipeg, Mb., Canada, R3B 2E9.

Many chemicals used as pheromones by bark beetles are derivatives of monoterpenes (alcohols, ketones, or rearrangement products). It has been known for over two decades that monoterpene alcohol pheromones (e.g., ipsdienol and *cis*- and *trans*-verbenol) can be produced through the allylic oxidation of monoterpenes found in the resin of the host tree (e.g., myrcene and  $\alpha$ -pinene, respectively). These biosynthetic pathways were widely believed to be relatively simple adaptations of pathways designed for the detoxification of the resin monoterpenes. However, the subtleties underlying the biosynthesis of bark beetle pheromones are only just beginning to emerge. For example, ipsdienol may not be formed solely through the oxidation of host monoterpenes but may, in fact, involve a *de novo* pathway (Ivarsson, Schlyter and Birgersson, 1993, *Insect Biochem. Molec. Biol.* 23:665-662). Additionally, it is important to consider the stereochemistry of pheromone biosynthesis: chirality is an important feature which differentiates coleopteran pheromones. For example, the results of several investigations (too numerous to list here) indicate that both the (S)-(+)- and (R)-(-)-enantiomers of ipsdienol can serve as pheromones, and that different beetles respond differently to the two enantiomers. This has very interesting biochemical implications: how do the beetles manipulate the chirality of the ipsdienol that they produce? The results of recent studies to explore the biosynthesis of the monoterpene pheromones will be summarized.

## AGGRESSIVE CHEMICAL MIMICRY BY BOLAS SPIDERS.

*Kenneth V. Yeorgan*, University of Kentucky, Lexington, KY 40546 USA.

Bolas spiders are so named because late-stadia immature and adult females swing a droplet of adhesive on a silk thread at their flying prey, certain species of male moths. These spiders, which belong to five genera in the large orb-weaver family (Araneidae), are widespread but not abundant; they are known from most parts of the world except temperate Eurasia. The best studied genus, *Mastophora*, occurs in North and South Africa; five species of *Mastophora* have been described from the USA, namely *archeri*, *bisaccata*, *cornigera*, *hutchinsoni*, and *phrynosoma*. The species of moths captured by bolas spiders have been extensively documented for a few species of *Mastophora*, and semiochemicals emitted by bolas spiders have been identified (by researchers in Florida) for one species, *Mastophora cornigera*. A review of sex pheromones and sex attractants of moth prey species suggests that at least some species of bolas spiders have the biosynthetic capability to produce diverse chemical attractants, including compounds that are seldom produced in combination by a single moth species. Young *Mastophora* spiderlings generally do not hunt with a bolas. Instead they use the first two pairs of legs to capture prey that come within their reach. Field experiments in 1993 demonstrated that *M. phrynosoma* spiderlings attract adult flies of the genus *Psychoda*.

## MOLECULAR DETERMINANTS OF THE PLANT/FUNGUS INTERACTION.

*Olen C. Yoder* and B.G. Turgeon. Department of Plant Pathology, Cornell University, Ithaca, NY 14853 USA.

The goals of this talk are to: (I) Describe molecules produced by fungi which have been critically evaluated for roles in pathogenesis to plants, and (ii) Illustrate how molecular and conventional genetic technologies have been used to assess the pathological function



of certain fungal metabolites. Case studies from several different laboratories are presented: 1) Many fungi, including pathogens of both plants and animals, produce a dark brown pigment called **melanin**. For some (but not all) of these fungi, mutations in the melanin biosynthetic pathway (which cause albinism) result in loss of ability to penetrate through the plant epidermis. In one case a cloned gene from the pathway was transformed into the corresponding nonpenetrating albino mutant; both melanin production and penetrating ability were restored to wildtype levels. 2) Some fungi are hypothesized to penetrate their hosts enzymatically. One candidate for this role is **cutinase**, an esterase which degrades cutin, a major part of the plant epidermis. Several lines of circumstantial evidence have been advanced to support the view that cutinase can mediate penetration. However, a recent experiment employed gene cloning technology to produce site-specific mutants lacking cutinase; they were found to be just as virulent as wildtype. 3) Many plants, upon microbial attack, produce antimicrobial secondary metabolites called phytoalexins, which have been proposed as defense mechanisms. For pea, the characteristic phytoalexin (pisatin) is induced by the pathogen *Nectria haematococca*. Pathogenic strains of this fungal produce **pisatin demethylase (PDA)**, which degrades pisatin to a nontoxic compound; nonpathogenic strains do not produce PDA. The cloning of PDA encoding genes made possible site-specific gene disruptions. Transformants specifically lacking PDA have recently found been found to retain their pathogenicity to pea, thus indicating that PDA is not essential for pathogenicity. 4) *Cochliobolus heterostrophus* and a polyketide (**T-toxin**) it produces both specifically affect corn carrying the mitochondrial *T-urf13* gene. When a fungal locus (*Tox1*) which controls production of T-toxin is mutated, both T-toxin production and high virulence to *T-urf13* corn are reduced or lost.



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## ABSTRACTS — CONTRIBUTED PAPERS

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### INDUCED DEFENSE IN COTTON PLANTS AS A RESPONSE TO DAMAGE BY *SPODOPTERA LITTORALIS* LARVAE.

*Hans T. Alborn*, Department of Chemical Ecology, University of Göteborg Sweden.

It has earlier been shown that when given a choice between cotton seedlings that in the cotyledon stage had been damaged by feeding insects, and non-damaged seedlings, lepidoptera larvae prefer to feed on and develop slightly better on the latter. To investigate if "anti-feeding" could be induced also in fully developed leaves on full grown cotton plants *Spodoptera littoralis* larvae were allowed to feed overnight on a single leaf on cotton plants. In a choice test, several days later, new *S. littoralis* larvae were fed non-damaged leaves of equal age and size from damaged and previous non-damaged plants. The larvae showed a clear avoidance of leaves from damaged plants if these came from a position on the plant above the previous damaged leaf. Fourth instar larvae could in no-choice tests barely survive on leaves from "induced" plants while young second instar larvae died. The same degree of anti feeding defense seemed to exist in damaged leaves, leaves that were fully developed when the damage occurred, and in leaves that developed after that the damage occurred. A component in insect saliva has been shown to induce the production of volatile, parasitoid attractants in several plants. The same component might be responsible for the induction of an anti-feeding defense in cotton plants, in that case being a very useful tool for inducing natural defense in plants.

### TANNIN ACTION IS A REAL FEENOMENON: PROTEIN PRECIPITATION IN THE MIDGUT FLUID OF GYPSY MOTH CATERPILLARS.

*Heidi M. Appel* and Jack C. Schultz, The Pennsylvania State University, University Park, PA 16801 USA.

Since Paul Feeny suggested 25 years ago that tannins bind nutritionally important proteins in insect herbivores, there has been no evidence to date that tannins bind proteins in the digestive tract of insects. We report here for the first time the precipitation of proteins in the midgut fluid of gypsy moth caterpillars by ecologically relevant tannins at naturally occurring concentrations. Incubating red oak tannin in midgut fluid of fifth-instar gypsy moth caterpillars reared on artificial diet resulted in 1) a loss of midgut fluid proteins on SDS-PAGE gels of midgut fluid extracts, 2) a precipitate containing tannin and nitrogen, and 3) a loss of tannins from HPLC profiles of gut fluid extracts. As much as half of the nitrogen in gut fluid is removed from solution by tannin. We suggest that protein precipitation represents the mechanism of reduced growth and fecundity and reduced susceptibility to disease of gypsy moths on high-tannin foliage. The pH and Eh conditions of the midgut of gypsy moth caterpillars indicate that protein precipitation by tannins is most likely achieved by the formation of covalent and not hydrogen bonds.

## TANNIN SENSITIVITY AND TOLERANCE IN LARVAL LEPIDOPTERA.

*Raymond V. Barbehenn*, Michael M. Martin. University of Michigan, Ann Arbor, MI 48109, USA.

Tannic acid (TA) has no detrimental effects on late-instar larvae of *Orgyia leucostigma* when ingested in artificial diet (5% DW). *Malacosoma disstria*, by contrast, develops lethal pupal deformities when less than 1% TA is ingested. We examined several mechanisms potentially responsible for their tannin-tolerance and -sensitivity, respectively. (1) The peritrophic envelopes (PE) of both species are impermeable to TA. (2) Virtually 100% of ingested TA was recovered in the frass of *O. leucostigma*, but only 19-21% of ingested TA was recovered from *M. disstria*. (3) Little hydrolysis of TA was detected in either species, and no oxidation of TA was found in *O. leucostigma*, but TA is oxidized in the midguts of *M. disstria*. (4) Both species maintain similar oxidizing redox parameters in their midguts. We conclude that an impermeable PE may be necessary to protect the midgut epithelium from TA, but that PE impermeability is not sufficient to protect an insect from the detrimental effects of the oxidation of TA in its midgut. Factors in addition to redox parameters are necessary to explain the occurrence of phenol oxidation in larval Lepidoptera.

## DO BRASSICA-PHYTOALEXINS HAVE AN EFFECT ON HOST-PLANT SELECTION IN THE CABBAGE ROOT FLY?

*Robert Baur*, Erich Städler, Kenji Monde\* and Mitsuo Takasugi\*, Swiss Federal Research Station, CH-8820 Wädenswil, Switzerland; (\*): Div. Material Science, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060, Japan.

In the plant genus *Brassica* (Cruciferae) microbial infection induces the production of phytoalexins, i.e., compounds with antimicrobial properties. Identified phytoalexins usually contain an indole—or indole-related (oxindole)—system linked to a N- and S-containing moiety. To date, there is no information on effects of these compounds on herbivorous insects associated with the *Brassica* plants producing them. For the cabbage root fly, *Delia radicum* (Anthomyiidae), which feeds exclusively on Crucifers, the major groups of non-volatile oviposition stimulants on the leaf surface are known to be glucosinolates and a group of novel compounds (identification in progress). While these compounds for the ovipositing female provide basic information on the identity and suitability of a plant species as host, additional compounds, like phytoalexins, may be used to assess the suitability of individual plants among potential hosts. In oviposition behavior assays with *D. radicum*, the screening of 11 *Brassica*-phytoalexins yielded 3 compounds (methoxybrassinin, cyclobrassinin, brassitin) which stimulated oviposition. However, compared to the already known oviposition stimulants, these compounds had only moderate activity. The other phytoalexins tested had no effect and no evidence for inhibition was found. Our preliminary results suggest, that oviposition behavior in the cabbage root fly may be influenced by compounds other than the oviposition stimulants known to date.

## CHEMICAL DEFENSES OF THE THYSANOPTERA.

*Murray S. Blum*, Henry M. Fales, Tappey H. Jones, University of Georgia, Athens, GA 30602, USA.

Many species of Phlaeothripidae discharge anal droplets during confrontations with a variety of adversaries. These exudates are fortified with natural products that can function as contact irritants, repellents, or in some cases fumigants. The allomonal products include

esters, acids, hydrocarbons, aromatic compounds, and monoterpenes. Mixtures of compounds characterize most of the exudates and it is likely that the defensive efficacies of these secretions reflect synergistic interactions. These anal exudates are effective repellents for ants which probably constitute the major predators of thrips.

#### PHARMACOPHAGOUS RELATION BETWEEN ZONOCERUS GRASSHOPPERS AND THE INTRODUCED WEED CHROMOLAENA ODORATA IN AFRICA.

*Michael Boppré*, Ottmar W. Fischer, Forstzoologisches Institut der Universität Freiburg, D-79085 Freiburg i.Br., Germany.

*Zonocerus variegatus* (L.) (Pyrgomorphidae) is a polyphagous African grasshopper. In parts of West Africa its dry-season population has reached pest status, apparently in coincidence with the spread of the introduced weed, *Chromolaena odorata* (L.) K. & R. (Asteraceae: Eupatorieae), which, however, is not a foodplant for *Zonocerus*. Knowledge of pharmacophagous utilization of pyrrolizidine alkaloids (PAs) by *Zonocerus elegans* has suggested that flowers of *Chromolaena* which are attractive for and consumed by *Zonocerus variegatus* in a special manner do not have a nutritional function but rather serve solely as a PA-source. Field studies demonstrate that *Z. variegatus* is attracted to various PA-containing plants more strongly than to *Chromolaena* flowers, and pure PAs are also effective lures. In conclusion, *Zonocerus* seems to enjoy a non-nutritional association with *Chromolaena* which only provides PAs. These secondary plant compounds are stored and chemically protect the grasshoppers and particularly their diapausing eggs from predation and parasitism, and thus cause increased fitness and influence the population density of dry-season *Zonocerus*. Our ongoing behavioral, physiological and chemical studies strongly support this hypothesis and give an example of the hidden and unpredictable effects which the introduction of a foreign plant can have on population dynamics of a native insect species and on (agro-) ecosystems. This knowledge on the pharmacophagous behavior and its chemoecological context leads us to develop a PA-based bait to poison *Zonocerus* and/or infect the insects with pathogens, thus controlling them specifically in a way harmless to man and environment.

#### SOME THERMODYNAMIC CONSIDERATIONS FOR THE ANAEROBIC DEGRADATION OF HYDROCARBONS.

*John M. Brand*, David T. Gibson and Allen J. Markovetz, Department of Biochemistry, University of Fort Hare, Alice 5700, South Africa, and Department of Microbiology, University of Iowa, Iowa City, IA 52242.

A large number of hydrocarbons are ubiquitous in nature. Some arise from biological sources, some from oil deposits, and some from man-made sources or contaminating spills. As a considerable amount of energy is available from the reaction of hydrocarbons with oxygen, many organisms are able to grow on and degrade a variety of these highly reduced substances under aerobic conditions. Soil microorganisms offer the richest and the greatest biodiversity of all natural environments. Many soils and sediments are anaerobic and it has been questioned for many years as to whether aliphatic hydrocarbons can be degraded under these conditions. A recently obtained isolate has confirmed that hexadecane can be degraded completely to carbon dioxide under anaerobic conditions. In order to establish the most favorable conditions for such a process to occur, a cursory look at the thermodynamics of certain likely reactions under various conditions is valuable. It is shown that sufficient energy is available when either sulfate or Fe (III) act as terminal electron

acceptor in an environment that has a hydrogen partial pressure below about  $10^{-6}$  atm. This criterion of hydrogen partial pressure is often met in anaerobic soils and sediments.

### **STRUCTURE-ACTIVITY RELATIONSHIPS (SAR) OF AVIAN CHEMOIRRITANTS: IMPLICATIONS FOR PLANT ANIMAL EVOLUTION AND CONSERVATION BIOLOGY.**

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There is little understanding of avian chemosensory biology. Common misperceptions are that birds generally have poorly developed senses of taste and smell. Several recent studies have shown this not to be the case, demonstrating the ecological significance of both sensory modalities. Even less well described is avian perception of chemoirritants. This aspect of sensory biology may be of even greater importance to the biology of birds, given that many plant and insect defensive metabolites directly act on chemoreceptive fibers of the trigeminal nerve and somatosensory system. I report on the structure-activity relationships of avian trigeminal irritants. The critical features of an avian repellent are (1) the presence of an aromatic ring, (2) the basicity of the molecule in general, and specifically, when an electron withdrawing group is present, the absence of an acidic function within the electron withdrawing functionality, (3) electronegativity of the aromatic ring, (4) steric effects and extreme delocalization of lone pairs of electrons (as occurs with meta isomers and aromatic multiply substituted with electron donating groups) tend to interfere with repellency. These studies have two equally important implications. First, there are apparent taxonomic differences between birds and mammals as to what constitutes an aromatic chemoirritant. For example, birds are insensitive to a variety of mammalian irritants, e.g., mustard oil, capsaicin, ammonia, whereas mammals are insensitive to avian irritants, methyl anthranilate, o-aminoacetophenone. Systematic SAR studies lead us to believe that a simple difference in receptor mechanism is responsible for the different perception of chemoirritation between the two taxa. This may have significant consequences for the evolution of plant-animal interactions, e.g. exploiting the sensory stems of seed predators (mammals) vs. dispersers (birds). Second, we are also exploiting our more detailed understanding of avian chemoirritants to design environmentally benign, nonlethal repellents for protecting birds against accidental poisoning from waste water or granular pesticides and the protection of crops from depredation.

### **INTER AND INTRA-SPECIES CHEMICAL RECOGNITION IN SOCIAL INSECTS: EFFICIENCY AND PLASTICITY OF THE CHEMICAL SIGNATURE.**

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Social insects use non volatile pheromones to identify each other. Behavioral tests using lures and involving chemical and mathematical analysis (PCA, FCA...) have shown that these chemical signatures which are carried not only by ants but also by termites and wasps consist of a particular set of cuticular hydrocarbons. Inter species recognition is based on qualitative differences in the composition of these substances (in the case of termites belonging to the genus *Reticulitermes*<sup>1-2</sup>). When mixed colonies are set up experimentally between two species of ants or termites, the original chemical signatures show adaptative changes which serve to reduce the level of inter-species aggression<sup>3</sup>. In

this case each of the two species acquires intermediate cuticular hydrocarbon profiles except in the complex slaves/slave-making ants<sup>4</sup>. Within a single species, variations in the relative proportions of these substances serve to distinguish one colony from another<sup>5</sup>. These proportions vary synchronously with time<sup>6</sup> in all the members of the same caste of a colony. Differences in the chemical pattern characterize each caste in termites, ants and wasps<sup>7-8</sup>. Experiments in which larvae were transferred from one colony to another have shown that in *Camponotus vagus* ants, the chemical signatures characterizing a colony tend to adapt to the new social environment<sup>9</sup>.

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#### THE INFLUENCE OF PHENOLIC DEFENSES OF EUCALYPTUS ON HABITAT USE BY ARBOREAL FOLIVOROUS MARSUPIALS.

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Balance trials on several arboreal folivorous marsupials (ringtail possums *Pseudocheirus peregrinus*, greater gliders *Petauroides volans* and koalas *Phascolarctos cinereus*) have shown condensed tannins in *Eucalyptus* foliage have little demonstrable effect on digestion in these species but that absorbed phenols have severe effects on acid-base balance and excretion of nitrogen. We have used broad-scale surveys in south-eastern Australia to investigate inter-relationships between foliar phenolics and nutrients, climatic variables, structural attributes of forests and presence/absence of arboreal folivorous marsupials. In forests below an apparent threshold in the ratio of nitrogen or phosphorus to phenolics, arboreal folivorous marsupials are rare or absent. Above the nutrient/phenolic threshold, concentrations of nutrients and phenolics, especially condensed tannins, still explain a significant amount of the variation in probability of finding folivores, but structural and climatic factors are equally important. We postulate two broad components to habitat choice by arboreal folivorous marsupials: If potential toxicity of the foliage is too high (i.e., nutrient/phenolic ratios low), the forest is not used as habitat regardless of other factors; if toxicity is below a critical threshold, the use of the forest as habitat is determined by interactions between leaf quality, climate and the structure of the forest.

#### FAST GROWTH VERSUS SUPERIOR DEFENSE: EVIDENCE FOR A TRADE-OFF IN THE LEAF BEETLE *OREINA ELONGATA*.

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*Oreina elongata* is a chemically defended leaf beetle. If its food plant contains pyrrolizidine alkaloids (PAs), all life stages of the beetle recycle these toxins to use as defensive compounds. However, one of the two hostplant genera these beetles use does not contain any pyrrolizidine alkaloids. In this paper, I compare two populations, which in the field are each restricted on one host, the other host not being present. The field host of one of

the populations is *Adenostyles alliariae*, which contains PAs, the host of the other population is *Cirsium spinosissimum*, devoid of PAs. In the laboratory, larvae of both populations were raised on either host and their performance and defensive toxin content measured. Although larvae of both populations were equally able to sequester PAs from *A. alliariae*, the larval performance on this plant differed between the two populations. Larvae which do not associate with this plant in the field, had poorer growth and survival on it than larvae of the population which naturally lives on *A. alliariae*. However, larvae of both populations grew and survived best on the alkaloid free *C. spinosissimum*. The data therefore suggest a trade-off between better growth on one plant and better defense on the other.

#### DO SPECIALIST BEES ORIENT TO KEY VOLATILES IN POLLEN?

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Earlier behavioral studies with the flower-specialist solitary bee, *Chelostoma florissomne* (Megachilidae), suggest that pollen odors of its host plant, *Ranunculus acris* (Ranunculaceae), contain key chemicals used by the bee's in host-flower recognition. Chemical analyses of *R. acris* floral volatiles confirm these observations, showing the volatile profile of pollen to be distinct from that of the whole flower: it comprises fewer and different volatiles, most prominent among which is the lactone protoanemonin. This compound is characteristic of the Ranunculaceae, and is thus a likely key substance used by the bees in flower selection. To test this, bee responses to floral odors with and without added protoanemonin were investigated in 2-choice behavioral bioassays. Sample attractiveness was measured by the frequency of bee feeding-attempt responses. Although protoanemonin is not necessary to elicit feeding responses, its presence clearly increased the attractiveness of natural floral odors to *C. florissomne*. For all 4 flower and pollen species tested, the bees showed a preference for samples containing protoanemonin. Other sensory modalities, particularly gustatory perception of protoanemonin, may be necessary to elicit the strong, clear-cut preferences exhibited by foraging bees in the field.

#### COMPARATIVE ANTIFEEDANCY OF GABA/GLYCINERGIC ANTAGONISTS TO DIABROTICITE BEETLES.

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An earlier quantitative-structure-activity study of many plant derived and synthetic neurotoxicants suggested that  $\gamma$ -aminobutyric-acid (GABA) or glycine receptors mediate chemoreception in the western corn rootworm, *Diabrotica virgifera virgifera*. Only those compounds known to be GABA/glycinergic antagonists applied to a preferred food source (disks punched from squash blossoms) were rejected by this beetle species. It is unknown if these same compounds are deterrent to related beetles that have similar and different host plant affinities. I collected common Diabroticite beetles found in Pennsylvania and screened several of these GABA antagonists to determine their relative antifeedant activity against these beetles. All the compounds deterrent to the western corn rootworm were also deterrent to the oligophagous and closely related Northern corn rootworm (*D. barberi*), polyphagous Southern corn rootworm (*D. undecimpunctata howardi*) and the striped cucumber beetle, a cucurbit specialist (*Acalymma vittata*) at roughly the same range of concentrations. This suggests that taste is mediated by a similar mechanism in all these beetle species.



## MICROSCALE ACTIVITY BIOASSAYS FOR THE ISOLATION OF ARTHROPOD NEUROTOXINS.

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The renewed interest in natural products in the past decades, has mainly focused on plant and microbial compounds. However, compounds of animal origin have received less interest until the recent years, where antimicrobial and neurotoxic compounds have been successfully isolated from frogs or spiders. One of the reasons for the paucity of previous research in this field is the difficulty of bioassaying and analyzing samples of very small size, such as venom secretions. If chemical tools have drastically improved, activity bioassays still lag behind in terms of sensitivity and amount requirements, except for target-directed, molecular-level assays. In order to identify and monitor the isolation of potentially useful constituents in arthropod venoms, we have implemented several microscale bioassays. They include the brine shrimp microscale assay (Solis *et al.*, 1993, *Planta Med.* 59:250-252), and two novel methods: a toxicity microinjection assay using *Drosophila melanogaster* and a microscale smooth muscle myogenic activity bioassay using *Gryllus bimaculatus* crop. The latter is a notable improvement over existing methods, which use large-volume assay chambers (ca. 2 ml). We have successfully developed a micro-chamber of 100  $\mu$ l, and further improved the system by coupling it with a digital-based data-acquisition system. These bioassays have been successfully used in the study of neurotoxic substances from several arthropod venoms (scorpion, centipede) and these results will be presented.

## TRENDS IN THE EVOLUTION OF CONTACT OVIPOSITION STIMULANTS IN SWALLOWTAIL BUTTERFLIES.

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Like many taxa of phytophagous insects, swallowtail butterflies (family Papilionidae) feed as larvae on plants belonging to a variety of families, not all closely related. Several of these families have long been known to share various classes of secondary compounds, leading Dethier in 1941 to suggest that use of such compounds as behavioral cues by swallowtails may have acted as facilitators or constraints on host shifts during the family's history. In recent years, suites of contact oviposition stimulants have been wholly or partially characterized for seven swallowtail species. Here we examine these findings in the light of a new phylogeny of the swallowtails, based on mtDNA sequencing, to assess the degree to which oviposition stimulants have remained conservative during swallowtail evolution and hence likely to have served as long-term behavioral constraints on host shifts.

## QUANTIFICATION OF CANTHARIDIN IN CANTHARIPHILOUS CERATOPOGONIDAE (DIPTERA), ANTHOMYIIDAE (DIPTERA) AND CANTHARIDIN-PRODUCING OEDEMERIDAE (COLEOPTERA).

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Cantharidin contents were determined in several canthariphilous insects by means of quantitative gas chromatography. Usually the ceratopogonids *Atrichopogon oedemerarum* and *A. trifasciatus* caught in the field contained low concentrations of cantharidin, with concentrations in males, in most cases, being lower than in females. When fed in the

laboratory with synthetic cantharidin, these species concentrated cantharidin by as much as 100-fold (males) and 40-fold (females). Accumulation in the different body tagmata (head, thorax, abdomen) of these species is similar. Maximal concentrations of cantharidin in tissues of *Atrichopogon* are comparable to those known from oedemerid and meloid beetles. In *A. trifasciatus* about 90% of total cantharidin content is bound in tissues. Investigations using the canthariphilous anthomyiid fly *Anthomyia pluvialis* and three cantharidin-producing oedemerid species revealed the same pattern of distribution in different body tagmata as in *Atrichopogon*.

#### HOST RECOGNITION BY MONARCH BUTTERFLIES, *DANAUS PLEXIPPUS*.

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The post-alightment behavioral sequence, after natural alightment leading to oviposition by the laboratory reared Monarch butterflies, *Danaus plexippus* and the chemoreceptors involved have been studied. Fractionation and bioassays of extracts of *Asclepias curassavica* have led to the isolation of active components and have indicated the possible involvement of flavonoids. Cardenolides are not responsible for stimulating oviposition.

#### ATTRACTIVENESS OF PLANT PYRROLIZIDINE ALKALOIDS (PAs) AS DEFENSIVE COMPOUNDS FOR INSECTS: BIOCHEMICAL ANSWERS.

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A great number of insects belonging to different orders such as Lepidoptera, Orthoptera, Coleoptera and Homoptera are known to sequester PAs from plants. In respect to their attractiveness for specialized insects PAs are unique among the some 10,000 known plant alkaloids. Plants synthesize, translocate and store PAs as polar salt-like *N*-oxides. These *N*-oxides are metabolically nontoxic, but they are easily reduced in the guts of insect or mammalian herbivores giving the more lipophilic and toxic tertiary bases. Tracer experiments revealed that tertiary PAs are passively taken up into the hemolymph by non-sequestering insects, e.g., larvae of *Spodoptera littoralis*, as well as sequestering species, e.g., larvae of *Cretonotos transiens* (Arctiidae) and *Zonocerus variegatus* (Pyrgomorphidae). In *Spodoptera* the ingested PAs are rapidly and metabolically unchanged removed from the hemolymph by excretion; in the two sequestering species the ingested PAs are rapidly *N*-oxidized by a mixed functional NAD(P)H-dependent oxygenase localized in the hemolymph (*Cretonotos*) or the fat-body (*Zonocerus*). The *N*-oxides are kept in the body. Experiments using <sup>14</sup>C/<sup>18</sup>O-double-labeled PA-*N*-oxide revealed that in the sequestering species the direct uptake of PAN-ox is less than 15%. The results indicate that in the insects studied: (i) uptake of PAs occurs as free base and is an unspecific process; (ii) sequestering species transform the toxic free base into the nontoxic *N*-oxide; (iii) sequestering species "lost" the ability to excrete the PAN-oxide. Thus, *N*-oxidation and unability of PA excretion are biochemical prerequisites for the evolutionary selection of PA sequestration.

## CAN SMALL RARE PREY BE CHEMICALLY DEFENDED? THE CASE FOR MARINE LARVAE.

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Marine larvae are small, widely dispersed, and consumed by a wide variety of generalist fishes and invertebrates that would rarely consume many larvae of any particular species. Because most consumers produce enzymes that allow them to consume small quantities of noxious compounds with impunity, and because larvae would usually comprise a very small proportion of the diet of any of these consumers, it is unclear that larval secondary metabolites would be consumed in quantities sufficient to negatively affect consumers, and thus select for larval recognition and avoidance. Despite this, chemically defended larvae do occur. Using chemically defended larval mimics, we show that secondary metabolites from a Caribbean tunicate dramatically decrease the growth (by >80%) and reproduction (by about 40%) of an anemone that preys on larvae even when defended larvae constitute <2% of its daily diet and represent the equivalent of consuming no more than 15 larvae/day. Thus, consumption of even small numbers of chemically defended larvae can decrease consumer fitness and select for predators that recognize and avoid consuming these larvae.

## THE POSTPHARYNGEAL GLAND AS A "GESTALT" ORGAN FOR NESTMATE RECOGNITION IN THE ANT *CATAGLYPHIS NIGER*.

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Exudates of the postpharyngeal glands were found to modify nestmate recognition in *Cataglyphis niger*. Application of an alien glandular exudate elicited aggression towards nestmates, and vice versa, application of a nestmate's secretion reduced the aggression that is generally exhibited towards alien ants. Since the glandular secretion is composed predominately of hydrocarbons, they could be used as a model for the distribution of recognition cues within and among nestmates. The postpharyngeal gland sequester, rather than synthesize, hydrocarbons both from an internal pool and from the cuticular surface by grooming. These hydrocarbons are also exchanged between nestmates mostly by trophalaxis independently of the food flow. Thus the postpharyngeal gland can be described as a "Gestalt organ." It is responsible for pooling recognition chemicals emanating from other members of the colony, provides this mixture for reapplication onto the ants' cuticle by self and allogrooming, and by that maintain a unified colony odor which is mandatory for creating an efficient recognition system in large ant colonies.

## EGG PARASITOIDS OF GALERUCINAE (COLEOPTERA; CHRYSOMELIDAE): ROLE OF CHEMICAL CUES FOR HOST FINDING AND HOST ACCEPTANCE.

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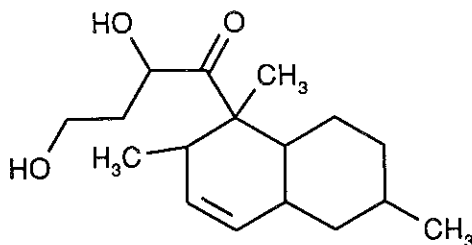
*Oomyzus galerucivorus* and *O. gallerucae* (Chalcidoidea, Eulophidae) parasitize only eggs of those species of the subfamily Galerucinae (Coleoptera, Chrysomelidae), which are known to contain anthraquinones. Hosts of *O. galerucivorus* are eggs of *Galeruca tanacetii*, *O. gallerucae* is parasitoid of eggs of *Galerucella* and *Xanthogaleruca* spp. Investigations of the host finding behavior of these parasitoids revealed that they find their hosts randomly, when in addition to the eggs no host plant or any other stimuli are offered. After host

location, the following behavioral sequences were observed both in *O. galerucivorus* and *O. gallerucae*: first, drumming with their antennae on the egg surface, then tapping with the abdominal tip, drilling with the ovipositor into the egg, complete insertion of the ovipositor, oviposition, and finally host feeding. By drumming and tapping on the egg surface, the parasitoids recognize the eggs as hosts, while drilling into the eggs represents a behavioral element just prior to host acceptance. Anthraquinones applied onto glass pearls failed to elicit drumming behavior in both parasitoid species. A detailed investigation of the exact location of anthraquinones in galerucine eggs revealed that these compounds are present only inside the eggs, but not in the (extra)chorion. Thus, the parasitoids are able to contact these compound only by drilling or inserting the ovipositor into the eggs, or by host feeding. Therefore, the anthraquinones are no key compound for host recognition. However, it remains to be examined whether they play a role for host acceptance. Behavioral bioassays with *O. galerucivorus* showed that host eggs (*G. tanacetii*) are neither recognized by the surface structure nor by the color of the extrachorion, but by chemical cues which are soluble in dichloromethane. *O. galerucivorus* shows drumming behavior on filterpaper treated with a dichloromethane extract of the extrachorion of *G. tanacetii* eggs. We are currently fractionating this extract and analyzing the active fractions by GC-MS.

#### PHOMODIOL, A NOVEL COMPOUND PRODUCED BY *PHOMOPSIS*.

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This work was done as part of an ongoing study to discover biologically active compounds from the fungus *Phomopsis*—specifically those fungal isolates found growing endophytically on willows. An isolate of *Phomopsis* was obtained from twigs of *Salix lasiolepis* by placing surface sterilized twigs on agar in petri dishes and incubating; *Phomopsis* was identified by microscopic analysis of the conidia. The isolate was then fermented on millet, the fermentation extracted, and the extract tested for antibiotic activity. A single activity, against *Candida tropicalis*, was discovered. The active compound, phomodiol, was isolated by bioassay guided preparative HPLC chromatography. The structure of phomodiol was elucidated using MS and NMR spectroscopy. Pure phomodiol was found to be active against *C. tropicalis* in a disk diffusion assay at a minimum concentration of 100 µg/ml



## ONTOGENY OF DROSOPHILA CUTICULAR SIGNATURE AND COURTSHIP BEHAVIOR.

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The ontogenies of cuticular pheromones of four strains of *Drosophila melanogaster* are described. Two of the strains belonged to the African race (Jallon and Pechine, 1989), while the other two were of the non-African type. There is always a switch between a young fly pattern common for all strains and both sexes and different patterns for mature flies. Surgical experiments the use of mutants suggest the importance of known hormones, ecdysone and juvenile hormone and a cephalic factor of an unknown nature. Parallel behavioral studies are also reported.

## SYNTHETIC ATTRACTANTS FOR WESTERN CORN ROOTWORM LARVAE IN LABORATORY BIOASSAYS.

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Dichloromethane ( $\text{MeCl}_2$ ) has been demonstrated to attract western corn rootworm larvae, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), with choice-test bioassays in our laboratory. We propose that dichloromethane is a mimic of carbon dioxide ( $\text{CO}_2$ ), which is released by corn roots and has previously been demonstrated to attract the larvae. Experiments performed with our bioassay confirm attraction of larvae to  $\text{CO}_2$  and also demonstrate the dose dependent behavioral activity of several different chloroalkane analogs of  $\text{MeCl}_2$ , including chloroform ( $\text{MeCl}_3$ ); carbon tetrachloride ( $\text{MeCl}_4$ ); 1,1-dichloropropane; 1,1-dichloroethane; 1,1-dichlorobutane; and ethyl dichloroacetate. Dramatic attraction of western corn rootworm larvae has been demonstrated with  $\mu\text{l}$  volumes of  $\text{MeCl}_3$ ;  $\text{MeCl}_4$ ; 1,1-dichloropropane; and ethyl dichloroacetate. The same amounts of 1,1-dichlorobutane and ethyl dichloroacetate have been demonstrated to repel western corn rootworm larvae. An intermittent contaminant of  $\text{MeCl}_2$ , orthodichlorobenzene, has also been demonstrated to repel larvae. The same volumes of solvents including diethyl ether, hexane, and ethyl acetate did not demonstrate any attraction or repulsion. Results of these experiments suggest that at least some of the chloroalkanes tested may mimic  $\text{CO}_2$  based upon size of the functional groups, electronegativity and bond angles.

## INFORMATION IN SCENT COUNTER-MARKS: A STRIKING NEW PHENOMENON IN HAMSTERS AND VOLES.

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Among mammals it is common for individuals to scent mark in places that other conspecifics have previously marked (counter-marking). We investigated the functions of such marking by determining what an animal remembered after it investigated a place in which the scents of two individuals had been deposited. In a large series of experiments using both golden hamsters (*Mesocricetus auratus*) and meadow voles (*Microtus pennsylvanicus*) we have discovered that both species preferentially remember the individual's scent that is on top. The same effect is obtained when the two stimulus scents are directly on top of one another, in a crossed pattern, or even when some exemplars of the "bottom scent" are not covered by the "top scent." These results suggest that both

species detect the existence of two individual scents, determine which one is on top, and selectively remember the top scent. As far as we know, this ability has never been described or even suggested before. Such an ability is likely to be highly advantageous, because it would allow an animal to focus its attention on the individual that was most recently in an area.

#### THE PARADOX OF POLYMORPHIC PLANT DEFENCE AND THE LOW COSTS OF DEFENCE.

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Whereas it has been possible to use chemical variation within plant species to prove chemical defence, we are left with the question — If chemical defence is important, why are these species not monomorphic for the defensive compound(s)? When the costs of being cyanogenic are examined in some leguminous plants, it appears that the costs are rather unimportant. Furthermore, cyanogenic individuals often outperform acyanogenic plants from the same population. Some aspects of this paradox will be explored and explained.

#### ARRESTMENT BY IXODID TICK SPECIES ON CONTACT CHEMOSTIMULI FROM STEER.

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Strong arrestment by larvae, nymphs and adult stages of ixodid ticks is obtained to a dichloromethane extract of steer hair when applied to an artificial membrane in a "host-simulating" arena. The tick's arrestment on the treated area is mediated by returns at the border of the treated area by walking along it. Detailed video analysis (video clip will be shown) and electrophysiology experiments on contact chemosensilla indicate that leg sensilla as well as the palps are actively employed to sample the substrate. Fractionation of the extract by TLC permitted the isolation of behaviorally active polar and a non-polar constituents.

#### LONG-RANGE FEMALE SEX PHEROMONE OF THE LONGHORN BEETLE *MIGDOLUS FRYANUS*.

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The first known long-range female-released sex pheromone for the family Cerambycidae is reported from *Migdolus fryanus*, a sugarcane pest in South America. Although two female-specific compounds, namely, N-2'-(S)-methylbutanoyl 2-methylbutylamine and N-formyl L-isoleucine methyl esters were identified, field tests with synthetic chemicals revealed that only the amide was active and that the amino acid derivative neither increased or decreased trap catches by the amide. This is the first identification of an amide as a sex pheromone.

## 17. RE-EVALUATION OF DIMETHYL DISULFIDE AS A SEX ATTRACTANT IN GOLDEN HAMSTERS.

Robert E. Johnston and Aras Petrusis. Cornell University, Ithaca, USA.

Vaginal secretions of female hamsters are the source of odors that are highly attractive to males, and dimethyl disulfide (DMDS) has been identified as an important, attractive component of this secretion. If DMDS is a sex attractant, it should be more attractive to males than to females and the response of males should be influenced by gonadal hormones. Using a better method for measuring attraction than was previously used, we found that males spent very little time investigating DMDS. They spent more time than females investigating vaginal secretions, but there was no sex difference in investigation of either DMDS or a mixture of mercaptans. In a second experiment males' responses were investigated after castration and replacement therapy with testosterone or empty capsules. Males implanted with testosterone investigated vaginal secretions more than males with empty capsules, but hormone treatment did not affect responses to either DMDS or the mercaptan mixture. These results suggest that DMDS by itself has no special significance as a sexual attractant in hamsters.

## 18. PHOTOAFFINITY LABELING AND CDNA CLONING OF THE PHEROMONE BINDING PROTEIN OF THE GYPSY MOTH, *LYMANTRIA DISPAR*.

Siana LaForest<sup>3</sup>, Gehua Du<sup>1</sup>, C. Rikard Unelius<sup>4</sup>, Richard G. Vogt<sup>5</sup>, Daniel Kluger<sup>6</sup>, and Glenn D. Prestwich<sup>1,2</sup>. <sup>1</sup>Department of Chemistry, <sup>2</sup>Department of Biochemistry and Cell Biology, <sup>3</sup>Department of Ecology and Evolution, <sup>4</sup>SUNY at Stony Brook, Stony Brook, NY 11794, KTH, Sweden, <sup>5</sup>Univ. of South Carolina, and <sup>6</sup>Univ. of Rochester Medical School.

In insects, empirical evidence suggests that the process of pheromone reception is mediated by solubilized pheromone binding proteins (PBPs). Antennal sensory hairs of the male gypsy moth *Lymantria dispar* (Lepidoptera, Lymantriidae) contain two homologous proteins belonging to the class of PBPs. One of these, Lyd-PBP2, has been found to have significant affinity for the principal pheromone component, (+)-disparlure. PBP2 can be specifically photoaffinity labeled with a non-pheromone compound, [3H]-9,10-epoxytetradecyl diazoacetate, in a fashion which shows selectivity for chain length and for the location and presence of the epoxide moiety. We describe the isolation, cloning, and sequencing of two cDNAs encoding Lyd-PBP2. This novel PBP shares a high biochemical homology with PBPs from *Heliothis virescens*, *Manduca sexta*, and two *Antheraea* species. It contains 6 conserved cysteines and seven conserved Arg, Lys, and His residues characterizing all PBPs and general odorant binding proteins, but is two amino acids longer than the Hev-PBP (to which it is the most similar).

## 19. THE APHID SEX PHEROMONE NEPETALACTONE: AN INTERSPECIFIC HOST-LOCATION CUE FOR *PRAON* SPP. PARASITOIDS.

Richard Lilley<sup>1</sup>, Jim Hardie<sup>1</sup> and Lester J. Wadhams<sup>2</sup>. <sup>1</sup>Imperial College at Silwood Park, Ascot, Berks SL5 7PY U.K. <sup>2</sup>Institute of Arable Crops Research, Harpenden, Herts AL5 2JQ, UK.

An aphid sex pheromone (+)-(4aS, 7S, 7aR)-nepetalactone, acts as a kairomone for female aphid parasitoids of the genus *Praon*. In olfactometer assays female *P. volucre* were attracted to nepetalactone and to sexual females of two species of cereal aphid, *Sitobion avenae* and *S. fragariae* which release this pheromone to attract males. The effectiveness of nepetalactone

fraction of the plant contains two sesquiterpenic lactones: isoalloalantolactone and elema-1,3-11-trien-8,12-olide. Isoalloalantolactone completely inhibited *A. hypochondriacus* growth at the three test concentrations, while elema-1,3-11-trien-8,12-olide caused a total inhibition of this species at 100 and 200 ppm. *E. crusgalli* was resistant to both compounds. Sesquiterpenic lactones are implicated in allelopathy and possess a wide biological activity spectra, but their effects on phytopathogenic fungi are scarcely studied. Isoalloalantolactone and elema-1,3-11-trien-8,12-olide strongly inhibited radical growth of *Helminthosporium* sp., while growth of *Pythium* sp. was marginally inhibited. *Fusarium*, the most resistant of the fungi, was inhibited 50% by Isoalloalantolactone.

#### 15. INDUCED FEEDING BY *PIERIS RAPAE* LARVAE ON NASTURTIUM (*TROPAEOLUM MAJUS*) WITH ALLELOCHEMICALS.

*Xinpei Huang* and J.A.A. Renwick. Boyce Thompson Institute, Tower Road, Ithaca, NY 14853, USA.

Neonate larvae of *Pieris rapae* fed and grew normally on nasturtium, *Tropaeolum majus*. However, cabbage-reared larvae refused to feed on this plant and starved to death. When neonate larvae were reared on cabbage leaves treated with chemical fractions from nasturtium foliage, they accepted nasturtium as a food plant. These fractions contained strong feeding deterrents to cabbage-reared larvae, and one of the deterrents has been identified as a caffeic acid derivative. Individual allelochemicals, including cardenolides, two glucosyl cucurbitacins, chlorogenic acid and related compounds also induced larvae to feed on nasturtium to some extent, and structure-activity and dose-activity relationships were demonstrated.

#### 16. INFLUENCE OF HOSTPLANT ON MIDGUT PH AND REDOX POTENTIAL IN THREE LEPIDOPTERANS.

*Kelly S. Johnson* and Gary Felton, Department of Entomology, University of Arkansas, Fayetteville, AR 72701.

The pH and redox conditions in the insect midgut are thought to influence the digestion and assimilation of nutrients as well as oxidative activation of quinones, phenolics and possibly tannins. Large interspecific differences in pH and redox potential have been measured in herbivorous insects, but the extent to which gut conditions are determined by dietary pro- or anti-oxidants or active regulation by the insect is not known. To assess the importance of hostplant on midgut conditions, we measured the pH and redox (Eh) of three Lepidoptera (*Helicoverpa zea*, *Heliothis zea*, and *Hyphantria cunea*) reared on four hostplants (cotton, soybean, clover and wild geranium). All three species had alkaline pH's (9.3 to 10.6) and reducing midguts (-100 to -240 mV). ANOVA of host and insect species effects indicated that midgut pH was not affected by hostplant, but redox values were influenced by both insect species and hostplant. Larvae fed wild geranium had significantly lower redox potentials (-200 to -240 mV), followed by those fed cotton, than larvae fed soybean and clover (-100 to -160 mV). Although larvae fed the four hosts had similar rankings of midgut redox values, these values did not correspond to redox values of foliar juices (soybean>wild geranium>cotton>clover).



## 12. MODE OF ACTION OF *SENECIO PALMENSIS* BIOACTIVE COMPONENTS ON *LEPTINOTARSA DECEMLINEATA*.

*Carmen Gutierrez*<sup>1</sup>, *Matias Reina*<sup>2</sup> and *Azucena Gonzalez-Coloma*<sup>2</sup>. <sup>1</sup>Centro de Investigaciones Biologicas, CSIC, Velazquez 144, 28006 Madrid, SPAIN. <sup>2</sup>Instituto de Productos Naturales y Agrobiologia, CSIC, Ave. Astrofisico F. Sanchez 2, 38206 La Laguna Tenerife, SPAIN.

The sesquiterpenes 2,10-bisaboladien-1-one and 11-*acetoxy-5-angeloyloxy-silphinen-3-one*, isolated from *Senecio palmensis* as antifeedants against *Leptinotarsa decemlineata*, were evaluated for food consumption and growth depression against this insect. Exposure of fourth-instar larvae to these compounds over a 24-h period resulted in reduced feeding and growth rates. To distinguish between antifeedant and toxic effects, growth efficiencies were calculated as the slope of the regression of relative growth rate on relative consumption rate. The comparison of these results with those of antifeedant simulation bioassays indicates that feeding inhibition is the primary mode of action of the bisabolene, while the silphinene shows both antifeedant and toxic effects.

## 13. PROSPECTING OF ECDYSTEROIDS IN MYCOPHYTA (FUNGI)

*Juraj Harmatha*, *Karel Vokac* and *Milos Budesinsky*. Institute of Organic Chemistry and Biochemistry, Academy of Sciences, 16610 - Prague, Czech Republic.

Ecdysteroids are widely distributed in plant species belonging to a large set of families throughout the majority of classes from the subkingdom Embryophyta, but they have not been found in Thallophyta. However, occurrence of ecdysteroid-related compounds have been reported in Rhodophyta and Mycophyta (fungi). Insect moulting bioassays, which did not indicate evidence ecdysone activity in fungi, retarded probably their discovery in fungi up to the present time. A chemical screening of 78 species of mushrooms indicated the presence of ecdysteroids in two species. From *Paxillus atrotomentosus* (Batsch) Fr. there was isolated and identified a new ecdysteroid, paxillosterone, a 24-methyl homolog of a 20-hydroxyecdysone derivative. From *Tapinella panuoides* (Fr. ex Fr.) Gilb., syn. *Paxillus panuoides* six ecdysteroids were isolated: paxillosterone, panuosterone and 25-hydroxypanuosterone, together with two previously reported higher plant constituents, 20-hydroxyecdysone (20E) and turkesterone, and one 16-hydroxy derivative of 20E, malacosterone, known as an animal (Mollusca, Gastropoda) constituent.

## 14. PHYTOTOXIC COMPOUNDS OF *RATIBIDA MEXICANA*.

*B. E. Hernandez-Bautista*<sup>1</sup>, *Anaya, A.L.*<sup>1</sup>, *Soto, D.F.*<sup>2</sup>, *Sanchez, P.*<sup>2</sup>, *Mata, R.*<sup>2</sup>, and *Cocoletzi, A.*<sup>2</sup>. <sup>1</sup>Instituto de Fisiologia Celular, UNAM, Apdo. Postal 70-243, Mexico 04510, D.F. <sup>2</sup>Facultad de quimica, UNAM. Edificio B. Ciudad Universitaria, Mexico 04510, D.F.

*Ratibida mexicana* (Wat.s) Sharp (Asteraceae) is an endemic Mexican plant that has a dispersion growth in inaccessible area along the Sierra Madre Occidental mountains in the State of Chihuahua. Tarahumara Indians named the plant "Howinowa" and use its roots for the treatment of rheumatism and as an antiseptic agent. Considering that many species of the Asteraceae family contain secondary metabolites that play an important role in biochemical interactions, a bioactive directed study of organic extracts of *R. mexicana* was performed. Bioassays were made to evaluate the effects of organic extracts and isolated compounds from the root of *R. mexicana* on the radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli*, and on the growth of phytopathogenic fungi. The active hexane

putative pollinator attractant in response to pollination and the interplay between the attractant and a floral defense compound, nicotine, in response to simulated herbivory to examine the coordination between floral "advertisement" and defense. The emission of a single compound, benzyl acetone (4-phenyl-2-butanone), from flowers of *Nicotiana attenuata* increased dramatically at night. Extractable pools of benzyl acetone were found only in the lip of the corolla and diurnal changes in the size of the pool closely tracked those seen in headspace sampling. Extractable pools of nicotine in the corolla did not vary statistically throughout the day except during the period of peak benzyl acetone emission when the nicotine pool decreased significantly. Hand pollinated flowers emitted less benzyl acetone than unmanipulated flowers. Flowers that received self or out-cross pollen did not differ in their emissions. Plants that received extensive leaf damage had significantly greater nicotine pools in the corolla but benzyl acetone pools were not changed. These results suggest that regardless of the potential increased "apparency" to herbivores this plant does not alter its floral "advertisement" in response to herbivory.

#### 10. BIOACTIVE COMPONENTS FROM *SENECIO PALMENSIS*.

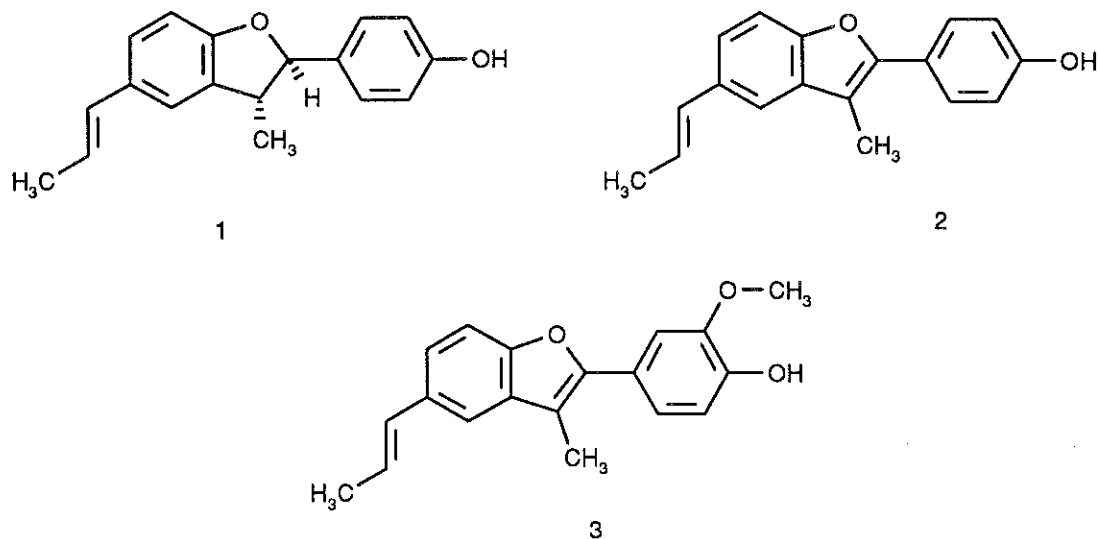
*Azucena Gonzalez-Coloma*<sup>1</sup>, Carmen Gutierrez<sup>2</sup>, Raimundo Cabrera<sup>3</sup> and Matias Reina<sup>1</sup>.  
<sup>1</sup>Instituto de Productos Naturales y Agrobiologia, CSIC, Ave. Astrofisico F. Sanchez 2, 38206 La Laguna Tenerife, SPAIN. <sup>2</sup>Centro de Investigaciones Biologicas, CSIC, Velazquez 144, 28006 Madrid, SPAIN. <sup>3</sup>UDI Fitopatologia, Universidad de La Laguna, Tenerife, SPAIN.

The sesquiterpenes 2,10-bisaboladien-1-one and 11- $\beta$ -acetoxy-5- $\alpha$ -angeloyloxy-silphinen-3-one were isolated from *Senecio palmensis*. As antifeedants against *Leptinotarsa decemlineata* larvae and adults in choice and no-choice assays. Juglone was included in the experiment as a reference antifeedant. The bisabolene and the silphinene represented  $1.2 \times 10^{-2}$  and  $2.4 \times 10^{-2}$ % of the plant dry weight respectively. The beetles were more sensitive to these compounds in choice than in no-choice assays with a gradient of increasing sensitivity from second-instar larvae to adults. The bisabolene antifeedant activity was up to 45 times lower than juglone. The silphinene was more active than the bisabolene with a range of activity similar to juglone. Both compounds also had mild antifungal activity against six species of phytopathogenic fungi.

#### 11. DIVERSITY OF PHOTOTOXINS AND THEIR INSECTICIDAL MODES OF ACTION IN ASTERACEAE.

*Gabriel Guillet*, J. Thor Arnason, Bernard J.R. Philogene. University of Ottawa, Ottawa, Ontario K1N 6N5 Canada.

The effect of phototoxins (light-activated toxins) of the Asteraceae on insects was studied. Insecticidal studies were conducted on three related polyacetylenes, tridecapentayne and two of its thiophene and thiarubrine derivatives, extracted from *Rudbeckia hirta* (Black-eyed Susan). Although each compound was toxic in presence of near-UV light against larvae of *Manduca sexta* (Lepidoptera: Sphingidae), only the first and the third allelochemicals were also active without near-UV light or in the dark. The dark toxicity of tridecapentayne could be related to the very high instability and reactivity of its 5 conjugated acetylene bonds. However, thiarubrine seems to act via a non degradative process independent of light stimulation. As these compounds are representative of the diversity of phototoxins found in Asteraceae, such results suggest that diversification of the mode of action could constitute an important evolutionary trend of phototoxins in this plant family.



These lignans, along with the original crude ethanol extract, were bioassayed for insecticidal activity on the European corn borer, *Ostrinia nubilalis*, and the rock hole breeding mosquito, *Aedes astropalpus*. As well, bioassays were performed on several cancer cell lines.

#### 8. RESPONSES OF FREE-RANGING BEAVER TO PREDATOR ODORS.

Axel Engelhart<sup>1</sup> and Dietland Müller-Schwarze. College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210 USA. <sup>1</sup>Tierphysiologie, Tübingen University, D-72076 Tübingen, Germany.

Extracts from excrements of otter (*Lutra canadensis*), wolf (*Canis lupus*), coyote (*C. latrans*), lynx (*Lynx canadensis*), black bear (*Ursus americanus*), dog (*C. familiaris*), and lion (*Felis leo*) were applied to sticks of aspen, the beaver's preferred diet, and presented at beaver colonies in two New York State populations. Predator odors significantly reduced feeding. Of the differences between the predator odors, only otter odor was significantly more active than the others. Odors of allo- and sympatric predators did not differ in their effects. The two populations differed significantly in their responses: the one with a richer predator fauna avoided the predator odors more. Predator odors appear to be a viable choice for beaver repellents.

#### 9. THE INFLUENCE OF POLLINATION AND HERBIVORY ON FLORAL VOLATILES IN *NICOTIANA ATTENUATA*.

Michael Euler and Ian Baldwin. State University of New York, University at Buffalo, Buffalo, NY, USA.

Floral volatiles attract pollinators and diurnal patterns of volatile emission are frequently correlated with periods of pollinator activity. The emission of floral volatiles may also increase the "apparency" of plants to plant predators. We examined the plasticity of a

## 5. CHEMICAL ATTRACTION OF THE PINEAPPLE BEETLE, *CARPOPHILUS HUMERALIS* (F) (COLEOPTERA: NITIDULIDAE).

*Robert J. Bartelt* and Bruce W. Zilkowski. USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, Illinois 61604, USA.

The pineapple beetle is a member of the nitidulid species complex that infests date gardens in southern California and in other parts of the world. The species also attacks crops such as pineapples and corn; ripe or decomposing fruits are preferred. *C. humeralis* is cross attracted to the male-produced aggregation pheromones of related species such as *C. hemipterus* and *C. obsoletus*, especially when these pheromones are synergized by fermenting bread dough, but production of an analogous pheromone in *C. humeralis* has not been demonstrated. However, in the laboratory, volatiles collected from male and female *C. humeralis* feeding on pineapples or oranges were very attractive in wind-tunnel bioassays. The active compounds included a substituted phenol and a pyrazine, which are apparently produced by microorganisms. Slight modifications of the chemical structures rendered them inactive. The compounds are active in the wind tunnel at pheromone-like levels (sub-nanogram to low nanogram amounts) and are synergized by more abundant fermentation volatiles such as small esters.

## 6. TYRAMINE, AN OVIPOSITION STIMULANT FOR THE BLACK SWALLOWTAIL BUTTERFLY FROM THE LEAVES OF WILD PARSNIP.

*Maureen Carter*, Kusum Sachdev-Gupta and Paul Feeney. Cornell University, Ithaca, NY 14853 USA.

Earlier work isolated two oviposition stimulants for the black swallowtail butterfly, *Papilio polyxenes*, from the leaves of one of its host plants, wild carrot. The stimulants were identified as trans-chlorogenic acid and 7-O-(6"-O-malonyl)- $\beta$ -D-glucopyranoside. Here we report an additional stimulant from the leaves of a second host species, wild parsnip, *Pastinaca sativa*. Column chromatography of an extract of the leaves of this host plant yielded three fractions that, when combined, were stimulatory to female butterflies. Activity of the first fraction was due to trans-chlorogenic acid, that of the second to tyramine, while the third fraction contained yet undetermined neutral compounds. Our results are discussed in the light of other work on the oviposition chemistry of swallowtail butterflies.

## 7. ISOLATION AND BIOLOGICAL ACTIVITY OF THREE NEW LIGNANS FROM *PIPER DECURRENS*.

*D. C. Chauret*, B.C. Bernard, J.T. Arnason, T. Durst, H.G. Krisnamurty, C. Hasbun, P. Sanchez, J. Poveda. Department of Biology, University of Ottawa, Ottawa, Ontario K1N 6N5 Canada.

The crude ethanol extract of the leaves of *Piper decurrens*, a member of the Piperaceae (pepper) family, was subjected to column chromatography, yielding three new lignans (1,2, and 3).

were measured in the bioassays. Greenhouse experiments with dry *I. tricolor* mixed with soil in pots were performed in sterile and non-steril soil. Seeds of *A. hypochondriacus* were planted in pots containing *I. tricolor*, 2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine (Gesaprim, Ciba-Geigy) and 1-glyphosphate and the glyphosphate salt of isopropylamine (Faena, Monsanto), two different commercial herbicides used as a comparison to *I. tricolor*. Number and dry weights of monocots and dicots weeds and *A. hypochondriacus* growing in different treatments were measured. Monocots were inhibited by *Ipomoea* in steril soil. *Ipomoea* had a similar inhibitory effect on monocots in comparison to Faena.

### 3. CHEMICAL DEFENSES OF THE TROPICAL BUTTERFLY, *HELICONIUS* SPP.

*Manuel Aregullin* and Eloy Rodriguez. Phytochemistry and Toxicology Laboratory. Developmental and Cell Biology. School of Biological Sciences. University of California, Irvine, CA 92717, USA.

Some moths and butterflies (Lepidoptera) contain cuticular venoms either in the larval, pupal, and/or adult stages stored in hairs or spines used in their defense against potential predators, but can cause epidemics of contact dermatitis in various tropical parts of the world. The symptomatology of human exposure to these Lepidoptera range from skin eruptions to severe pain, hemorrhage, and in some cases, death. The chemistry of the venoms found in toxic butterflies and moths appears to be varied. Thus, little is known about the chemistry and mode of action of many of the venoms. We examined the chemistry and toxicology of the venoms from the larval stages of the Costa Rican butterflies, *Heliconius ismenius clarescens*, *H. melpomene rosina* and *Agraulis vanillae* (Heliconiinae) whose larvae are covered with irritating spines. Chemical analysis of these toxins indicate that the major toxic component is histamine. To establish the potency of this substance, spin extracts were prepared and tested using the Guinea Pig Irritancy Test for contact venoms. The venoms isolated produced an analogous reaction to histamine, consisting of localized redness, flare, and edema ("triple response") when applied topically.

### 4. CHEMICAL BIOLOGY OF A RESIN USED IN GROOMING BY COATIS.

*Manuel Aregullin* and Eloy Rodriguez. Phytochemistry and Toxicology Laboratory. Developmental and Cell Biology. School of Biological Sciences. University of California, Irvine, CA 92717. USA.

A major research focus of our laboratory is the study of zoopharmacognosy, i.e., the use of plant products by animals for possible therapeutic purposes. The white-nosed coatis (*Nasua narica*, Procyonidae) of Barro Colorado Island in Panama have been observed by Gompper to exhibit a peculiar group behavior where resin exuded from scratches and cuts made by the coatis on the trunks of *Trattinnickia aspera* (Burseraceae), are used in fur rubbing. This behavior has suggested to us the possible use of the resin as an ectoparasite repellent. Phytochemical studies on the resin exudates of *T. aspera* showed the major constituents of the resin to be  $\alpha$ -amyrin,  $\beta$ -amyrin, the sesquiterpene  $\beta$ -selinene, and the unexpected sesquiterpene lactone  $\delta$ -hydroxyasterolide. Details on the isolation, structure elucidation, and preliminary insect repellency evaluation of the resin will be presented.

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# ABSTRACTS —

## POSTER PRESENTATIONS

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### 1. PHYTOTOXIC ACTIVITY OF COMPOUNDS ISOLATED FROM *METOPIMUM BROWNEI* (JACQ.) URBAN (ANACARDIACEAE).

Ana Luisa Anaya<sup>1</sup>, Blanca Estela Hernandez-Bantista<sup>1</sup>, J. Fausto Rivero-Cruz<sup>2</sup>, Daniel Chavez-Velasco<sup>2</sup>, and Rachel Mata<sup>2</sup>. <sup>1</sup>Instituto de Fisiología Celular. UNAM. Ciudad Universitaria. Apdo. Postal 70-243. Mexico 04510, D.F. <sup>2</sup>Facultad de Química. Departamento de Farmacia. UNAM. Ciudad Universitaria, Mexico 04510, D.F.

*Metopium brownei* (Jacq.) Urban (Anacardiaceae) is a very abundant tree that grows in tropical forests of the coast of the Gulf of Mexico. This species forms pure populations favored by fire and produced a very irritant exudate, it has also some medicinal use. The effect of the MeOH extracts (wood, stem, bark, and leaves) of *M. brownei* on the radial growth of *Fusarium oxysporum*, *Helminthosporium* sp. and *Phytium* sp. was evaluated. The stem bark and wood extracts cause a significant inhibition of the radial growth of these phytopathogenic fungi. The antifungal principle from the stem bark turn out to be a mixture of 3 substituted alkylcatechols with zero, one and two double bonds. GCMS analysis of the bistrimethylsilyl derivatives indicated that the mixture contains mainly n-C15 substituted catechols. Furthermore, small quantities of n-C17 homologs were also detected. On the other hand the major antifungic agents from the wood were characterized as dihydroquercetin and eriodictyol. The phytogrowth activity of the organic extracts and isolated compounds were also evaluated by measuring their effect on germination and radical growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli*.

### 2. EFFECT OF *IPOMOEA TRICOLOR* ADDED TO STERIL AND NON STERIL SOIL IN POTS ON THE GROWTH OF WEEDS AND *AMARANTHUS HYPOCHONDRIACUS*.

A.L. Anaya<sup>1</sup>, D.J. Sabourin<sup>2</sup>, B.E. Hernandez-Bautista<sup>1</sup>, C. Flores<sup>1</sup>, R. Pereda-Miranda<sup>3</sup> and I. Mendez<sup>4</sup>. <sup>1</sup>Instituto de Fisiología Celular, UNAM., Apdo. Postal 70-243, Mexico 04510, D.F. <sup>2</sup>The University of Michigan-Flint, Biology Dept., 303 East Kearsley Street, Flint, Michigan 48502-2186. <sup>3</sup>Facultad de Química, UNAM. Edif: E. Cd. Univ. Mexico 04510, D.F. <sup>4</sup>Instituto Investigaciones Matemáticas Aplicadas, UNAM. Cd. Univ., Mexico 04510, D.F.

The allelopathic potential of *Ipomoea tricolor*, a ruderal plant used in Mexican Agriculture to control weeds and Tricolorin A, the major phytogrowth inhibitor present in the resin glycoside of this plant has been demonstrated by bioassays of aqueous leachate composed of plant material and bioassays made from the compound. Germination and radical growth of *Amaranthus hypochondriacus*, *Echinochloa crusgalli*, *Senna uniflora*, *I. tricolor*, and *I. purpurea*

## ULTRAVIOLET RADIATION INFLUENCES PROTECTIVE SHIELD ON THE PLANT SURFACE.

Alicja M. Zobel, C. Silva, J. Chen and J. Snelgrove, Chemistry Department, Trent University, Peterborough, ON, Canada K9J 7B8.

In leaves of *Ruta graveolens* irradiated with 366 nm ultraviolet, different peaks were visible on HPLC chromatograms compared to those from control leaves. Quantitative and qualitative differences were visible both in the fraction removed from the surface, thus directly influenced by UV, and from the interior of the leaf. Gas chromatography-mass spectrometry enabled some of the peaks to be identified as pyranocoumarins and furanocoumarins. UV may change the physiology of the cells, resulting in the production of new compounds simultaneously with quantitative changes in the concentrations of furanocoumarins in the control plants. Enhanced activity of 5-O-methoxytransferase may be responsible for the increased ratio of bergapten (5-MOP) to xanthotoxin (8-MOP), and it can explain the appearance of isopimpinellin, which contains a 5,8-disubstituted nucleus, in much higher concentrations than any of the others.

males and females. Both in field cage and greenhouse experiments, we could capture with tansy essential oil about 30% of the released females. Different types of synthetic reduced mixtures (always with thujone  $\alpha$  and  $\beta$  as major constituents, concentration ratios corresponding to the essential oil) also allowed for interesting rates of trapping (between 28% and 15% of females). No males could be found in traps and most of the trapped females were mated. The characteristics of this relationships raise exciting ecological questions. Our recent data indicate the possibility of pharmacophagy (females visiting non-host plant to acquire chemical defense for their progeny)<sup>4</sup>. We also try to develop EGVM female attractants that could be used in vineyards.

<sup>1</sup>Gabel B. (1992) - *J. appl. Entomol.*, 113, 153. <sup>2</sup>Gabel B. & Thiery D. (1994) *J. insect Behav.*, 7 (in press). <sup>3</sup>Gabel B., Thiery D., Suchy V., Marion-Poll F., Hradsky P., Farkas P. (1992) -*J. Chem. Ecol.*, 18, 693. <sup>4</sup>Thiery D., Gabel B., Benedet F. (1994) - *24<sup>e</sup> Journees Entomophages*, Aussois, France.

### OVIPOSITIONAL RESPONSES OF THE POLYPHAGOUS HERBIVORE, EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* (HÜBNER)), TO CHEMICAL CONSTITUTENTS OF HOST PLANTS.

*Sujaya Udayagiri* and Charles E. Mason, University of Delaware, Newark, USA.

Chemically mediated ovipositional responses of *O. nubilalis* females to host plants were investigated in the laboratory using two-choice bioassays. Foliar extracts of corn, pepper and potato were prepared using pentane, acetone and methanol. Results indicate that non polar chemicals in these host plants, that are soluble in pentane, and slightly polar chemicals in potato, that are extractable in acetone, stimulate oviposition. Females laid greater numbers of eggs on substrates containing extracts of corn leaves from plants at early whorl and tassel (pre-pollen shedding) stages of development but not to those containing extracts from plants at 2-leaf or blister stages indicating that plant phenology affects chemically-mediated ovipositional responses in European corn borer. Besides leaf extracts, pentane extracts of husks, tassels and silk were also attractive. When presented a choice between pentane extracts of corn and pepper, females exhibited a preference for corn. No preferences were exhibited between pentane extracts of corn and potato or between similar extracts of pepper and potato. These results suggest tactics by which plant chemicals that function as kairomones can be used for disrupting oviposition behavior of *O. nubilalis* in the field.

### EXTREME DIFFERENCES IN ALKALOID CONCENTRATIONS WITHIN ROSETTE PLANTS OF *CYNOGLOSSUM OFFICINALE*: ARE PLANTS SMART INVESTORS?

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The youngest leaves of rosette plants of *Cynoglossum officinale* L. (Boraginaceae) contain up to 100 times higher levels of pyrrolizidine alkaloids (PAs) than the oldest leaves. Generalist herbivores avoid the youngest leaves and diets with high PA levels, which shows that PAs can act as chemical defences against herbivores. Specialist herbivores were not deterred by high PA levels in their food. PAs are not broken down but can, and are, reallocated from the ageing leaves to the youngest leaves. We hypothesize that the plant distributes its PAs matching the expected photosynthesis in future, in order to maximize total growth. This hypothesis was tested in a model, which predicts the optimal distribution of PAs in rosette plants.



## THE ADAPTATION OF THE DEFENSIVE SECRETION OF *BLEDIUS* (COL., STAPH., OXY.) TO NATURAL PREDATORS.

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Rove beetles of the genus *Bledius* possess an abdominal defensive gland. The adaptation of the defensive gland secretion to the natural target organism (i.e., the natural predators of the *Bledius* species) was examined with the burrowing, shore inhabiting species *Bledius arenarius*, *B. furcatus* and *B. spectabilis*. The main predators are different insect species in *B. arenarius* and *B. furcatus* and wading birds in *B. spectabilis*. The defensive gland secretion of the *Bledius* species contains the toxin p-toluquinone. In *B. arenarius* this toxin is dissolved in octanoic acid and octyloctanoate. *B. furcatus* and *B. spectabilis* use  $\gamma$ -dodecalactone and 1-undecene as solvents. The ratio of these solvents is species-specific. The secretion of *B. arenarius* and *B. furcatus* contains a remarkable surplus of octanoic acid and 1-undecene as compared to octyloctanoate and respectively. Application experiments revealed that these specific solvent ratios provided a more effective deterrent to the natural insect predators than other solvent ratios. On the other hand the secretion of *B. spectabilis* contains more  $\gamma$ -dodecalactone than the secretion of *B. furcatus*. Feeding experiments using solution coated mealworms showed that mixtures with a high content of  $\gamma$ -dodecalactone are particularly distasteful to wading birds. Thus by combining the solvents in certain ratios, the defensive secretions of the three *Bledius* species are adapted to their specific target organisms.

## EFFECT OF *APHANAMIXIS POLYSTACHYA* ON INSECTS AND FUNGAL PESTS OF STORED-PRODUCTS.

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Ground leaves, bark and seeds of *Aphanamixis polystachya* (Meliaceae) were tested for lethal and sub-lethal effects on three species of stored-product pests (*Tribolium castaneum*, *Callosobruchus chinensis* and *Sitophilus oryzae*). Crude seeds extracts were also tested on insects and micro-organisms for their infestation and progeny/growth inhibition efficacy. The ground plant materials provided good protection for wheat flour, pulses and rice grains by reducing insect oviposition and seed infestation rates. The crude seed extracts showed strong repellent, feeding deterrent and toxic effects on insects and strong growth inhibition effects on various fungal species in laboratory cultures.

## THE INFLUENCE OF TANSY ODOUR ON EUROPEAN GRAPEVINE MOTH BEHAVIOR.

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European grapevine moth (EGVM) females (*Lobesia botrana*) are strongly attracted by flowers of a non host-plant: the wild chrysanthemum *Tanacetum vulgare*<sup>1</sup> which is a toxic plant for EGVM first larval instars. There seems to exist mechanisms to reject this unsuitable plant: tansy odor severely reduces egg-laying, mating and also male lifetime<sup>2</sup>. We first intended to define which molecules from the floral aroma could mimic the attractiveness. EAG recording directly coupled to GC allowed the screening of less than 13 monoterpenes well detected by females<sup>3</sup>. The attractiveness of different mixtures of these monoterpenes has been assayed by releasing hundreds of males and females in field cages or in green houses equipped with yellow odorized sticky traps. Non odorized traps did not attract

## PHEROMONAL MEDIATION OF NEST LOCATION IN HONEY BEES.

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For long-term survival and reproduction, honey bees must locate a safe and reliable nest cavity. Microenvironment, size, and location are important physical factors affecting suitability of cavities, and pheromones coordinate the processes of selection, communication, and movement of swarms to cavities. Honey bees operate according to two hierarchies during nest seeking activities: one relates to physical condition and properties of the potential nest cavity; the other relates to pheromonal and olfactory cues that mediate the behavioral processes. Experimental designs were devised to determine which physical and pheromonal factors were governing honey bee behavior. Cavity size was shown to be important in temperate honey bees, but not in tropical honey bees. Nasonov pheromone plays the dominant role in communication and is the single long-range attractant pheromone of honey bees. Queen pheromone acts only as a short-range pheromone indicating the location of the queen. Synthetic Nasonov pheromone is the heart of all major swarm trap survey and detection systems used throughout the U.S. by public research and regulatory agencies. The pheromonal system also has proven useful for controlling unwanted [=potentially Africanized] bees via elimination trapping and currently is being used by private companies for protection of country clubs and recreational areas in Tucson, Arizona.

## BIOACTIVITY OF MAJOR PHENOL AND NEUTRAL COMPOUNDS FROM BEAVER (*CASTER CANADENSIS*) CASTOREUM.

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North American beaver (*Castor canadensis*) mark their territories with castoreum, a chemically complex secretion from their castor sacs. When placed on human-made mud mounds, castoreum elicits sniffing and land visitation from beaver, sometimes resulting in destroying and/or overmarking the odor source. Our objective was to identify specific compounds/mixtures from the biologically active phenolic and neutral fractions that evoked similar responses. Biological activity was measured by the elicitation of and extent of specific responses and their strength (duration, frequency, and proportion of beavers responding). Principal components analysis (PCA) was used to linearly combine six related measures of observed responses and one index of overnight visitation. The first principal component was positively correlated with all the response parameters, permitting the samples to be ranked based on their composite bioactivity. Generally, single compounds stimulated fewer responses than mixtures. A 26-compound mixture of phenolic and neutral compounds elicited responses in a similar proportion of trials as castoreum. However, responses to castoreum were stronger than to any synthetic sample. Beavers showed more variation in their likelihood to respond than in response completeness or strength to the synthetic samples. A measure of multiple visits by beavers to the experimental scent mound revealed that response completeness should be measured at the family and not just the individual level. These findings may be relevant to studies of communication in other social mammals.

avoided walking onto the polygodial-treated half. Close-up video recording of behavior revealed that the aphids turned away from polygodial-treated areas following antennal tip contact. Aphids with both antennal tips removed showed no response to the antifeedant in the choice test, suggesting that polygodial is detected by contact chemoreceptors located on the antennal tips.

#### MUSTH CHEMOSIGNALS AMONG ASIAN ELEPHANTS.

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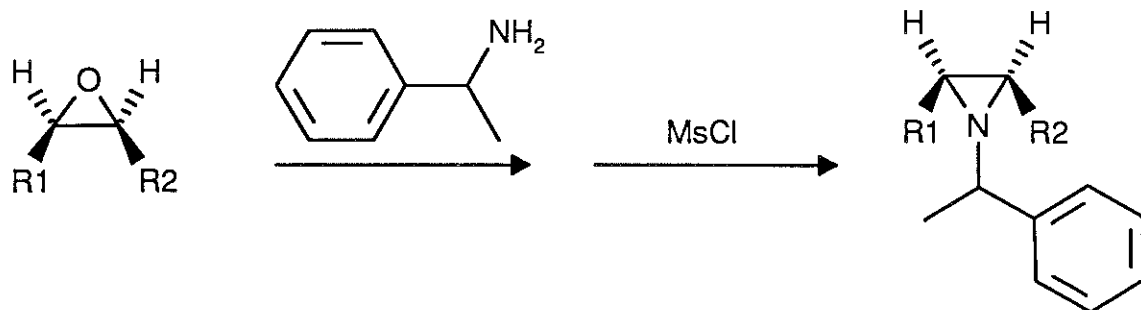
Temporal gland secretions (TGS) are exuded by male Asian elephants (*Elephas maximus*) during their musth cycles, periods of heightened aggression. Conspecifics, especially females, detect natural components of these temporal gland secretions. Two discrete chemical types that elicit different behavioral responses are among the chemical signals released from TGS. First, among the natural, dichloromethane-soluble volatiles of TGS is a ketone, cyclohexanone. Presented as a single synthetic compound, cyclohexanone evoked four types of bioresponses, including flehmen, that were individually variable in occurrence, type and intensity among 23 female elephants. There was an apparent relationship between degree of responsiveness to cyclohexanone and social hierarchal position. Cyclohexanone may be a musth alerting chemical signal. A second set of conspecific chemical signals contained in the light volatile headspace fraction from TGS elicited avoidance responses by female elephants. We modified methodology used for measuring atmospheric trace gases to sample, store and analyze the headspace volatiles of low (~1 ppm) concentrations. 850 ml internally electropolished stainless steel canisters were used to store certain classes of volatile compounds, in stable form, for long periods of time prior to analysis or use in multiple sequential bioassays. The efficacy of this method was demonstrated by 1) the identical gas chromatographic/mass spectrometric identification of thirty compounds from TGS headspace samples at the time of collection and after storage for one year, and 2) the retainment of the biological activity over several years. Bioassays of identical groups of female elephants over time suggested that some of the light volatile uniquely present in the active samples may be musth-avoidance chemical signals.

#### DIET-DEPENDENT SENSITIVITY OF LARVAE TO A HOST PLANT FEEDING DETERRENT.

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*Nasturtium*, *Tropaeolum majus*, is normally an acceptable host plant for the cabbage butterfly, *Pieris rapae*. However, larvae transferred from cabbage or other host plants to nasturtium refused to feed, and starved to death. This extreme rejection behavior is explained by the presence of strong antifeedants in nasturtium foliage. Larvae reared on nasturtium develop only limited sensitivity, and larvae reared on artificial diet remain completely insensitive to these antifeedants. Sensitivity to the deterrents is gradually acquired by first instars as they feed on other plants. Thus sensitization or habituation resulting from dietary experience can determine the insect's response to plant semiochemicals.

making quality control impossible. Conversion of disparlure enantiomers to diastereomeric aziridines has made it possible to detect as little as 0.1% of the (-)-enantiomer and to correlate enantiomeric purity with trapping efficiency.



#### FACTORS AFFECTING PRODUCTION OF PHEROMONE AND DEFENSIVE SECRETIONS IN THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*, (COLEOPTERA: TENEBRIONIDAE).

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The red flour beetle, *Tribolium castaneum*, is a cosmopolitan pest of cereal grains and milled products. Male *T. castaneum* produce the aggregation pheromone 4,8-dimethyldecanal and both sexes secrete benzoquinones and hydrocarbons as defensive semiochemicals. The effects of age, mating, feeding, and beetle density on pheromone production were assessed in a series of studies in which volatiles were analyzed from single or multiple beetles. Individual virgin male *T. castaneum* produced about 600 ng/day at two days after emergence and maintained this level of production over the course of a 30-day experiment. Mating or the presence of a female had no effect on pheromone production. Feeding and direct contact with food was required for pheromone production, and no pheromone was produced when males were exposed to food volatiles only. Males held in groups of five or more produced no pheromone, but secreted various quantities of methyl benzoquinone, ethyl benzoquinone, and 1-pentadecene, presumed defensive chemicals. Males produced significantly more of these defensive compounds than females. Single males exposed to the volatiles from 50 males produced pheromone at the same level as a single male exposed to clean air. Thus reduction of pheromone in crowded males is not due to volatiles from other males, but is likely caused by some effect from direct physical contact with other beetles.

#### APHID-REPELLENT EFFECTS OF POLYGODIAL, A PLANT SESQUITERPENOID.

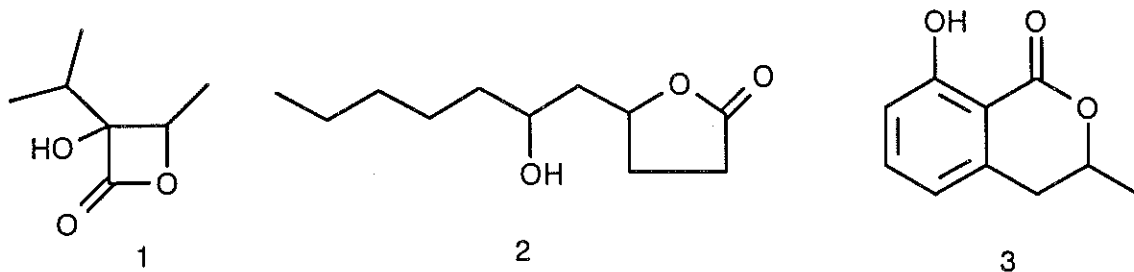
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Polygodial is a plant-derived sesquiterpenoid with particularly high aphid antifeedant activity. Aphids (apterous *Myzus persicae*) were presented with areas of floating leaf discs treated with 0.1% (+)-polygodial in choice and no-choice tests. In no-choice conditions, aphids on discs treated entirely with polygodial showed no apparent differences in behavior compared with insects on solvent-treated control discs. However, when leaf discs were treated with polygodial on one half and solvent on the other, i.e. a choice situation, aphids

## HAIRPENCIL VOLATILES OF A GIANT DANAINE BUTTERFLY, *IDEA LEUCONOE*.

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Unlike most other danaines, *Idea leuconoe* acquires pyrrolizidine alkaloids (PAs) from its host, *Parsonsia laevigata* (Apocynaceae), during the larval stage. The hairpencils were found to contain a complex mixture of volatiles, including PA fragments (danaidone,  $\delta$ -lactone 1), aromatics (phenol, p-cresol, benzoic acid), *E,E*-farnesol,  $\gamma$ -lactones (2 etc.), hydrocarbons (Z9-tricosene etc.). In addition, wild males contained mellein (3) in varying quantities. The "pharmacophagous" affinity of *I. leuconoe* males to mellein suggested a biological significance similar to that found in most other danaine males to PAs.



## TOBACCO IS UNABLE TO RECOVER NITROGEN INVESTMENT IN DEFENSE.

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*Nicotiana sylvestris* is a tobacco species that utilizes an inducible nicotine defense against herbivores. Given that they are capable of taking up large quantities of nicotine hydroponically, and that nicotine supposedly has a metabolic half-life of less than 1 d, we allowed plants to take up nicotine by adding nicotine at a physiologically realistic concentration (1 mM) to the hydroponic solutions in which they were growing in order to test the hypothesis that tobacco could utilize the nitrogen from the metabolism of nicotine for growth under nitrogen-limited conditions. Nicotine feeding more than double nicotine pools at the time of feeding (an additional 0.86 mg N), only slowed down root growth during the 3 d feeding period and not thereafter, and did not statistically alter nitrogen pools. Nicotine-fed plants did not statistically differ from unfed plants in whole plant biomass or nitrogen pools. However, by 5 d after feeding, a pool of nicotine (~1 mg N), equivalent in size to that fed, was rapidly demethylated to nornicotine (~1 mg N) and myosmine, but not appreciably further. Impressive is the fact that even under severe nitrogen stress, plants remained unable to recover the nitrogen in nicotine to further growth. These results stress that metabolic turnover is not equivalent to resource recovery.

## CHIRALITY PURITY OF DISPARLURE SAMPLES.

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For many years it has been recognized that male gypsy moths (*Lymantria dispar*) respond to (+)-disparlure and that the (-)-enantiomer can be inhibitory. Other than bioassay, however, there has been no way of evaluating commercial disparlure samples for enantiomeric purity,

Very little is known about subterranean termite (Rhinotermitidae) chemical ecology. These economically and ecologically important insects are found in association with numerous species of fungi. We have isolated strongly phagostimulatory compounds from fungi in association with *R. hesperus* colonies in northern California. Populations of termites were maintained in the lab on Whatman filter paper, which was colonized by multiple species of fungi. Organic solvent extracts of the infected paper were strongly phagostimulatory. Efficient quantitative feeding bioassays were achieved using cellulose TLC plates as an edible substrate. Separation of active components was monitored by bioassays throughout a multistep chromatographic sequence, including ion exchange, gel filtration, and HPLC. Phagostimulatory HPLC peaks were analyzed spectroscopically. Alternatives to broad spectrum insecticides for control of subterranean termites will become practical only when baits can be made more attractive to termites than other potential food sources. Better understanding of the ecological influences on feeding behavior will lead to development of new control strategies.

#### **ECOLOGICAL THEORY: A USEFUL GUIDE TO THE PRESENCE/ABSENCE OF TANNINS AND OTHER SECONDARY METABOLITES?**

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The systematic distribution of tannins is used to predict their occurrence in the plant community of a Nebraska Sandhills Prairie. A good fit ( $p < 0.001$ ) between expected and observed distributions is seen at the family level, suggesting an importance for macroevolutionary pattern in determining the occurrence of tannins. In contrast, the ecological characteristics of these species (abundance, habitat preference, flowering phenology) are poor predictors. Systematics based predictions are also used to predict the occurrence of tannins in the flora of the Sheffield region (England) and these predictions are compared with extensive species level data about plant ecological characteristics. An overlap between systematics and ecology based predictions is seen, however, this overlap is not complete. For example, stress-tolerators and ruderal species are not associated with significantly high or low expected tannin contents. A hierarchical model for integrating systematic and ecological information into a coherent and more accurately predictive theory for the occurrence of tannins and other secondary metabolites is proposed.

#### **FEEDING STRATEGIES OF THE APHID SPECIES, *RHOPALOSIPHUM PADI* AND *R. MAIDIS* (HOMOPTERA: APHIDIDAE), IN WHEAT.**

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Hydroxamic acids (Hx), present in mesophyll tissue and sieve elements of wheat seedlings, are involved in resistance to aphids via antibiosis and antixenosis. The differences in performance between the aphids *R. padi* and *R. maidis* in wheat seedlings prompted us to study the feeding behavior of these aphid species by electropenetration graphs. While *R. padi* was very sensitive to feeding deterrence by Hx in wheat seedlings, *R. maidis* was insensitive. However, both species were equally sensitive to Hx in artificial diets. The insensitivity of *R. maidis* to Hx in seedlings was attributed to a feeding strategy avoiding contact with the compounds by decreasing the number of cellular punctures in live tissues other than sieve elements during its way to the phloem.

In addition, combinations of ouabain and digitoxin had a dose-dependent, negative and synergistic impact on pupal weights of armyworms. These results show that cardenolides are toxic to two generalist herbivores and that plants may benefit from the differentiation of such biologically active secondary metabolites.

#### **CAPSAICIN DETECTION BY TRAINED STARLINGS: THE IMPORTANCE OF OLFACTION AND TRIGEMINAL CHEMORECEPTION.**

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Unlike mammals, naive birds do not respond to capsaicin (the pungent principle in *Capsicum* peppers). This lack of response does not reflect insensitivity. The present experiments show that European starlings (*Sturnus vulgaris*) can be trained to detect capsaicin, and further, that an intact trigeminal nerve is required for response acquisition. These findings suggest the intriguing possibility that the mammalian trigeminal system encodes capsaicin as a chemically painful stimulus while the avian trigeminal system encodes it as something else (e.g., a warm stimulus). These results provide additional confirmation that the chemosensory world of birds, and in particular, the perception of mammalian chemical irritants, is fundamentally different from the chemosensory world of mammals.

#### **WHY DO COURTING MALE GARTER SNAKES CHIN-RUB BACKWARDS? EVIDENCE FOR A COPULATORY PHEROMONE.**

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Red-sided garter snakes, *Thamnophis sirtalis parietalis*, are known for their large vernal mating aggregations. In Manitoba, Canada up to ten thousand males seek unmated females that emerge from hibernation single or a few at a time over a four to five week breeding season. Males are attracted to females by skin-derived sex attractiveness pheromones produced across the female's dorsal surface. Mating balls of 10 to 100 males are common. During and immediately after copulation by one of these males, the female becomes transiently unattractive to further courtship by males. In addition, these females become unreceptive. This change in attractivity is not due to a loss of the attractiveness pheromone. Although recently mated females themselves were unattractive, hexane extracts of these same recently mated females were as attractive to courting males as were hexane extracts of unmated females. Behavioral tests in the field indicate that cloacal secretions associated with mating were inhibitory to courting males. This copulatory pheromone is unlike the attractiveness pheromones in that it is relatively short-lived, usually lasting for 24-48 hours. In field trials, unmated females smeared with these secretions were found to be significantly less attractive to males than untreated, unmated females. Additionally, treated females were not significantly different in their attractivity than recently mated females.

#### **PHAGOSTIMULANTS ISOLATED FROM FUNGI ASSOCIATED WITH THE WESTERN SUBTERRANEAN TERMITE, *RETICULITERMES HESPERUS* BANKS L.**

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Associations between fungi and termites are common in nature. Known interactions range from pathogenic to symbiotic, with most such ecological relationships still unexplored.

## CHEMICAL CAMOUFLAGE IN AN APHID-ANT-PARASITOID SYSTEM.

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Parasitoids of the aphidiid species *Lysiphlebus cardui* forage in ant-attended colonies of *Aphis fabae* without being molested by the guarding ants. By contrast, the aphidiid wasp *Trioxys angelicae* is rapidly recognized and vigorously attacked when discovered in aphid colonies. The cues for recognition are located on the cuticle of the species. Lure experiments showed that dead hexane-washed individuals of *T. angelicae* when treated with the hexane soak of *L. cardui* did not release aggressive behavior in ants. Vice versa, dead *L. cardui* were immediately recognized when the hexane extract of *T. angelicae* was applied. Chemical analysis of the cuticular hydrocarbons revealed that the profiles of *L. cardui* and its host *A. fabae* are similar mainly in n-alkanes and some branched alkanes. By contrast, *T. angelicae* possesses additionally Z-11-alkenes of C<sub>27</sub>, C<sub>29</sub>, C<sub>31</sub>, and C<sub>33</sub>. Bioassays demonstrated that washed aphidiids on which a hexane solution of Z-11-heptacosene or Z-11-nonacosene was applied were more aggressively treated by ants when offered in an aphid colony than were control individuals that were only hexane washed. The absence of such alkenes might be the reason why *L. cardui* is "invisible" for ants.

## THE APHID SEX PHEROMONE NEPETALACTONE: AN INTERSPECIFIC HOST-LOCATION CUE FOR PRAON SPP. PARASITIDS.

Richard Lilley,<sup>1</sup> Jim Hardie<sup>1</sup> and Lester J. Wadhams,<sup>2</sup> <sup>1</sup>Imperial College at Silwood Park, Ascot, Berks SL5 7PY, UK; <sup>2</sup>Institute of Arable Crops Research, Harpenden, Herts AL5 2JQ, UK.

An aphid sex pheromone (+)-(4aS, 7S, 7aR)-nepetalactone, acts as a kairomone for female aphid parasitoids of the genus *Praon*. In olfactometer assays female *P. volucre* were attracted to nepetalactone and to sexual females of two species of cereal aphid, *Sitobion avenae* and *S. fragariae* which release this pheromone to attract males. The effectiveness of nepetalactone as a means of manipulating parasitism levels among aphid populations was examined in the field. The presence of nepetalactone increased parasitism in colonies of *S. avenae* on individual barley plants when compared to colonies where pheromone was absent. The possible exploitation of these observations in aphid control is discussed.

## EFFECTS OF PLANT CARDENOLIDES ON GENERALIST HERBIVORES.

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Cardenolides sequestered by aposematic insect herbivores from their milkweed host plants provide some of the best known examples of chemical defense against natural enemies and continue to offer a didactic example of "ecological chemistry." Nevertheless, we know almost nothing about whether these toxic steroids are in fact plant chemical defenses against herbivores—despite their bitter taste and ubiquitous inhibition of Na<sup>+</sup>, K<sup>+</sup> ATPases. In this paper I examine whether two plant cardenolides influence the survivorship, growth rate or rate of feeding of two generalist herbivore species: the fall armyworm, *Spodoptera frugiperda*, and the Syrian hamster *Mesocricetus auratus*. Results of laboratory experiments using cardenolide-dosed, artificial diets showed that the less polar cardenolide digitoxin slowed the growth rates of both armyworms and hamsters, but had no effect on survivorship. In contrast, armyworm survivorship was reduced when fed the more polar cardenolide ouabain, but this cardenolide had little effect on growth of either herbivore.



as a means of manipulating parasitism levels among aphid populations was examined in the field. The presence of nepetalactone increased parasitism in colonies of *S. avenae* on individual barley plants when compared to colonies where pheromone was absent. The possible exploitation of these observations in aphid control is discussed.

## 20. THE ROLE OF PHENOLIC PHEROMONES IN THE BROWN EAR TICK, *RHIPICEPHALUS APPENDICULATUS* NEUMANN — A REAPPRAISAL.

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We reinvestigated volatile emissions of the brown ear tick, *Rhipicephalus appendiculatus*, the vector of the deadly livestock disease, East Coast fever, during the non-parasitic phases. Airborne volatiles emanating from live, newly moulted, unfed *R. appendiculatus* ticks were trapped by passing charcoal-filtered air over the ticks and trapping the volatiles on activated charcoal. The trapped volatiles were eluted with solvent and analyzed by GC and GC-MS. Six phenolic compounds, phenol, salicylaldehyde, *p*-cresol, 2,6-dichlorophenol, 2,6-dibromophenol and 2-bromo-6-chlorophenol were identified among the trapped volatiles. Authentic samples of the identified compounds were tested behaviorally using a tick climbing aggregation bioassay. Our results suggest an aggregation role for these compounds during the non-parasitic phases.

## 21. SEX PHEROMONES IN THE GENUS *HEMILEUCA* (LEPIDOPTERA: SATURNIIDAE).

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The genus *Hemileuca* is the most diverse of the U.S. Saturniidae, with 18 species described to date. Some species are of economic importance, such as *H. oliveae*, the range caterpillar, which occurs in sporadic large outbreaks. Larvae of all species may be a nuisance due to their stinging spines. The large and colorful dayflying moths tend to be locally abundant, but are short-lived as adults. To date, sex pheromones of *Hemileuca* species have not been identified, although field studies have demonstrated interesting patterns of inter-attraction between quite dissimilar species (Tuskes 1984). Furthermore, differences in pheromone blends appear to be used by sympatric species for reproductive isolation. We report here the identification of E10, Z12-hexadecadienyl alcohol, acetate, and aldehyde, and hexadecanyl acetate as female-produced sex pheromone components for *H. electra*, and discuss the cross-attraction or inhibition of attraction of other *Hemileuca* species.

## 22. RELATIONSHIPS BETWEEN CUCURBITACIN CONTENT AND THE INSECT AND PATHOGEN COMMUNITY IN ZUCCHINI HYBRIDS.

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Both the nature and the mechanisms of generalized plant resistance to insect and pathogen communities are poorly understood, in spite of the wealth of information on the effects of insects and pathogens in isolation on their plant hosts. In squashes and other cucurbit plants, cucurbitacins have been linked to resistance to many insect exploiters, and to susceptibility to diabroticite beetles, but little information exists on the effects of these

compounds on plant pathogens. We attempted to quantify insect and pathogen resistance in twelve families of F2 progeny of hybrids of 'Black Beauty' zucchini (*Cucurbita pepo*) and the gourd *Cucurbita texana*. We counted *Diabrotica* spp. and *Acalymma vittata* cucumber beetles, aphids and squash bugs (*Anasa tristis*) on leaves of mature field-grown plants. Leaf area removed and area covered with powdery mildew fungus were estimated, and we counted the number of leaves showing chlorosis, thickening, vein-clearing, and wilting symptoms of pathogen infection. Finally, we chose four families of plants with relatively extreme levels of insect and pathogen infestation for quantification of cucurbitacin E glycoside using TLC. Cucurbitacin E glycoside was detected in leaf blade and petiole samples, as well as in fruits, an unexpected result based on past data for related plants. Leaf blade cucurbitacin was negatively correlated to leaf damage, as was fruit concentration with a sum of a five pathogen symptoms, and petiolar concentration was positively associated with total insect infestation. The data indicate relationships among the members of the exploiter community of zucchini hybrids. Cucurbitacins may not enhance resistance in the complex milieu of community pressures acting on our plants.

### 23. NEEM AS LAGOMORPH REPELLENT.

*Dietland Müller-Schwarze*, College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, USA.

Extracts from seeds and other parts of the neem tree (*Azadirachta indica*) inhibit feeding and/or reduce growth in over 200 species of insects. But effects on vertebrate pests are little investigated. Neem extract, containing 0.3% of the bitter-tasting tetranortriterpenoid (limonoid) *azadirachtin* (trade name Morgosan-o™) was applied to twigs from apple trees and presented to free-ranging Eastern cottontails (*Sylvilagus floridanus*). Under the 1993 and 1994 winter conditions in upstate New York, neem proved to be an effective repellent, but only in high concentration. Three dilutions,  $10^{-1}$  to  $10^{-3}$ , were not active.

### 24. BEAVER AND RED MAPLE.

*Dietland Müller-Schwarze*, Bruce A. Schulte, Lixing Sun, Annette Muller-Schwarze, Christine Muller-Schwarze, College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, USA.

In our study area, beaver (*Castor canadensis*) avoid red maple (*Acer rubrum*). It ranks with conifers near the bottom of their feeding preferences. In cafeteria-style feeding experiments beaver rejected red maple. When extracts from the bark of red maple were applied to small aspen (*Populus tremuloides*) logs, they were accepted less by beaver. Conversely, red maple was rendered more palatable by applying extract from the bark of aspen. Colonies that consumed more of the samples were also less selective. To test whether beaver can leach out undesirable compounds, we soaked red maple in a pond for 2 to 36 days. Feeding experiments showed little effect of such treatment.

### 25. NEURORECEPTOR MECHANISMS FOR GUSTATION IN ADULT *DIABROTICA*.

*Christopher A. Mullin*, Sylwester Chyb, Herb Eichenseer, Ben Hollister & James L. Frazier. Pennsylvania State University, University Park, PA 16802 USA.

Taste chemoreception is essential for insect selection of suitable foods. Gustatory sensilla concentrated on mouthparts or other external surfaces are responsible for transduction of

chemical stimuli into nerve impulses that lead to behavioral acceptance or rejection of a potential nutrient source. Taste receptors have not been successfully purified or cloned from any animal, and the molecular basis for action of feeding deterrents and stimulants in insect gustatory systems is unknown. Our recent work with adult western corn rootworm, *Diabrotica virgifera virgifera* LeConte, implicates  $\gamma$ -aminobutyric acid (GABA)/glycine receptor in the perception of phago-stimulants and -deterrents. A pharmacological action profile using established ligands of major neuroreceptors linked taste chemoreception with GABA/glycine-gated ion channels. Plant isoquinoline and indole alkaloids and terpenoid epoxides structurally related to classical antagonists of GABA<sub>A</sub> or glycine receptors were also strongly antifeedant. The antifeedants  $\alpha$ -hydrastine, strychnine and picrotoxinin and the phagostimulants cucurbitacin B, GABA and glycine strongly elicited action potentials in galeal cells on rootworm maxilla. Good correlation occurred between feeding consumption and gustatory cell responses. A template for a shared amino acid/alkaloid binding site on taste receptor proteins was constructed based on computer molecular modeling in three-dimensional space and electrophysiological and behavioral correlations. The molecular basis for action of feeding deterrents and stimulants, when determined, would allow a rationale basis for insecticidal bait design.

## 26. CHEMICAL AND ECOLOGICAL INTERRELATIONSHIP BETWEEN OLIVE TREE (*OLEA EUROPAE* L.) AND OLIVE WEEVILS (*DYSCERUS PERFORATUS* (ROELOFS))

Shuhei Nakajima, Toshio Kitamura, Toshihide Ichikawa\*, Naomicha Baba, Faculty of Agriculture, Okayama University, Okayama, Japan. \*Faculty of Agriculture, Kagawa University, Kagawa, Japan.

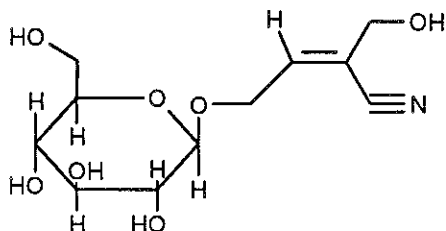
The olive trees have been cultivated in the Inland Sea Area of Japan, mainly in Okayama and Kagawa Prefectures. On the other hand, the olive weevils (*Dyscerus perforatus* (Roelofs); Curculionidae, Coleoptera), are indigenous to Japan and widely distributed throughout Honshu, Shikoku, and Kyushu Islands. They originally feed on *Ligustum japonicum* Thunb. and *L. obtusifolium* Sieb. et Zucc., both of which belong to the same family, Oleaceae. However, the insects began to shift their host plants to the olive trees after they were planted on large scale in Japan, and the population of the weevils increased very rapidly. Therefore, they become most serious pests of an olive in Japan so far. On the course of our study on the interrelation between an olive and the weevils, the insect feeding stimulant from the olive bark was isolated and identified as oleuropein, one of the plenty seco-irridoides in the olive trees. We also found that the weevils release a sweet odor when they are fed on the olive twigs. Since the preliminary bioassay showed that the weevils were attracted by a filter paper with this odor, we tried to isolate and identify such attractant(s). In this paper, the isolation and identification of these semiochemicals will be discussed.

## 27. A CYANOGLUCOSIDE, SARMENTOSIN, FROM THE MAGPIE MOTHS, *ABRAXAS* SPP. (GEOMETRIDAE).

Ritsuo Nishida<sup>1</sup> and Miriam Rothschild.<sup>2</sup> <sup>1</sup>Pesticide Research Institute, Kyoto Univ., Kyoto 606, Japan, and <sup>2</sup>Ashton Wold, Peterborough, PE8 5LZ, UK.

A group of geometrid moths in the genus *Abraxas* exhibit very similar aposematic wings with brilliant yellow colored bodies, and are unpalatable to predators. A single bitter compound was isolated in high concentration from adult body tissues of five *Abraxas* species studied (*A. miranda*, *A. nipponibia*, *A. latifasciata*, *fulvobasalis* and *A. grossulariata*),

and identified to be a cyanoglucoside, sarmentosin. The compound appeared to be sequestered regardless of their host plants: former three species are *Euonymus*-feeders, *A. fulvobasalis* is a *Salix*-feeder and *A. grossulariata* is polyphagous. The presence of sarmentosin in the individual host plants remains to be clarified. It is strongly suggested that sarmentosin is a defense substance commonly used by these closely related aposematic moths.



## 28. OVIPOSITION STIMULANTS IN THE CUTICULAR WAX OF THE COMMON ARMYWORM, *PSEUDALETIA SEPARATA*, TOWARD THE BRACONID WASP, *COTESIA KARIYAI*.

*Yoshitsugu Ohara*, Junji Takabayashi and Shozo Takahashi, Pesticide Research Institute, Faculty of Agriculture, Kyoto University, Kyoto 606-01, Japan.

*Cotesia kariyai* Watanabe (Hymenoptera: Braconidae) is one of the dominant monophagous parasitoid of the common army worm, *Pseudaletia separata* Walker (Lepidoptera: Noctuidae). After a brief searching behavior, *C. kariyai* found larvae by antennal contact and jumped onto the larvae. Then she immediately inserted her ovipositor into the larva. This behavior was elicited toward the 2nd to early 6th instar larvae. However, when a host larvae was covered with fine meshed nylon gauze, the wasp did not elicit the oviposition behavior to the object. The contact chemical kairomone was extracted from the body surface of the larvae and the extract was bioassayed using a glass rod on which a sample was impregnated. When the wasp showed insertion behavior with her ovipositor to a dummy, the sample was judged to be active. Purification of the extract according to the bioassay result and chemical analysis of the active fraction will be presented.

## 29. LOW MOLECULAR WEIGHT PHENOLICS OF *BETULA PUBESCENS* LEAVES.

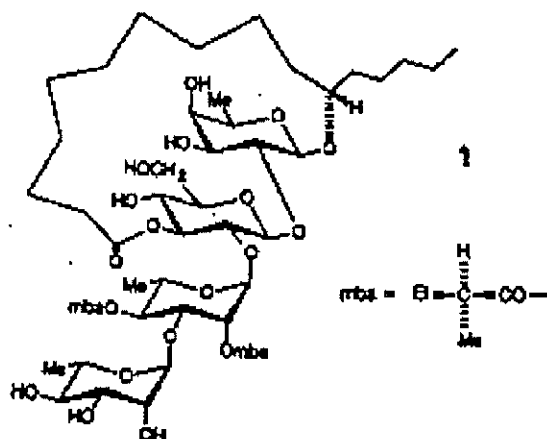
*V. Ossipov*, K. Nurmi, J. Lopenen, K. Pihlaja and E. Haukioja. University of Turku, FIN-20500, Turku, Finland.

We report data on the composition and content of low molecular weight phenolics in birch leaves. In methanol extract, 36 phenolic compounds were found by RP-HPLC. The main compounds were isolated by preparative HPLC and their structures determined on the basis of chromatographic (analytical HPLC), chemical (hydrolysis) and spectroscopic (UV, MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR) techniques. Myricetin-3-O- $\beta$ -L-(acetyl)-rhamnopyranoside, quercetin-3-O- $\beta$ -L-(4''-O-acetyl)-rhamnopyranoside, 5-O-caffeoylquinic (neochlorogenic) acid, *cis*- and *trans*-isomers of 3- and 5-O-(*p*-coumaroyl)-quinic acids and 1-O-galloyl-(2-O-acetyl)-glucose were detected in birch leaves for the first time. Phenolics were quantified by HPLC. Chlorogenic acid is the chief phenolic constituent of leaves, *ca* 4.7 mg g $^{-1}$  dry wt or 16% of total content. Interrelation between structure and ecological activity of phenolic compounds will be discussed.

### 30. TRICOLORIN A, MAJOR PHYTOGROWTH INHIBITOR FROM *IPOMOEA TRICOLOR*.

Rogelio Pereda-Miranda,<sup>1</sup> Ana Luisa Anaya<sup>2</sup> and Rachel Mata.<sup>1</sup> <sup>1</sup>Laboratorio de Fitoquímica, Departamento de Farmacia, División de Bioquímica y Farmacia, Facultad de Química. Universidad Nacional Autónoma de México, Coyoacán 04510, México, D.F. México. <sup>2</sup>Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.

The allelopathic potential of *Ipomoea tricolor* (Convolvulaceae), used in Mexican traditional agriculture as a weed controller, has been demonstrated by measuring the inhibitory activity of organic extracts on seedling growth of *Amaranthus leucocarpus* and *Echinochloa crusgalli*. Bioactivity-directed fractionation of the active CHCl<sub>3</sub> extract led to the isolation of the allelopathic principle, which turned out to be a mixture of the so-called "resin glycosides" of convolvulaceous plants. The structure of tricolorin A, the major phyto growth inhibitor present in the active fraction, was elucidated as that represented by formula 1, based on spectroscopic and chemical methods. The isolation of this bioactive constituent in a pure state was successfully achieved by a high resolution preparative HPLC methodology. Bioassays showed that radicle elongation of the two weed seedlings tested was inhibited by tricolorin A with IC<sub>50</sub> values ranging from 12-37 μM.



### 31. POLYMORPHISM IN SEX PHEROMONE BLEND IN TWO POPULATIONS OF THE BLACK CUTWORM MOTH, *AGROTIS IPSILON* (LEP., NOCTUIDAE).

Jean-Francois Picimbon,<sup>1</sup> Christophe Gadenne,<sup>2</sup> Jean-Marc Becard,<sup>2</sup> Leam et Srene<sup>1</sup> and Jean-luc Clement.<sup>1</sup> <sup>1</sup>CNRS-Laboratory of Neurobiology, Marseille, France. <sup>2</sup>INRA-Station of Zoology, Montfavet, France. <sup>\*</sup>Present address: Department of Chemistry, Stony Brook, USA.

The sex pheromone of *A. ipsilon* was determined for the American population as a blend of (Z)-7- dodecenyl acetate (Z7-12: Ac) and (Z)-9 tetradecenyl acetate (Z9-14: Ac). The combination of the two compounds has not been effective in the field trials in France. However, the addition of (Z)-11 hexadecenyl acetate (Z11-16: Ac), augments by 90% the trap catch. The chemical analysis of the hexane extracts of pheromone glands by gas chromatography coupled with mass spectrometry effectively revealed the presence of Z7-12: Ac, Z9-14: Ac and Z11-16: Ac in the picogram range. In addition, other compounds: (Z)-5 decenyl acetate (Z5-10 : Ac), (Z)-8 dodecenyl acetate (Z8-12 : Ac), (Z)-11 tetradecenyl acetate (Z11-14 : Ac), (Z)-11 hexadecenyl aldehyde (Z11-16 Al) and (Z)-11 hexadecenol (Z11-16 : OH) were also detected. These products are not present in decapitated females,



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