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Program and abstracts

**Laboratorio de Química Ecológica
Departamento de Ciencias Ecológicas
Facultad de Ciencias
Universidad de Chile**



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Sudelab S.A.

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Program schedule

Monday October 2

14:00-19:00 **Registration**

14:00-16:00 **ISCE Executive meeting**

16:00-18:00 **ISCE Executive and Council meeting**

19:00-21:00 *Mixer: barbecue*

Tuesday October 3

08:30-09:30 **Registration**

09:30 **Symposium 1: Aphid-Plant Interactions**

09:30-10:00 **Victor Eastop**
Aphids, plants and other organisms

10:00-10:20 *Coffee break*

10:20-10:50 **Jan Pettersson**
Plants - aphids, odour communication and interaction

- 10:50-11:00 **Ana María Castro**
Ethylene: a new tool for breeding tolerance to aphids in cereals?
- 11:00-11:30 **John Pickett**
Exploiting chemical ecology in controlling sucking insect pests
- 11:30-11:40 **Andrés Quiroz**
Odour communication of *Rhopalosiphum padi* L. (Hom.:Aphididae) feeding on cereals
- 11:40-12:10 **Freddy Tjallingii**
Stylet penetration of plants by aphids in a chemical context
- 12:10-12:20 **Beata Gabrys**
Behavioural responses of *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulz.) (Homoptera, Aphididae) to glucosinolates in their host plant
- 12:20-12:50 **Yvan Rahbé**
The *Cucumis melo* / *Aphis gossypii* model: biochemical and behavioural characterisation of aphid-plant interactions and their alteration by a resistance gene (vat) in melon

13:00-15:00 **Lunch**

15:00 **Symposium 2: Chemical ecology of social insects**

- 15:00-15:30 **David Morgan**
New aspects of recruitment and trail following in ants
- 15:30-15:40 **Ruth do Nascimento**
The chemistry of the metapleural gland secretions of three leafcutters species, *Atta Sexdens rubropilosa*, *Atta cephalotes* and *Acromyrmex octospinosus* (Hymenoptera: Formicidae)
- 15:40-15:50 **José Hernández**
Ethological and chemical studies of the abdominal glands extracts of the leaf-cutting ant *Atta laevigata*
- 15:50-16:00 **Horst Baumann**
Monoterpenes and alkaloids in the ant *Myrmica*: how do both interact during predation?
- 16:00-16:10 **Hiromi Sasagawa**
Flower kairomone mimics Nasonov pheromone of the Japanese honeybee, *Apis cerana japonica* Rad.

16:10-16:20 **Shigeru Matsuyama**
Identification of linalool oxide in Nasonov gland extract of the Japanese honeybee, *Apis cerana japonica* Rad.

16:20-16:30 **Jean-Luc Clément**
Regulation of the chemical signature of social insects in natural and artificial mixed societies

16:30-16:40 **Lester Wadhams**
Discrimination of plant volatiles in the honeybee

17:00-17:20 *Coffee break*

17:20 Symposium 3: Bioactive compounds of natural origin

17:20-17:30 **Francisco Macías**
Potential allelopathic compounds from cultivar sunflowers (*Helianthus annuus* L.)

17:30-17:40 **Carlos Céspedes**
Alkaloids in perennial ryegrass growing in soils of Southern Chile

17:40-17:50 **Francisco Cruz**
In vitro production of an exudate from nodal explants of mesquite tree (*Prosopis laevigata*)

17:50-18:00 **Bárbara Moreno**
Insecticidal activity of some native plants from the highlands of Colombia

18:00-18:10 **Peter Baeckström**
Aspects on bioassay guided fractionation of complex plant extracts using adsorption chromatography and accelerating gradient elution

18:10-18:40 **Pierre Escoubas**
Microscale bioassays and their application to the isolation of neuroactive compounds from arthropod venoms

19:30-21:00 *Dinner*

Wednesday October 4

08:30 Symposium 4: Synthesis, separation and biological activity of enantiomers

- 08:30-09:00 **Kenji Mori**
Synthesis of chiral insect pheromones
- 09:00-09:30 **Wittko Francke**
Structure assignment of chiral semiochemicals
- 09:30-09:40 **Walter Leal**
Identification of scarab beetle *Anomala osakana* sex pheromone clarifies the ecological significance of interruption in the japanese beetle sex pheromone system
- 09:40-09:50 **Tércio Ferreira**
Enantioselective syntheses of methyl 2,6,10-trimethyldodecanoate, the male-produced pheromone of the north american stink bug
- 09:50-10:00 **Rikard Unelius**
Synthesis and characterization of stereoisomers of nepetalactone and nepetalactoles, sex pheromones for host-alternating aphids

10:00-10:20 *Coffee break*

10:20-10:50 **Torbjörn Norin**
Chiral chemodiversity and its role for biological activity - some observations from studies on insect/insect and insect/plant relationships

10:50-11:20 **Gerhard Gries**
Identification and field testing of chiral insect pheromones

11:30-18:00 *Excursion*

19:30-21:00 *Dinner*

21:00 Special lecture series "Social Interactions in Scientific Development"

21:00-22:00 **John Pickett**
From fossils to chemical ecology

Thursday October 5

08:30 Symposium 5: Plant-herbivore interactions

08:30-09:00 **Ralph Mumma**
The genetic basis of small arthropod resistance of geraniums

09:00-09:30 **David Jones**
Positive and negative costs: a paradox in chemical defence

09:30-09:40 **Ernesto Gianoli**
Environmental constraints on the expression of constitutive and induced wheat chemical defenses

09:40-09:50 **Bernd Hägele**
The choice and performance of *Callimorpha dominula*, *Cylindrotoma distinctissima*, *Oreina cacaliae* and *O. speciosissima* (two generalist and two specialist herbivores on Senecioneae plants) towards cacalol and seneciphyllin, a sesquiterpene and a pyrrolizidine alkaloid found in *Adenostyles alliariae* and *A. alpina* (Asteraceae, Senecioneae)

09:50-10:00 **Ingrid Markovic**
Volatile and non-volatile chemical bases for gypsy moth, *Lymantria dispar*, larval rejection of green ash, *Fraxinus pennsylvanica*, foliage as food

10:00-10:20 *Coffee break*

- 10:20-10:50 **Alicja Zobel**
Histological localization of secondary metabolites in relation to their protective role for the plant
- 10:50-11:00 **Pachagounder Palaniswamy**
Some factors affecting the antixenotic resistance in crucifers to flea beetles, *Phyllotreta* spp.
- 11:00-11:30 **Thor Arnason**
Insect herbivores that cope with extreme stress of phytotoxic host plants

11:40 Symposium 6. Multitrophic interactions

- 11:40-12:10 **Helmut van Emden**
Plant chemistry and natural enemies of aphids
- 12:10-12:20 **Eduardo Fuentes**
Influence of different wheat and oat cultivars on the development in the cereal aphid *Sitobion avenae* of the cereal aphid parasitoid *Aphidius rhopalosiphi* and the generalist parasitoid *Ephedrus plagiator*
- 12:20-12:30 **Ritsuo Nishida**
Aphid chemicals affecting feeding behaviour of a ladybird beetle
- 12:30-12:40 **Caroline Liepert**
Chemical mimicry of aphid parasitoids of the genus *Lysiphlebus* (Hymenoptera, Aphidiidae)
- 12:40-12:50 **Dale Norris**
Glycine max intrinsic and extrinsic chemical defense against herbivory
- 12:50-13:00 **Roberto Trigo**
Chemoecology in plant-herbivore-parasitoid interactions *Aristolochia arcuata* x *Battus polydamas* x *Areoscelis rufa*

13:00-15:00 *Lunch*

15:00 Symposium 7: Pheromones: isolation, mechanisms, uses

- 15:00-15:30 **Glenn Prestwich**
Why is pheromone perception so specific?
- 15:30-15:40 **Baldwyn Torto**
Sex pheromone studies in the desert locust *Schistocerca gregaria* (Forsk.) [Orthoptera: Acrididae]

- 15:40-15:50 **Aivlé Cabrera**
Study of the aggregation pheromone of the banana weevil *Cosmopolites sordidus* (Germar)
- 15:50-16:00 **Gal Yarden**
(Z,E)- α Farnesene - a sex/aggregation pheromone component of the *Maladera matrida* beetle
- 16:00-16:10 **Walter Leal**
Scarab beetle *Anomala japonica* utilizes a more complex sex pheromone blend than a similar species, *A. cuprea*
- 16:10-16:20 **Cam Oehlschlager**
Chemical communication system of the sugarcane weevil, *Metamasius hemipterus*
- 16:20-16:30 **Cam Oehlschlager**
The aggregation pheromone of the coconut rhinoceros beetle, *Oryctes rhinoceros*
- 16:30-16:40 **Jeremy McNeil**
Receptivity of males from two host races of the fall armyworm, *Spodoptera frugiperda* to different sex pheromone lures
- 16:40-16:50 **Francis Webster**
GC-EAD analysis and chemical ecology of the white pine weevil *Pissodes strobi*
- 16:50-17:00 **J. D. Hardege**
Sex pheromones in marine polychaetes: heterospecificity of chemical signals

17:00-17:20 *Coffee break*

17:20-19:20 Poster session

19:30-21:00 *Dinner*

21:00 Silverstein-Simeone Plenary Lecture

21:00-22:00 **Wilhelm Boland**
How to survive with volatiles: plante contre herbivore

Friday October 6

08:30 Symposium 8: General topics in chemical ecology

- 08:30-09:00 **Luz María Pérez**
The role of secondary metabolites and gene products in the hypersensitive response developed by higher plants upon fungal attack
- 09:00-09:10 **Horacio Heinzen**
The chemical basis of the resistance of cereal seeds to pathogenic fungi
- 09:10-09:20 **Scott Smedley**
Vitamine E derivatives in the defensive secretion of pupae of *Epilachna borealis* (Coleoptera: Coccinellidae)
- 09:20-09:30 **Christa Holz**
The multifunctional role of cantharidin
- 09:30-09:40 **Rupert Kellner**
Maternal pederin transfer in *Paederus* rove beetles and its ecological significance
- 09:40-09:50 **Alvaro Eiras**
The behavioral responses of *Aedes aegypti* (L.) (Diptera: Culicidae) to carbon dioxide plumes
- 09:50-10:00 **Gordon Hamilton**
Chemical ecology of the new world sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae)

10:00-10:20 *Coffee break*

- 10:20-10:30 **Rosângela Epifanio**
Chemical defenses of Brazilian octocorals
- 10:30-10:40 **Manoj Rai**
Semiochemicals mediating oviposition-aggregation in the gregarious desert locust, *Schistocerca gregaria*
- 10:40-10:50 **Anita Marsaioli**
The ecological role of *Chusia* floral resins and their detailed chemical composition
- 10:50-11:20 **Miguel Alonso**
Neotropical bracken fern: are establishment strategies dependent upon plant variety?

11:20 Symposium 9. Nitrogen in plant defence

- 11:20-11:50 **Michael Wink**
Chemical defense of alkaloid producing plants
- 11:50-12:00 **Roberto Trigo**
Activity of pyrrolizidine alkaloids in chemical defense against the orb-weaving spider *Nephila clavipes*. I. Senecionine free base and N-oxide
- 12:00-12:10 **Dietrich von Baer**
Alkaloids and anthracnose in *Lupinus albus*
- 12:10-12:20 **Vijaya Kumar**
Role of caffeine in the resistance of tea clones to the shot hole borer, *Xyleborus fornicatus*
- 12:20-12:50 **Keith Brown**
The ecological activity of alkaloids in natural systems
-

13:00-15:00 *Lunch*

15:00 Symposium 10: Chemical ecology of vertebrates

- 15:00-15:30 **Dietland Müller-Schwarze**
The role of chemical ecology in vertebrate biodiversity conservation
- 15:30-16:00 **Francisco Bozinovic**
Chemical and digestive ecology of foraging in vertebrates
- 16:00-16:30 **Klaus Jaffé**
Human odors. A basis for human sexual selection and host recognition by hematophagous arthropods: a chemical-ecological approach
- 16:30-17:00 **Bets Rasmussen**
Identification and bioactivity of an estrous pheromone of Asian elephants: a surprise, perhaps a trend
-

21:00-23:00 *Banquet*

Poster presentations

- 1 **Alberto Fereres**
Aphid settling behavior modifying terpenes: effect on *Myzus persicae* (Homoptera: Aphididae)
- 2 **Alberto Fereres**
Characterization of the resistance of melon lines to *Aphis gossypii* (Homoptera: Aphididae) and cucumber mosaic virus (cmv)
- 3 **Wilf Powell**
Using aphid sex pheromones to manipulate parasitic wasp populations
- 4 **Jacques Pasteels**
The evolution of host-derived defense in *Oreina* leaf beetles
- 5 **Roberto Trigo**
Floral scents in *Passiflora* species pollination
- 6 **Roberto Trigo**
Geometry optimization and conformational analysis of pyrrolizidine alkaloids by molecular mechanics. A chemoeology approach
- 7 **Pilar Marivil**
Alkaloid profile during the development of sweet varieties of *Lupinus albus* plants
- 8 **Uta Hashagen**
Breeding cereals for increased self-defence against aphids through increases in their hydroxamic acid content
- 9 **Ivanildo de Lima**
Volatile components from the salivary glands of calling males of the South American fruit fly *Anastrepha fraterculus* (Wied.): partial identification and behavioural activity
- 10 **Ritsuo Nishida**
Ecological association of new and old world chrysomelid leaf beetles with cucurbitacins
- 11 **Jean-Luc Boevé**
Volatiles emitted by larvae of the European apple sawfly and by the fruit they infest: potential significance for foraging natural enemies
- 12 **Marta del Campo Couratier**
Developmental effects of an acquired alkaloid and its adaptive consequences in a moth (*Utetheisa ornatix*).
- 13 **Masahiko Tokoro**
Food searching behavior of the pleasing fungus beetle, *Dacne picta* (Crotch)

- 14 **Ximena Arias**
"Head space" composition and surface topography of two varieties of *Lycopersicon esculentum*: correlation with the oviposition preferences of the fruitmoth, *Scrobipalpuloides absoluta* meyrick (Gelechiidae)
- 15 **Adela María Sánchez**
Comparison of allelopathic potential of four forestry soils
- 16 **Manuel Reigosa**
Allelopathic effects in *Capsicum annuum* L.. Some considerations about practical uses of allelopathy
- 17 **Julio Zygadlo**
Biochemical actions of volatile allelochemicals from *Tagetes minuta* L.
- 18 **Carolina San Martín**
Presence of allelopathic activity in callus of *Datura stramonium*
- 19 **Alicia Bardón**
Allelopathic effects and biological activity of constituents from *Vernonia* species (Compositae)
- 20 **Cristian Desmarchelier**
Wheat rootlet growth inhibition test of aqueous extracts of South-American plants
- 21 **Fernando Perich**
Effects of acetophenones on red clover (*Trifolium pratense* L.) growth
- 22 **L.M. Cunha Rebouças**
The application of aggregation pheromone of the *Rhynchophorus palmarum*
- 23 **Kiyoshi Nakamuta**
Male sex pheromone of the *Cryptomeria* twig borer, *Anaglyptus subfasciatus*
- 24 **Tadashi Nemoto**
Drosophila sex pheromones: structure and application
- 25 **Paulo Zarbin**
An expeditious synthesis of 1-hydroxy-5-nonanone: a volatile emission of *Bactrocera cacuminatus*
- 26 **Gal Yarden**
(Z,E)- α -Farnesene - an electroantennogram active component of the *Maladera matrida* volatiles
- 27 **Johanne Deslile**
Effect of temperature on the relative performance of light and pheromone traps to capture hemlock looper adults
- 28 **Federico Castrejón**
Sex pheromone glands in *Toxotrypana curvicauda* (Diptera: Tephritidae)

- 29 **Tadakazu Nakashima**
Phytoalexins in sapwood of *Cryptomeria japonica*
- 30 **David Morgan**
The natural insecticide azadirachtin, produced by plant tissue culture
- 31 **Larissa Collins**
Chemo-attractants for oviposition in *Toxorhynchites* mosquitoes
- 32 **Alison Blackwell**
Physiological and behavioural responsiveness of the biting midge *Culicoides nubeculosus* to semiochemicals in relation to age, physiological condition and nutritional status
- 33 **Alison Blackwell**
Sweet gale (*Myrica gale*): a natural repellent for pests of man and livestock
- 34 **Juraj Harmatha**
Occurrence and biogenetic relations of sesquiterpenes in Senecioneae plants and their role in a proposed chemical defence against herbivores
- 35 **Hassane Mahamat**
Maturation accelerating components of the adult male produced aggregation-maturation pheromone system of the desert locust *Schistocerca gregaria*
- 36 **Leonora Mendoza**
Localization of secondary metabolites on the surface of *Pseudognaphalium* spp.
- 37 **Brenda Modak**
Antimicrobial activity of the resinous exudates of *Heliotropium* spp.
- 38 **Claude Bernard-Bourret**
Collateral sensitivity of p-glycoprotein rich multidrug-resistant cells to lignans
- 39 **Mario Camino**
Effect of two terpenes from mexican plants on fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae)
- 40 **Lucila Aldana**
Biological activity of plants on *Spodoptera frugiperda* larvae
- 41 **María Elena Valdés**
Biological activity of extracts of *Ricinus communis* on fall armyworm *Spodoptera frugiperda* (Lep.: Noctuidae)
- 42 **Bárbara Moreno**
Antifeedant activity of the leaves extract of the native Colombian plant *Berberis montaguiensis* sp. n. L.A.C. (Berberidaceae)

- 43 **Peter Baeckström**
Aspects on bioassay guided fractionation of complex plant extracts using adsorption chromatography and accelerating gradient elution

Symposium 1. Aphid-plant interactions

APHIDS, PLANTS AND OTHER ORGANISMS

V.F. Eastop, Natural History Museum, London

The relationships between aphids, plants, other organisms and some physical components of the environment such as temperature are reviewed. Aspects considered include year cycles, polymorphism, fecundity, relationship of different groups of aphids with particular groups of plants and with other insects including other plant-feeders and ants, honeydew, alarm pheromones, aposematid colouring, camouflage both visual and tactile, colour variation within species, morphological variation within species, both 'inherent' environmentally induced and founder effects, multivariate analysis and problems of its interpretation, parasites, predators, stridulating mechanisms, co-evolution of plants and aphids, plant galls, trapping aphids and the interpretation of trap catches, and the curation of aphid collections. The taxonomic status of permanently parthenogenetic populations and the nature of aphid species, reproductive barriers, adaptive peaks and centripetal selection for gene combinations are discussed. The problems associated with the variation in insect pests and its influence on the future of the taxonomy of aphids and associated organisms is discussed.

Notes:

NOT a chemist (Victor)

Mites on aphids, 1st instar parasitic

Aphids - transmit many viruses - e.g. tobacco etch virus

Most aphids actually feed on trees.

3 generations packaged together - need 3 under constant conditions

Many parthenogenetic - when introduced into foreign habitats - often have only part of the genotype

Great color variation - not well understood

Great variation when reared at diff temps.

PLANTS - APHIDS, ODOUR COMMUNICATION AND INTERACTION

Jan Pettersson, Department of Entomology, Swedish University of Agricultural Sciences, P.O. Box 6077, S-750 07 Uppsala, Sweden

Aphid ecology is dominated by dynamic processes where aggregation and propagation is balanced by migration. This system is optimized by a finely tuned communication system between the aphids and their environment. Preliminary studies on a limited number of aphid species indicate that odour stimuli can be important for these processes. The communication consists of a network of stimuli where plant and aphid stimuli interact more or less connected to each other. Present studies can be summarized to following general hypotheses:

- host plant discrimination. Finding a host plant is an over all problem and in spite of a poor flying capacity trap catches indicate that host plant odours attract flying aphids.
- feeding site evaluation. Once the correct plant is found it is necessary to evaluate different available feeding sites. In this process coexistence with other aphids on the same plant seems important. Feeding together with other species seems to be less favourable and odours can be an indication of the presence of other species.
- density regulation. The basic element in social communication between aphids seems to be an intraspecific aggregation. However, food resource management means that a density optimisation has to be at work and possibly the contours of such mechanisms can be seen.
- seasonal migration. The seasonal migration in aphids is a process regulated by a range of different stimuli. Odour mechanisms, host plant odours and pheromones seem to contribute to the precision of this.

The interaction between the attacking aphid and the host plant is dynamic. It may also include an element of plant/plant interaction. In a pilot study aphid attacked oat plants produce volatile compounds that elicit an induced resistance in nearby plants. This rapidly induced resistance has a limited persistence and lasts only for a few days in the experiments but is costly for the plant and expressed morphologically in terms of retarded growth.

Notes: Philosopher
Descriptive phase
Hibernates as eggs.

ETHYLENE: A NEW TOOL FOR BREEDING TOLERANCE TO APHIDS IN CEREALS?

Castro, A.M., Giménez D.O., Brocchi, G, Almaraz L. and Montaldi, E. Departamento de Biología y Ecología y de Producción Vegetal, Facultad de Ciencias Agrarias y Forestales, U.N.L.P. CC31, CP1900-La Plata, Argentina

Plant growth inhibition in aphid infested plants has been well documented in susceptible cultivars: lower mitotic index, shorter coleoptiles and leaves, less radical volume, a reduced number of total leaves differentiated and a diminished aerial and radical biomass. Nevertheless, those cultivars selected by their tolerance against aphid feeding have displayed a higher growth rhythm under infestations.

Lower plant growth rate is a result of the decreased number of cells and shorter but wider cells. These symptoms are characteristic of the hormone ethylene. Ethylene is a hormone that induces the ageing of plants; it is synthesized under every stress condition, autocatalytically. Aphid feeding induces high ethylene synthesis in wheat, barley and oat susceptible and tolerant cultivars, this hormone being found in the volatiles of the infested plant.

Having developed a biological test, tolerance to aphids is likely to be identified without insects, subjecting the experimental lines to an enriched ethylene atmosphere. Since tolerant cultivars carry mechanisms to detoxify the increasing ethylene doses their growth is kept normal, despite the higher hormone synthesis.

Notes: Good talk

EXPLOITING CHEMICAL ECOLOGY IN CONTROLLING SUCKING INSECT PESTS

J. A. Pickett, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

Recent studies have shown that semiochemicals play a more important role in the chemical ecology of aphids and other sucking insects than originally thought, particularly in relation to longer range interactions. This presentation will describe studies ensuing from the identification of aphid sex pheromones and the discovery that these can effect long range attraction of aphids and also their parasitoids. An important aspect of recent research has been the contribution made by electrophysiological studies combined with behavioural investigations. These have elucidated the role of "redundant" olfactory cells in responding to components of unsuitable host plants, giving rise to a series of repellents active in field trials against aphids attacking cereals. The increased understanding of chemical ecology of sucking insects enhances prospects for aphid control by means of semiochemicals and promises similar developments against hoppers and thrips.

Notes: Push/Pull

Repellents/Antifeedants - Aggregation, traps

Negative lures

Lactone - Olls in parasitoid

Methyl salicylate → 30-50% reduction

Diff. species aphids on same olact produce diff. comods.

ODOUR COMMUNICATION OF *RHOPALOSIPHUM PADI* L. (HOMOPTERA:APHIDIDAE) FEEDING ON CEREALS

A. Quiroz¹, J. Pettersson², J.A. Pickett³, L.J. Wadhams³ and H.M. Niemeyer¹, ¹Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile; ²Swedish University of Agricultural Sciences, P.O. Box 7044, S-750 07 Uppsala, Sweden; ³AFRC IACR Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ, UK

The bird-cherry-oat aphid, *Rhopalosiphum padi* (L.) is an important pest that causes economic damage to cereals both as a virus vector and as a phloem feeder. It is a host alternating species, bird-cherry (*Prunus padus* L.) (Rosaceae) being its winter or primary host, and a wide range of cereals and grasses (Poaceae = Gramineae) its summer or secondary hosts.

In the present study we report on the odour communication of alatae and apterae of *R. padi* on two secondary hosts: oat and wheat. The occurrence of odour communication in apterous and alatae summer morphs of *R. padi* was investigated in olfactometer and in settling experiments. An arresting odour signal from alatae feeding on oats affecting alatae, and a repelling signal released by apterae feeding on oats and affecting apterae was demonstrated. This latter signal was density dependent above a density of ca. 4.5 aphids/cm² of available leaf area. It is suggested that the odour stimulus affecting apterae is part of a spacing mechanism related to food management.

Substances were isolated from the aphid-secondary host combinations and were identified by GC-MS. The nature and importance of these signals in aphid population dynamics will be discussed.

Notes:

Andreas
 2-tridecanone
 6-methyl 5-hepten-2-one } only in plants w high aphid density
 Both counteract of volatile (attractant) from undamaged wheat
 chem. w resistance - bred into wheat

STYLET PENETRATION OF PLANTS BY APHIDS IN A CHEMICAL CONTEXT

W. Freddy Tjallingii, Wageningen Agricultural University, Dept. Entomology. P.O.B. 8031, 6700 EH Wageningen, The Netherlands

In spite of the growing experimental evidence that aphids, or at least their antennal receptors, can be affected by plant volatiles, aphids still happen to arrive on non-host plants more or less randomly or predominantly using visual cues. The empirical evidence found by John Kennedy and his coworkers in the 50-ties still is of basic value. He nicely demonstrated that not landing, but taking off from plants was very selective.

Other work demonstrated that probing after landing was of great importance for host selection. During probing, *i.e.* stylet penetration, into plants 3 main phases can be distinguished on basis of studies using plant histology and the electrical penetration graph (EPG) technique: stylet pathway phase, xylem, and phloem phase. The stylets appeared to penetrate exclusively between the cells, *i.e.* outside the plasmalemma of living cells, making many brief intracellular punctures from which they withdraw to continue their extracellular pathway. Punctured cell instantly repair the disrupted area of their membranes leaving no visible damage in the cells. The xylem is occasionally used for drinking behaviour. The phloem is the food source. Phloem sieve elements are also delicately punctured and the pressure of its contents secures a constant and passive feeding of the insect. Tapping for hours and days seems not to harm the sieve element cells as we can observe, for example, by the maintenance of its transmembrane potential.

Plant cytochemistry has demonstrated that most allelochemicals occur in the vacuoles of specific cells and in a chemically form with reduced toxicity (glycosides, amides, etc.). The chemical war breaks out when these cells are disrupted and their contents become mixed with enzymes that considerably enhance toxicity of the allelochemicals. The damage of the stylet punctures seems too well controlled to evoke such activations of the plant defense system. Many correlation studies have demonstrated effects of allelochemicals on aphid behaviour as observed in specific assays and with aphid performance on plants containing different concentrations of them. Besides, aphids are phloem feeders, if any toxic component might occur in the extracellular fluids, as far as we know they will not ingest them, certainly not in amounts that will seriously affect them, which is in sharp contrast to biting insects. The foregoing knowledge on toxicity reduction of allelochemicals in the plant and their compartmentation in combination with stylet penetration details, makes it difficult to interpret the relevance of such correlation studies in the complete aphid-plant context. Some examples will be used to demonstrate these difficulties.

Plant damage caused by aphids is often devastating. This damage, however, is not directly caused by stylet penetration as discussed above. It seems to be mainly due to chemical interference in the plant metabolism and/or eliciting general or special biochemical reactions. Pure mechanical stimuli, possibly by stylet penetration, or, more likely, chemicals secreted into the plant with the aphid saliva may be responsible for these reactions. A number of authors have claimed that salivary compounds are used by aphids to detoxify the allelochemicals. Some of the enzymes secreted with the saliva have been identified and their assumed activity seems likely. Again, however, how relevant are these supposed detoxifications in the complete aphid-plant combination and, is our knowledge of the phenomena, the plant and salivary chemistry involved adequate? A case study will be discussed.

Notes: Incredibly boring
amides + glucosides

BEHAVIOURAL RESPONSES OF *BREVICORYNE BRASSICAE* (L.) AND *MYZUS PERSICAE* (SULZ.) (HOMOPTERA, APHIDIDAE) TO GLUCOSINOLATES IN THEIR HOST PLANT

B. Gabrys¹ and **W.F. Tjallingii**², ¹Department of Agricultural Sciences, Agricultural University, Cybulskiego 32, 50205 Wroclaw, Poland; ²Department of Entomology, Wageningen Agricultural University, POB 8031, 6700 EH Wageningen, The Netherlands

The chemical analysis of the plant material has shown differences in quantity and proportion of individual glucosinolates in different parts of the aphid's host plant, yellow mustard (*Sinapis alba* L.; Brassicaceae=Cruciferae). The glucosinolates were also detected in *Myzus persicae* honeydew.

The behaviour of the oligophagous cabbage aphid, *Brevicoryne brassicae* and polyphagous peach potato aphid, *M. persicae* during probing on preferred and avoided feeding sites was monitored using the EPG (Electro Penetration Graph) technique. It has been found that glucosinolates probably influence aphid behaviour long before they sample the phloem sap.

Notes:

THE *CUCUMIS MELO* / *APHIS GOSSYPII* MODEL: BIOCHEMICAL AND BEHAVIOURAL CHARACTERISATION OF APHID-PLANT INTERACTIONS AND THEIR ALTERATION BY A RESISTANCE GENE (VAT) IN MELON

Yvan Rahbé, Jian-Qun Chen, Nicolas Sauvion, Bernard Delobel, Michel Pitrat and Hervé Lecoq, INRA - INSA Lyon and INRA Avignon, France

The cotton/melon aphid *Aphis gossypii* is the main aphid pest of Cucurbitaceae. In addition to direct population damage, it is an important vector of many cucurbit viruses. A gene for resistance to this aphid was identified in melon at INRA Avignon, and was shown to govern species-specific resistance to the transmission of many non-persistent viruses. Isogenic melon lines carrying or not this vat gene (for virus aphid transmission) were used for a behavioural and biochemical characterisation of the resistance. The Electrical penetration graph technique allowed to show that this typical "non-acceptation" resistance was not inducible, and was readily detectable within two hours of contact with the resistant melon line. The resistance factors are probably present before phloem access, which is delayed on vat plants. However, when aphids reach their target phloem tissue on the resistant genotype, they precociously stop ingestion and start probing again, suggesting the presence of a deterrent factor in phloem sap. This was confirmed on artificial diets supplemented with phloem extracts. Biochemical composition of phloem sap was investigated in search for differences between isogenic lines. Phloem protein profiles were very similar, as well as levels in most protein amino-acids and other ninhydrin positive compounds. A group of *Cucumis* specific compounds was specially investigated. Pyrazole and its amino acid derivatives β -pyrazolyl alanine (BPA) and γ -glutamyl BPA were detected in melon phloem sap, but no quantitative differences appeared between susceptible and resistant genotypes. The effect of these compounds on the development and behaviour of melon host and non-host aphids was tested on artificial diets. Whereas their presence in plants could influence host recognition by the cucurbit strain of *Aphis gossypii*, their involvement in the determinism of the vat resistance was ruled out.

The only chemical differences detected in the phloem sap of the resistant line was the increase in a non-hydrolysable ninhydrin positive compound, correlated with the decrease of a peptide peak, currently under identification. The level of these peaks seems sensitive to the redox conditions of phloem sampling. The mechanisms of species-specific resistance to virus transmission will be discussed in view of this preliminary chemical characterisation and of short term (8 min) comparative EPGs between *Aphis gossypii* and *Myzus persicae* (respectively non vector and vector on vat plants).

Notes:

Chelating agents
pyrazole empds

Phe PAL Cinnamic acid

Symposium 2: Chemical Ecology of Social Insects

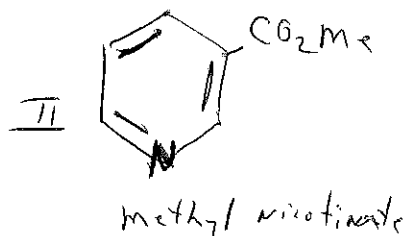
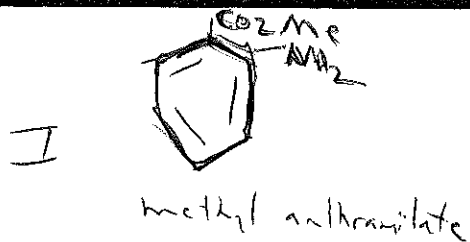
NEW ASPECTS OF RECRUITMENT AND TRAIL FOLLOWING IN ANTS

E.D. Morgan and N.J. Oldham, Keele University, Staffordshire, England ST5 5BG

A two-component pheromone operating on a new principle has been discovered as the trail pheromone of an army ant (*Aenictus* species). One component, methyl nicotinate, is required as a primer which prepares or alerts workers to follow a second component, methyl anthranilate, when it is introduced, even though it is introduced some hours later. Once primed, following of the second component on artificial trails is intense and persistent (with J. Billen and B. Gobin, University of Leuven).

The poison gland of the North American ant *Aphaenogaster cockerelli* contains two volatile components, one of which is used for trail-following, the other for recruitment. This two-component mixture explains the observation that the closely related species *A. albisetosus* is able to follow trails of *A. cockerelli* and why the reverse of this does not apply. The trail pheromone of *A. cockerelli* is a newly evolved character (with B. Hölldobler, University of Würzburg).

Notes:



Primer (P)
(primes them to follow I)

THE CHEMISTRY OF THE METAPLEURAL GLAND SECRETIONS OF THREE LEAFCUTTERS SPECIES, *ATTA SEXDENS RUBROPILOSA*, *ATTA CEPHALOTES* AND *ACROMYRMEX OCTOSPINOSUS* (HYMENOPTERA: FORMICIDAE)

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The chemical composition of the secretions from the metapleural glands of workers and soldiers of two *Atta* species, *Atta sexdens rubropilosa* and *A. cephalotes*, and workers of *Acromyrmex octospinosus*, have been studied. Proteins are the chief components of the secretion, which could be observed by infrared spectrometry and confirmed by the ninhydrin test. The non-proteinaceous portion of the secretion is composed by carboxylic acids, which are present as ionized salts. Phenylacetic acid is the major component in workers and soldiers of *A. s. rubropilosa* and *A. cephalotes*. Both *Atta* species also contain 3-hydroxydecanoic acid and its homologues as minor components together with indoleacetic acid. Despite the qualitative similarities of the acid portions in the secretion of both *Atta* species, they differ quantitatively. The secretion of *Acromyrmex octospinosus* contains 3-hydroxydecanoic and indoleacetic acids, but lack phenylacetic acid. The activity of the three major acids against bacteria and fungi has been confirmed.

Notes:

ETHOLOGICAL AND CHEMICAL STUDIES OF THE ABDOMINAL GLANDS EXTRACTS OF THE LEAF-CUTTING ANT *ATTA LAEVIGATA*

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In the leaf-cutting ants of the genus *Atta*, it is known that the Dufour's gland secretion is associated with territorial behavior and that the main function of the venom gland secretion is trail following. However little is known about the function and chemical composition of the rectal sac secretions. We found that the blends of abdominal gland extracts (Dufour's gland, poison gland and rectal sac), release agonistic behavior in *Atta laevigata*. Ants marked at the foraging area with blends of these extracts released strong agonistic reactions in their nest mate (biting and mutilation), if the extracts were taken from ant from a different colony. However if the extracts were from a nestmate, this agonistic behavior was absent. Additionally, the chemical composition of these extracts was characterized using GC and GC/MS. The fact that the blend of these extracts are necessary to release agonistic behavior, suggests that agonistic behavior is very complex and show multicomponent and multifunctional nature and that moreover these secretions showing colony specific effects.

Notes:

MONOTERPENES AND ALKALOIDS IN THE ANT *MYRMICARIA*: HOW DO BOTH INTERACT DURING PREDATION?

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²Inst. für Organische Chemie, Universität Hamburg, D-20146 Hamburg, Germany

During combat, the African ant *Myrmecaria eumenoides* extrude its blunt-tipped and membranous sting and releases poison gland secretion topically onto arthropod preys. The secretion is composed in approximately equal amounts of a high-volatile and a low-volatile fraction. (+)-Limonene is the main constituent (>97%) of the high-volatiles. It serves as recruitment-signal and attracts nearby nestmates to sites of combat. Scout ants returning to the nest mark their course with poison gland secretion. Limonene is highly volatile and the signal function would vane very quickly. The low-volatile fraction acts physico-chemically as a fixative by reducing the evaporation rate of the limonene. In an intraspecific context the low-volatiles elongate the durability of the chemical signal (Kaib & Dittebrand: Chemoecol. 1,3, 1990).

The low-volatile fraction is mainly made up by the (3*R*,5*S*,9*R*)- and (3*R*,5*R*,9*R*)-stereoisomers of 3-butyl-5-(1-oxopropyl)indolizidine, named Myrmecarin 237A and 237B (Francke *et al.*: Liebigs Ann. 965, 1995). These alkaloids serve in an interspecific context as toxin. When topically applied, isolated or synthetic Myrmecarins immobilize prey. 1:1 Mixtures of the alkaloids and limonene - equal to the ratio in the poison gland secretion - immobilize prey four times faster than the alkaloids on their own. On the cuticle of prey limonene increase both spreading velocity and spreading width of the alkaloids. In addition, limonene enhances the penetration of the alkaloids through the cuticle of prey.

Our results demonstrate in *Myrmecaria eumenoides* for the main constituents of the poison gland secretion, (+)-limonene and Myrmecarins, a dual function and that both physico-chemically increase the adaptative function of each other.

Supported by the Deutsche Forschungsgemeinschaft (Ka 526/4-4)

Notes:

Indolizidine alks - myrmecarin
alkaloids prolong effectiveness of limonene

FLOWER KAIROMONE MIMICS NASONOV PHEROMONE OF THE JAPANESE HONEYBEE, *APIS CERANA JAPONICA* RAD

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The Japanese honeybee (*Apis cerana japonica* Rad.: Acj) and the European honeybee (*Apis mellifera* L.: Am) share the same habitat in Japan. Our knowledge of the chemical ecology of Acj is scanty, as opposed to Am. We present the results of a comparative study on the role of semiochemicals on the social behavior of these two species.

Aggregation behavior, alarm behavior and recognition are controlled by semiochemicals from the Nasonov gland, sting apparatus, mandibular gland, feet and cuticles. Extracts from the Nasonov gland and sting apparatus of both species were analyzed by GC and GC/MS. Nasonov gland extracts induced aggregation behavior in both species, but the GC profiles of the extracts from Am and Acj were quite different.

The flowers of the oriental orchid (*Cymbidium pumilum* Rolfe: Cp) attract workers, drones, queens and also entire swarming colonies of Acj (Sasaki *et al.*, 1991), but not of Am. This is due to the fact that the flower scent mimics the Nasonov pheromone of Acj.

The scent of Cp flowers was collected with Teenax TA (70-270 mg) and analyzed by GC and GC/MS, and identified as acetone, acetophenone, 2-heptanone, 2-nonanone, n-heptanal, n-octanal, n-nonanal, benzaldehyde, linalool, furanoid type and pyranoid type linalool oxide (*cis*, *trans*), benzoic acid, fatty acid (C₂, C_{6-10,12,14,16,18}). When bioassayed by the polyethylene bag method (bag size: 340 x 250 x 0.03 mm), a real flower attracted 100% of workers and more than 93% of drones, whereas 0.1 flower-equivalent extracts attracted more than 90% of Acj workers, but was not active against workers (0%) and drones (0%) of Am. As the Acj workers gathered toward the Cp scent, they exposed their Nasonov glands. Acj Nasonov gland extract attracted 98% of workers and drones of Acj. Observing this, we conducted a chemical comparison between the Cp flower scent and the Nasonov gland extract. As a result, both furanoid type and pyranoid type of linalool oxide (*cis*, *trans*) were detected in both the scent and the extract. Synthetic linalool oxide (furanoid type and pyranoid type, *cis:trans*=1:1) attracted Acj workers and drones at the 0.5 to 500 ng level. On the other hand, linalool oxide did not attract Am workers or drones. In addition, 2-heptanone was also found in Cp scent and in the mandibular glands of both Acj and Am.

In conclusion, the Nasonov gland components were different between Acj and Am. In general, Acj is more sensitive to chemicals than Am. The oriental orchid was found to have the same components in its scent as Acj had in its Nasonov gland, that is, four types of linalool oxide. Differences between Acj and Am might be due to differences in semiochemicals, sensitivity, and recognition

Notes:

IDENTIFICATION OF LINALOOL OXIDE IN NASONOV GLAND EXTRACT OF THE JAPANESE HONEYBEE, *APIS CERANA JAPONICA* RAD.

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In marked contrast to the European honeybee, *Apis mellifera* L. (Am), our knowledge on the chemical ecology of the Japanese honeybee, *Apis cerana japonica* Rad. (Acj) is limited. We have now investigated the Nasonov gland chemistry and identified biologically active components.

Comparative studies on the Nasonov gland of Am and Acj by GC and GC/MS corroborated the occurrence of seven previously identified Nasonov pheromone in the former. On the other hand, Acj extract gave none of these compounds. Instead, four types of linalool oxide (*cis* and *trans* of both furanoid and pyranoid) were identified. In a laboratory bioassay, small groups of Acj workers placed in polyethylene bags were exposed to authentic linalool oxide (furanoid, racemate, 1:1 mixture of *cis* and *trans*). In response to 5-500 ng of linalool oxide on Teflon discs, workers were attracted (52-94%) and displayed their Nasonov gland. Neither Acj extract nor linalool oxide was attractive to Am workers. These results suggest that linalool oxide comprises the Nasonov pheromone of Acj.

Analysis of air above Acj workers exposing their Nasonov glands, absolute configuration of linalool oxide and their effects on pheromonal activity will be discussed.

Notes:

REGULATION OF THE CHEMICAL SIGNATURE OF SOCIAL INSECTS IN NATURAL AND ARTIFICIAL MIXED SOCIETIES

Clément, J.L., Bagnères, A.G., Bonavita-Cougourdan, A., Lorenzi, M.C.*, Vauchot, B., CNRS-Laboratoire de Neurobiologie, UPR 9024, 31, chemin J. Aiguier, F-13402 Marseille Cedex 20, France; *Dipartimento di Morfofisiologia Veterinaria, Università di Torino, Viale Mattoli 25, 10125 Torino, Italy

In social insect societies (ants, termites, wasps, bees, etc.), the importance of the role played by cuticular hydrocarbons in caste, nest and species recognition was clearly demonstrated. Each species possesses its own cuticular signature depending on the nature of the major hydrocarbons. Each nest and each category of individuals inside a nest has its own hydrocarbon pattern depending on the proportions of identical hydrocarbons.

Artificially mixed societies have frequently been used to investigate the mechanisms whereby the signal involved in recognition processes are regulated. Mixed colonies of the two ant species (*Manica rubida* and *Formica selysi*) showed that quantitative and qualitative changes occur in the chemical signature of both species as a result of their cohabitation. The new chemical signature is the result of a readjustment of the two species chemical pattern.¹ In two termite species (*Reticulitermes santonensis* and *R. (lucifugus) grassei*) mixed experimental colonies, after two hours of cohabitation, each species acquired all the hydrocarbons specific to the other species. But after 14 days of cohabitation a regulatory process involving quantitative changes was therefore at work.²

In naturally mixed nests of the slave making ant *Polyergus rufescens* and the slave ant *Formica spp.* on the contrary, the cuticular patterns differ between the two species.³ But the proportions of the common products between the slave and the slave making ants showed a tendency to adapt to those of the other species.⁴ *Polistes atrimandibularis* is an obligatory social parasite of *Polistes biglumis bimaculatus*. To take control of the host nest, the parasite changes very rapidly the chemical signature, in a sudden and complete disappearance of a whole chemical family.⁵

¹ Bagnères, A.G. *et al.* 1991. *J. Chem. Ecol.* 17: 1641-1664.

² Vauchot, B. *et al.* 1995. *J. Insect Physiol.* in press.

³ Haberstezer *et al.* 1993. *Physiol. Entomol.* 18: 160-166.

⁴ Bonavita-Cougourdan, A. *et al.* 1995. *Comp. Biochem. Physiol.* in press.

⁵ Lorenzi, M.C. *et al.* 1995. *Natural History and Evolution of Paper Wasps*, Chap. 10. Turillazzi, S. and Eberhard, M.J., eds. Oxford Univ. Press, in press.

Notes:

DISCRIMINATION OF PLANT VOLATILES IN THE HONEYBEE

M.H. Pham-Delègue,¹ F. Marion-Poll,² M.M. Blight,³ L.J. Wadhams,³ C.M. Woodcock,³ ¹INRA-CNRS (URA 1190), BP23, Bures-sur-Yvette, France, ²INRA, Route de Saint-Cyr, 78026 Versailles Cédex, France, ³IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

Foraging behaviour of honeybees is dependent on learning processes involved in the perception of plant volatiles. These substances play a major role in cueing orientation to food sources. In order to investigate complex odour recognition in the honeybee, the conditioned proboscis extension responses (CPE) of individual restrained bees were studied. As this response occurs naturally when foragers land on flowers, the CPE assay is considered to be a useful tool for characterising behaviourally active plant volatiles. The bees were stimulated either by synthetic samples of oilseed rape floral volatiles, presented individually or as a mixture, or by an air entrainment extract of oilseed rape flowers. A standard CPE assay or a novel combined gas chromatography (GC)-CPE technique was employed to stimulate the bees. With both procedures, it appeared that, after training to the synthetic mixture, bees consistently responded to three components out of the six present in the mixture. When the coupling technique was extended to include the simultaneous recording of electroantennogram responses (EAG), it was shown that all six components were detected at the sensory level, whereas discrimination between components appeared at the behavioural level. Further experiments using the air entrainment extract as the stimulus in the GC-CPE system confirmed that mixture recognition relied on a limited range of compounds, with only 5 components out of 50 eluting from the GC column eliciting CPE responses in more than 35% of the bees previously trained to the extract. The effects of prior olfactory experience on discrimination abilities was then evaluated by comparing the responses of naïve (no training), conditioned (paired training to the extract) and pseudoconditioned (unpaired training) bees. CPE activity was recorded in all bees for a limited number of components of the extract. Compared to the spontaneous responses of naïve bees, the level of CPE activity was increased in the conditioned group and depressed in the pseudoconditioned one. These data show that recognition of complex odours is based on a limited range of key components.

Notes:

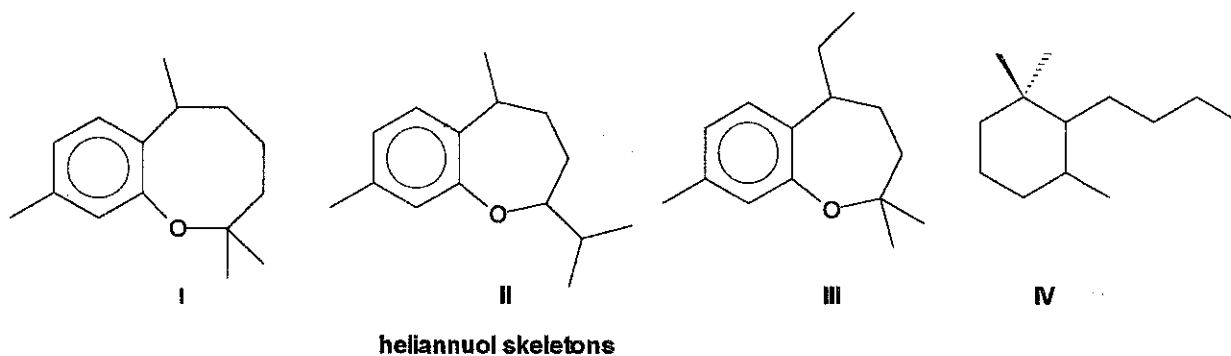
Mixture recognition based on a relatively few
compounds.

Symposium 3: Bioactive compounds of natural origin

POTENTIAL ALLELOPATHIC COMPOUNDS FROM CULTIVAR SUNFLOWERS (*HELIANTHUS ANNUUS* L.)

Francisco A. Macías, José M.G. Molinillo, Rosa M. Varela, Ascensión Torres and Diego Castellano, Department of Organic Chemistry, Faculty of Science, University of Cadiz, Apdo. 40, 11510 Puerto Real, Cadiz, Spain

The study of bioactive fractions of cultivar sunflowers (*Helianthus annuus* L.) varieties, SH-222 and VYP, afforded a large number of compounds. They are mainly sesquiterpenoids, along with some flavonoids, diterpenoids and phenols. These sesquiterpenoids are included in the following type of compounds: sesquiterpene lactones, heliannuols and bisnorsesquiterpenoids. Several compounds isolated from *Helianthus annuus* L. present the novel sesquiterpene skeleton heliannuol. There is no report of other compounds with such skeleton at this moment from any other species. We present the isolation and structural elucidation of 15 different heliannuols from these varieties with the basic structures shown in the figure. In addition, we have found five bisnorsesquiterpenoids that present the general structure IV.



The structure of these compounds were established by spectroscopic analysis of their ^1H and ^{13}C NMR, ^1H - ^1H , COSY ^1H - ^{13}C HETCOR and NOE-diff spectra. Some of these compounds have shown to be bioactive on the germination and root and shoot length on the dicotyledon species *Lactuca sativa*, *Lepidium sativum*, *Lycopersicon aesculentum* and the monocotyledon species *Hordeum vulgare*. The study of the allelopathic potential of the natural products involved in the above mentioned effects will be discussed.

Notes:

ALKALOIDS IN PERENNIAL RYEGRASS GROWING IN SOILS OF SOUTHERN CHILE

C. Céspedes¹, F. Perich¹, N. Butendieck² y O. Romero², ¹Depto. Cs. Químicas, Universidad de La Frontera, Temuco, Chile; ²Depto. Producción Animal, Estación Experimental INIA Carillanca, Temuco, Chile

Several perennial ryegrass cultivars used in southern Chile have recently been introduced from New Zealand. These varieties are specially used for feedings cows. Perennial ryegrass (*Lolium perenne* L.) infected with the fungi endophyte *Acremonium lolii* Latch, Christensen, Samuels (Fungi Imperfecti) is a highly active feeding deterrent to the Argentine stem weevil (*Listronotus bonariensis* Kuschel) and is involved in the resistance of endophyte-infected ryegrass and fescues to this insect pest. According to Harborne J.B., and Mullin Chr. (personal communication) the fungus/plant combination is a source of toxic alkaloids and high animal toxicosis including ryegrass staggers and fescue toxicosis. During the last few years ryegrass staggers has been detected in cows feeding on these forage varieties in Southern of Chile (Butendieck N., 1994). In this study we report data related to the detection of alkaloids in *L. perenne* var. Embassy.

The occurrence of alkaloids in these extracts was investigated by applying the common methodology used in phytochemical work: after an exhaustive extraction with methanol, solvent was removed in a vacuum rotary evaporator, and alkaloids were obtained by elution with solvents of increasing polarity. Some alkaloids were isolated from these extracts, which may correspond to the antifeedant signals observed at high endophyte infections. The structure, nature and properties of these signals will be discussed.

Acknowledgements. This work was supported by Depto. Cs. Químicas. Universidad de La Frontera, Temuco, Chile.

Notes:

Terrible!

☆ Te Strake

Indole & Pyrrolizidine alks

IN VITRO PRODUCTION OF AN EXUDATE FROM NODAL EXPLANTS OF MESQUITE TREE (*PROSOPIS LAEVIGATA*)

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Mesquite tree (*Prosopis leavigata*) affords a gum exudate with similar properties as gum arabic. The genus *Prosopis* is valuable to man because of its unusual resistance to drought and salinity, its usefulness as a wind-breaker, as a means of stabilizing drifting sands and a source of animal food and beans for human consumption, its ability to fix nitrogen in the soil and its use in minimal energy input agriculture. *Prosopis* gums have mainly been used by pharmaceutical and fine chemicals companies as a source of arabinose and galactose after hydrolysis or to a very limited extent for local use in areas where they are produced. Nevertheless, mesquite gum was proposed as a possible cheaper alternative for traditional water-soluble polymers (e.g. gum arabic) used in food products, although, as has been pointed out, the gums from *Prosopis* species have never been approved as a food additives in the USA or in the EEC.

Mesquite is the common name used in North America for several *Prosopis* species. In South America, it is named "algarrobo". Natural production of mesquite gum is unknown, there are trees with gum and trees without gum; maybe insects or some kind of stress induce exudate across bark. In this study, *in vitro* culture of *P. leavigata* was evaluated for propagation of chosen overproductive specimens. Explants with 1 or 2 nodes were taken from the youngest 6 nodes of the main stem and branches of 3-6 months old greenhouse grown stock plants. Explants were surface sterilized in 70% ethanol for 1 min and 75% commercial sodium hypochlorite for 10 min, and immersed in an anti-oxidant solution (100 mg/L citric acid, 50 mg/L ascorbic acid) for 15 min. Murashige and Skoog medium was used with 8 g/L agar, 30 g/L sucrose, 1.6 g/L glutamine, 100 g/L citric acid, 50 mg/L ascorbic acid, and 16 combinations of plant hormones, including benzylamine purine (BA) at 0-10 mg/L and naphthalene acetic acid (NAA) at 0-10 mg/L. One explant was placed on each medium vessel and incubated at room temperature with 16 h photoperiod and photon lux density of 2000 lux for 40 days. 5.0 mg/L BA and 1.0 mg/L NAA induced shoot proliferation giving 1 or 2 shoot/node (81.48 %). 0.0 mg/L NAA and 1.0-5.0 mg/L BA giving callus. All treatments afforded *in vitro* exudate, 43 explants (68%) yielded an exudate similar (0.3 mL) to mesquite gum. The results for *P. leavigata* provide the basis for a possible micropropagation system consisting of shoot proliferation and shoot growth; rooting is not yet concluded. Possible *in vitro* mesquite gum production was obtained. Exudate of explants and an authentic sample of mesquite gum IR spectra comparative will be carried out for structure determination.

Notes:

NADA

Low viscosity - rivals gum arabic

INSECTICIDAL ACTIVITY OF SOME NATIVE PLANTS FROM THE HIGHLANDS OF COLOMBIA

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In Colombia, there are a lot of problems in crops caused by pests. Its flora rich and diverse, offers a large number of economically significant plants that can produce bioactive compounds with insecticidal, antifeedant and other properties due to secondary metabolites with selective and specific activities¹. The ethnobotanical data help us to select species that can be applied as natural control agents, as part of Integrated Pest Management (IPM) strategies, and these methods can contribute to protect the environment and Human Health. The ethanolic and ethereal extracts of the following native and therapeutic species were tested against larvae of the insects *Spodoptera sunia* and *Achroia grisella* by contact and ingestion bioassays, using natural and artificial diets²; as general lethality assay we used the test with *Artemia salina* (Leach). The analyzed plants were: *Duranta mutisii*, *Chusia chiribiquitensis*, *Solanum lisyoides*, *Hyeronima moritziana* and *Ficus cundinamarcensis*, through the methodology reported previously^{3,4}. Bioassay guided chromatographic separation gave some fractions and compounds with antifeedant and toxic activities, which were statistically evaluated and their minimum inhibitory concentration (MIC) determined by appropriate methods. From the active fractions were isolated and purified some esteroidal and alkaloidal derivatives which were analyzed by spectroscopic techniques such as IR, UV, ¹H and ¹³C-NMR. We were mainly concerned for behavioral effects on *S. sunia* larvae of *D. mutisii* and *S. lisyoides* extracts and we will present and discuss the biological results and the preliminary structural analysis of the major active compounds, one of which is a novel benzylaminoalkaloid. We are deeply indebted to Colciencias (008-94) and Cindec (Universidad Nacional de Colombia-803209) for financial support.

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Notes:

ASPECTS ON BIOASSAY GUIDED FRACTIONATION OF COMPLEX PLANT EXTRACTS USING ADSORPTION CHROMATOGRAPHY AND ACCELERATING GRADIENT ELUTION

Peter Baeckström and Zandra Jirón, Department of Organic Chemistry, Royal Institute of Technology, KTH, S - 100 44 Stockholm, Sweden

For quite some time we have been studying the effect of polarity gradients in connection with liquid straight phase adsorption chromatography. Our results have lead us to higher loadings than is common practice in most laboratories. The method enables a rapid group separation of crude plant extracts in amounts sufficient for further investigations. Equipment and methodology will be presented as a poster. Examples including a highly efficient method for isolating azadirachtin from ground Neem seeds by combined extraction and chromatography in the same column will be shown.

The oral presentation of the poster will emphasize the advantages of using gradients with a strong eluant to enable the detection and isolation of constituents that are present in relatively small amounts in the extracts. The pit falls of using isocratic and stepwise gradient elution will be illustrated by computer simulated chromatography.

Notes: Commercial For His Columns / U&H!

MICROSCALE BIOASSAYS AND THEIR APPLICATION TO THE ISOLATION OF NEUROACTIVE COMPOUNDS FROM ARTHROPOD VENOMS

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The discovery of novel neuroactive compounds is of great importance for pharmacological and agrochemical applications. Arthropod neurotoxins are still a largely unexplored field and toxins previously isolated from spider or scorpion venoms have proven to be useful tools for the understanding of structure and function of neural receptors and ion channels, as well as for the development of genetically engineered microbial pesticides.

However, the amount of material available for structural and activity investigation is usually a limiting factor. We have therefore devised microscale assay techniques for the study of biological activity of these neurotoxins. Coupled with the most modern chemical techniques (Narrow-bore HPLC, Microsequencing, ESI-MS) they allow work at the nanomole level.

The first one is a microinjection technique, using *Drosophila*, for toxicological studies against insects. The second one is an insect smooth-muscle pharmacological preparation, using micro-chambers (100 μ l).

Results obtained with both assays for a series of scorpion venoms and isolated toxins will be discussed. In the microinjection assay, the gain in material used ranges from 10 to 100 times when compared with classical methods, using larger insects. Sensibility also appears higher for venoms belonging to the Chactoid group of scorpions.

The micro-pharmacological assay shows a specificity in the mode of action of different toxins and neurotransmitters, with the most active being detected at the nanomolar level. This assay has proved its usefulness in the study of fractionated scorpion venoms, where biological activity could be discovered in fractions otherwise devoid of toxic activity on either insects or mice.

Notes:

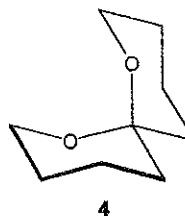
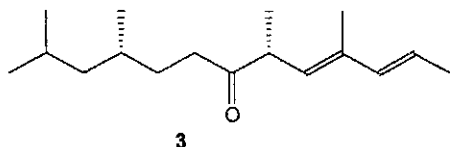
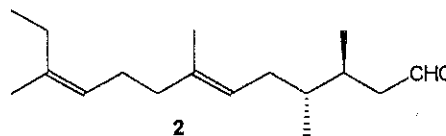
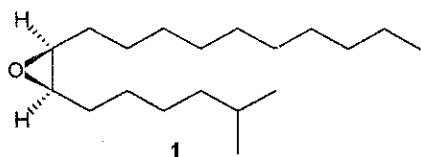
Peptides acting on Ca channels
Varying neurotoxicity - appear to be operation on H_2O targets.
By combining several microscale techniques
hope to find series of compounds.

Symposium 4: Synthesis, separation and biological activity of enantiomers

SYNTHESIS OF CHIRAL INSECT PHEROMONES

Kenji Mori, Department of Chemistry, Science University of Tokyo, Kagurazaka 1-3, Shinjuku-ku, Tokyo 162, Japan

Syntheses of optically active insect pheromones such as disparlure (1), faranal (2), matsune (3), olean (4), etc. will be discussed explaining the concept underlying each of the syntheses.



Notes:

- Review - History
Variety of approaches.
- Begin with natural product which is optically pure
 - Optical resolution - chemical or biochemical
 - Asymmetric synthesis - chemical or biochemical
- a) disparlure
c) faranal, matsune

STRUCTURE ASSIGNMENT OF CHIRAL SEMIOCHEMICALS

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

Structure elucidation of chiral semiochemicals will be reported. Modified cyclodextrins provide the ideal tool for enantiomeric separation by gas chromatography.

1. Unsaturated chiral secondary alcohols comprise species specific pheromone blends in leaf miner moths both with respect to enantiomeric and geometric composition of the components.
2. Chiral epoxy polyenes are widespread among Geometrid moths. Regioselective as well as enantioselective epoxidation of homoconjugated double bond systems are decisive for the formation of specific blends.
3. New bicyclic acetals of the 6,8-dioxabicyclo[3.2.1]octan-system could be identified from bark beetles, *Tephrocybus bicolor* and *Dendroctonus ponderosae*.
4. Common wasps, *Paravespula vulgaris* and *P. germanica* as well as *Dolichovespula saxonica* and *D. media* show species specific blends of chiral spiroacetals which represent cryptic straight chain keto-diols showing 9 or 11 carbon atoms. The absolute configuration at the alcohol functions differ between *Paravespula* and *Dolichovespula*.

Notes:

Cyclodextrins - not only useful for sep of enantiomers but also polar enols.

IDENTIFICATION OF SCARAB BEETLE *ANOMALA OSAKANA* SEX PHEROMONE CLARIFIES THE ECOLOGICAL SIGNIFICANCE OF INTERRUPTION IN THE JAPANESE BEETLE SEX PHEROMONE SYSTEM

Walter Soares Leal, Laboratory of Chemical Prospecting, National Institute of Sericultural and Entomological Science, Tsukuba, Japan

That chirality plays a pivotal role in chemical communication in scarab beetles was first demonstrated in the sex pheromone system of the Japanese beetle; the antipode of japonilure dramatically reduces aggregation of responding males. The biological significance of this interruption (*sensu* Wood) is now clarified with the identification of the sex pheromone of other species. A female-specific, EAD-active compound (undetected by FID) was found in *A. osakana*, which had the same retention time of japonilure. Indoor bioassays and field tests revealed that males did not respond to japonilure at all, but were highly attracted to its antipode (S,Z)-(dec-1-enyl)oxacyclopentan-2-one. In addition, interruption (now played by japonilure) was also observed. Although male antennae of the two species responded to both enantiomers in chiral GC-EAD, it was clarified that *A. osakana* females produced only the (S)-enantiomer.

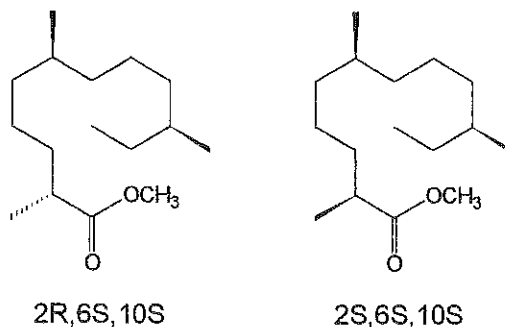
Notes:

Chiral GC-EAD - good col. for separation
Reciprocal antagonist of chiral enantiomers
"Brazilian Carnival"

ENANTIOSELECTIVE SYNTHESSES OF METHYL 2,6,10-TRIMETHYLDODECANOATE, THE MALE-PRODUCED PHEROMONE OF THE NORTH AMERICAN STINK BUG

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Methyl 2,6,10-trimethyldodecanoate was identified last year as a component of the male-produced pheromone of the North American stink bug, *Euschistus obscurus*¹. The absolute stereochemistry of this methyl branched ester remains to be determined. In this work, we report the first enantioselective syntheses of two stereoisomers (2R, 6S, 10S) and (2S, 6S, 10S) of methyl 2,6,10-trimethyldodecanoate out of 8 possible isomers.



The mass spectroscopic data of these two isomers was identical with that of the natural pheromone. These compounds will be available for further bioassay experiments with the insect which, we hope, could lead to the use of semiochemical for the management of this pest.

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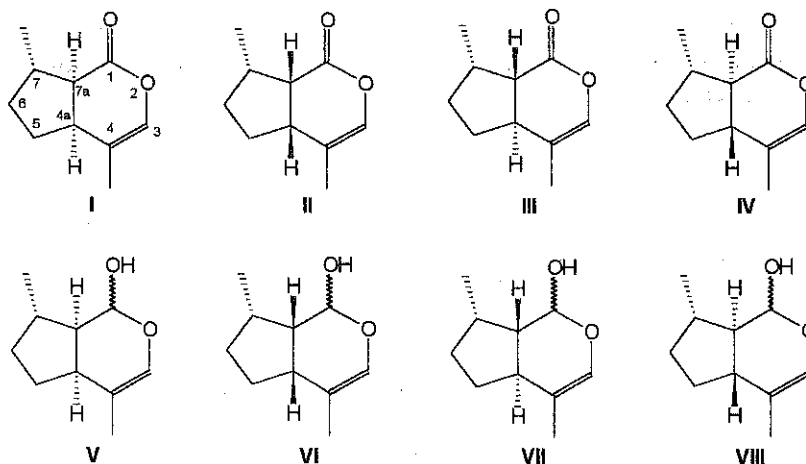
Notes:

Barking Dog - replaced snake

SYNTHESIS AND CHARACTERIZATION OF STEREOISOMERS OF NEPETALACTONE AND NEPETALACTOLES, SEX PHEROMONES FOR HOST-ALTERNATING APHIDS

C. Rikard Unelius, Piotr Prokopowic, Ulla Jacobsson and Per-Ola Norrby, Royal Institute of Technology, Department of Organic Chemistry, S-100 44 Stockholm, Sweden

Aphids are ubiquitous insects on earth. Some species cause severe damage to arable crops. With the objective to find environmentally safe methods to control the noxious aphid species, there is a prerequisite to interpret the chemical language of aphids. In recent years nepetalactones and related iridoids have been identified as sex pheromones for host-alternating aphids. The nepetalactone appear to act as an aphrodisiac and the nepetalactone as an attractant.



Some aspects of their chemistry and structure will be discussed in conjunction to the syntheses of these compounds.

Notes:

CHIRAL CHEMODIVERSITY AND ITS ROLE FOR BIOLOGICAL ACTIVITY - SOME OBSERVATIONS FROM STUDIES ON INSECT/INSECT AND INSECT/PLANT RELATIONSHIPS

Torbjörn Norin, Department of Chemistry, Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden

Many compounds occur in Nature in both enantiomeric forms and this *chiral chemodiversity* is very pronounced among certain classes of natural products whereas other classes of compounds usually occur in only one of the enantiomeric forms. Thus triterpenoids and steroids occur as pure enantiomers and only in one form, whereas many of the lower terpenoids are present in Nature in both of their enantiomeric forms and usually as mixtures.

The chiral chemodiversity among the terpenoids will be discussed in relation to the biological functions of the compounds. The discussion will refer to recent results on the enantiomeric compositions of monoterpenes from various natural sources and their possible role in insect/insect and insect/plant interactions (for references to our recent research on this topic see^{1,2}).

Some current studies on the use of enzymes for asymmetric synthesis of semiochemicals³ and the development of analytical techniques^{2,4} for the control of enantiomeric purity will also be discussed.

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Notes:

Diff e's operating for diff. components
Optical purity seems to be a "general" trait
i.e. if -pinene is, then -limonene will be,
Separate e's operating for each of the monoterpenes.

IDENTIFICATION AND FIELD TESTING OF CHIRAL INSECT PHEROMONES

Gerhard Gries, Simon Fraser University, Dept. of Biological Sciences, Burnaby BC V5A 1S6, Canada

The talk will present the identification and field testing of chiral candidate insect pheromones. Results will focus on pheromone components in geometrid moths, (mulberry looper, *Heterophila atrilineata*, and hemlock loopers, *Nepytia* and *Lambdina*), lymantriid moths (nun moth, *Lymantria monacha* and gypsy moth *L. dispar*), curculionid weevils (*Rynchophorus* and *Pissodes*) and scotyliid beetle *Pityogenes elegans*. Potential role of newly acquired pheromone components will briefly be outlined.

Notes:

Symposium 5: Plant-herbivore interactions

THE GENETIC BASIS OF SMALL ARTHROPOD RESISTANCE OF GERANIUMS

R.O. Mumma¹, D.J. Schultz², P.R. Paul¹, T.G. Grazzini⁴, D.L. Cox-Foster¹, J.I. Medford⁵, R. Craig³.
Pennsylvania State University ¹Dept. of Entomology, ²Intercollege Program in Genetics, ³Dept. of Horticulture,
⁴Centre Analytical Laboratories, State College, PA, ⁵Dept. of Biology

The small arthropod resistance of *Pelargonium xhrotorum* (garden geranium) is controlled by the glandular trichome excretion of ω 5 unsaturated anacardic acids in resistant geranium genotypes. The unique unsaturated fatty acids (C16:1 Δ 11, C18:1 Δ 13) have been shown to be the precursors for ω 5 unsaturated anacardic acids and are found only in the tall glandular trichomes. Our data suggest that the dominant Mendelian locus regulating resistance encodes a unique fatty acid desaturase. To isolate this desaturase, a two-directional approach was undertaken: tall glandular trichomes were analyzed for their protein content and for genes expressed in resistant *versus* susceptible genotypes. A unique protein was found only in trichomes of resistant plants (parent, F₁, F₂, backcross) when total trichome proteins were analyzed by western blot with a Δ 9C18:O- fatty acid desaturase antibody. A geranium cDNA library was probed with a gene for an acyl-ACP desaturase. Subsequently, two genes were isolated: one putatively corresponding to the ubiquitous Δ 9-stearyl desaturase; the other a unique fatty acid desaturase found (by Northern Blot analysis) to be expressed only in trichomes of resistant plants. This latter gene was cloned and expressed in *E. coli*, and resulted in the production of two new fatty acids.

Notes:

POSITIVE AND NEGATIVE COSTS: A PARADOX IN CHEMICAL DEFENCE

David A. Jones, Department of Botany, University of Florida

When a plant is as successful as *Pteridium aquilinum*, *Trifolium repens*, *Lotus corniculatus* or *Achillea millefolium* it must be doing most things right. These species are among the commonest and, today, the most widely distributed herbs in the world. All are cyanogenic species, but all are also polymorphic for cyanogenesis. That is, there are different proportions of cyanogenic individuals in different populations.

It is not easy to see why a common plant producing 'cheap' (in terms of glucose) chemical defence like cyanoglucosides should frequently be polymorphic when those species producing 'expensive' defensive compounds are almost exclusively monomorphic (with, perhaps, some quantitative variation). Furthermore, for compounds that are sporadic, cyanoglucosides are probably more widely distributed in nature than any others. This is emphasized by the fact that these polymorphic species have close relatives that are monomorphic for cyanogenesis (or acyanogenesis).

All this suggests that the costs, in terms of glucose at any rate, are unimportant to these plants. Examination of various 'fitness' characters, apparently not directly related to chemical defence, shows unexpected lack of trade-offs. In some situations there is even a negative cost for chemical defence: a good example of having the bun while retaining the penny.

Notes:

ENVIRONMENTAL CONSTRAINTS ON THE EXPRESSION OF CONSTITUTIVE AND INDUCED WHEAT CHEMICAL DEFENSES

Ernesto Gianoli and Hermann M. Niemeyer, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

The effects of temperature and photoperiod on constitutive and induced levels of hydroxamic acids (Hx) on wheat (*Triticum aestivum* L.) seedlings were evaluated under laboratory conditions. Induction of Hx was generated by controlled infestation by the bird cherry-oat aphid, *Rhopalosiphum padi* L. Constitutive levels of Hx were significantly affected by temperature; a non-significant trend was found for the photoperiod effect. Induction of Hx was also significantly affected only by temperature; no clear trend was found for the photoperiod effect. The significant effect of environmental conditions on growth rate of seedlings and the significant correlation between growth rate and both constitutive and induced levels of Hx, suggested that environmental effects on such defenses are partially mediated through their effect on plant growth rate. Interestingly, temperature affected constitutive and induced Hx levels in opposite ways, suggesting a tradeoff between such defenses on wheat seedlings depending on environmental conditions.

Notes:

THE CHOICE AND PERFORMANCE OF *CALLIMORPHA DOMINULA*, *CYLINDROTOMA DISTINCTISSIMA*, *OREINA CACALIAE* AND *O. SPECIOSISSIMA* (TWO GENERALIST AND TWO SPECIALIST HERBIVORES ON SENECEONEAE PLANTS) TOWARDS CACALOL AND SENECIPHYLLIN, A SESQUITERPENE AND A PYRROLIZIDINE ALKALOID FOUND IN *ADENOSTYLES ALLIARIAE* AND *A. ALPINA* (ASTERACEAE, SENECEONEAE)

Bernd F. Hägele and Martine Rowell-Rahier, Institut de Zoologie, Université de Neuchâtel, Switzerland

Potential deterrent or attractive effects of two chemicals (cacalol, seneciphyllin and the combination of both) were tested in a first set of three experiments in standard leaf disk choice experiments.

In a second set of experiments the same compounds were force fed to all species by painting them on leaf disks which were fed to the larvae over a period of ten to 15 days. The effects on the performance of the four species could be assessed by comparing the growth of the groups fed the leaf disks treated with the chemicals to those fed with disks treated with solvent only.

Larvae of the specialist leaf beetles *O. cacaliae* and *O. speciosissima* showed neither an attractive nor a repellent reaction towards the two chemicals. The larval growth of these species was not influenced by the experimental treatments.

Larvae of the generalist *C. distinctissima* (Diptera, Tipulidae) were repelled by the combination of cacalol and seneciphyllin in the high concentration treatment. All other treatment showed no effect on larval food choice. Larval growth however was not different between the treatments.

Caterpillars of the generalist *C. dominula* (Lepidoptera, Arctiidae) were attracted by seneciphyllin and repelled by cacalol. The combination of both was attractive to the larvae. In their growth only the groups which were fed seneciphyllin and cacalol differed significantly from each other.

Notes:

VOLATILE AND NON-VOLATILE CHEMICAL BASES FOR GYPSY MOTH, *LYMANTRIA DISPAR*, LARVAL REJECTION OF GREEN ASH, *FRAXINUS PENNSYLVANICA*, FOLIAGE AS FOOD

Ingrid Markovic and Dale M. Norris, Department of Entomology, University of Wisconsin-Madison

A major objective of this study was to contribute significantly to the understanding of the roles of chemicals in non-host decisions by extremely polyphagous insects. The studied tree, green ash (*Fraxinus pennsylvanica*), is one of the few tree (plant) species rejected by the generalist, gypsy moth (*Lymantria dispar*). Findings indicate that volatile green ash chemistries (e.g., linalool) are repellent and/or deterrent to gypsy moth larvae (GML) as measured by locomotory orientation to Super Q trapped and isolated ash chemicals. Results further demonstrated that such locomotory repulsion can prevent larvae from experiencing the severe negative (lethal) effects of even "test consumption" of ash tissues. This situation should be adaptive to the generalist because net effects would be less expenditure of energy in pursuing a negative, even lethal, plant destination and a greater channeling of larval dispersal to the host(s). Non-volatile green ash chemicals (e.g., ethyl acetate extractables) are perceived only upon GML arrival at, and consumption of, the plant tissue. Their effects thus are usually expressed as reduced feeding and/or disrupted ingestion, digestion, absorption and metabolism by the affected larvae.

Finally, our findings showed that the effects of volatile and non-volatile green ash chemistries are either larval repulsion (repellency) or highly detrimental limited intake and utilization of green ash food by the insect. The combined ash chemistries thus protect this plant from significant herbivory by this extreme polyphage.

Notes:

HISTOLOGICAL LOCALIZATION OF SECONDARY METABOLITES IN RELATION TO THEIR PROTECTIVE ROLE FOR THE PLANT

Alicja M. Zobel, Chemistry Department, Trent University, Peterborough, Ont. CANADA K9J 7B8

Some secondary metabolites (allelochemicals, phytoalexins) play a protective role in the plant which produces them. They can be antimicrobial agents, antifeedants, screens against ultraviolet radiation, and agents preventing germination immediately after seed maturation. They can be located either outside a plant (over the cuticle or in the surrounding air), or inside a plant. Inside the plant these compounds are located either in a water environment or in an air compartment. Compounds soluble in water, mostly glucosides, fill the hydrophilic part of the vacuole, which can contain as well hydrophobic part, filled with lipid-soluble secondary metabolites. Some secondary products (mostly aglycones) are extruded through the plasma membrane onto the surface of the cell and prevent microbial invasion, which could occur either in the intercellular spaces or on the surface of the plant. Epicuticular deposits as antimicrobials are antimicrobial, but, as they absorb ultraviolet, they can act as screens against UV radiation. Deposits of antimicrobial agents on the embryo offer protection against microbes and also prevent a seed from germinating under sub-optimal conditions.

Notes:

SOME FACTORS AFFECTING THE ANTIXENOTIC RESISTANCE IN CRUCIFERS TO FLEA BEETLES, *PHYLLOTRETA* SPP.

P. Palaniswamy, Agriculture and Agri-Food Canada, Winnipeg Research Centre, 195 Dafoe Road, Winnipeg, Canada, R3T 2M9

Flea beetles, *Phyllotreta cruciferae* (Goeze) and *P. striolata* (F.), are the two most serious pests of canola in Canada. A number of cruciferous plant species with varying degrees of antixenotic resistance to flea beetles were identified. Laboratory tests were conducted to determine the effect of water stress on flea beetle feeding in flea beetle resistant and susceptible crucifers. Flea beetles were allowed to feed on plants in choice arenas containing wilting and non-wilting plants and the density of beetles on plants and the amount of feeding damage were estimated. More number of beetles were found on non-wilting than on wilting plants but the feeding damage was always greater on wilting than non-wilting plants. On stinkweed (*Thlaspi arvense* L.), a crucifer which is highly antixenotic, the beetles feed more readily on excised than intact leaves. False flax, *Camelina sativa*, is even more antixenotic than stinkweed and almost no flea beetle feeding occurs on young leaves of this plant. Noticeable amount of feeding damage, however, could occur on senescent leaves.

Notes:

INSECT HERBIVORES THAT COPE WITH EXTREME STRESS OF PHYTOTOXIC HOST PLANTS

J. Thor Arnason, Gabriel Guillet, R. Aucoin and B.J.R. Philogène, Department of Biology, University of Ottawa, Ottawa, Ont. Canada K1N 6N5

Plants of the Asteraceae and Hyperaceae possess secondary compounds that induce photooxidation in insect herbivores that consume them, with a well established mode of action that involves the peroxidation of membrane lipids. Some specialist herbivores, such as *Chrysolina* spp. on *Hypericum perforatum* or *Chlorochlamys chloroleucaria* on *Rudbeckia hirta* counteract these defences by complex behaviors, such as night feeding, avoidance of phototoxic plant parts and attaching cut leaves to their dorsum. These and other herbivores have the ability to detoxify the phototoxic secondary substances. The PSMO systems involved, the metabolic products and the new p-glycoprotein based toxin pump have now been described. Dietary antioxidants (β -carotene, α -tocopherol, ascorbate) are additional defences against phototoxicity. They reduce mortality in herbivores exposed to phototoxins and some specialist herbivores have higher constitutive levels. Adapted specialist insects also have higher constitutive levels of superoxide dismutase (SOD) and respond to phototoxins in their diet by induction of catalase (CAT), glutathione reductase (GR) and reduced glutathione (GSH). Artificial inhibition of the enzymes SOD and CAT had little effect on phototoxicity but inhibition of GSH synthesis in herbivores enhanced photooxidative effects of administered phototoxins on lipid peroxidation. While insects have many mechanisms to overcome plants photooxidants, the Asteraceae appear to have a second group of secondary substances, the sesquiterpene lactones, that can attack antioxidant defences and synergise phototoxins.

Notes:

Symposium 6: Multitrophic interactions

PLANT CHEMISTRY AND NATURAL ENEMIES OF APHIDS

H. F. van Emden, Department of Horticulture and Landscape, School of Plant Sciences, The University of Reading, Whiteknights, Reading, Berkshire, RG6 6AS, U.K.

Of the several pathways by which plant chemistry affects the natural enemies of aphids, the two most obvious are either that the aphid picks up chemicals from the plant which directly affect the natural enemy or that the impact of the natural enemy on the aphids is affected by modifications of its behaviour caused by chemicals in plants. Sometimes, secondary plant chemicals can be toxic to insects, though the mode of feeding of aphids may enable them to avoid or greatly reduce contact with such substances. When not so avoided, toxic plant chemicals may adversely affect the natural enemies, although high levels in the aphids may sometimes enable the natural enemies to detect and avoid such toxic prey.

Non-toxic plant chemicals are also important in relation to natural enemies. Thus lower levels of nutrients in plants will affect aphid size, which has deleterious effects on the fecundity and size of parasitoids, though the impact of both predators and parasitoids can also actually be increased. Non-toxic plant chemicals encountered while feeding as larvae within aphids are also 'remembered' by parasitoids in selecting between host plants (even cultivars of the same crop) when searching for prey, and semiochemicals used by many predators for locating aphids and which occur in the honeydew of aphids have a plant origin. There is also evidence that the nutrition of aphids may influence their ability to produce alarm pheromone in defence against natural enemies.

There appears to be a basic contrast in the effect of toxic and non-toxic plant chemicals on natural enemies which is highly relevant to the use of transgenic plant resistance against aphids. Also, the intense study in the past on chemical aspects of aphid-host plant relationships clearly needs extending to the third trophic level if it is to be truly relevant to aphid population dynamics.

Notes:

INFLUENCE OF DIFFERENT WHEAT AND OAT CULTIVARS ON THE DEVELOPMENT IN THE CEREAL APHID *SITOBION AVENAE* OF THE CEREAL APHID PARASITOID *APHIDIUS RHOPALOSIPHI* AND THE GENERALIST PARASITOID *EPHEDRUS PLAGIATOR*

Fuentes-Contreras, J.E.^{1,2}, Powell, W.², Wadhams, L.J.², Pickett, J.A.² and H.M. Niemeyer¹, ¹Laboratorio de Química Ecológica, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile; ²IACR-Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, UK

The effects of three wheat cultivars and two oat cultivars in the development of the cereal aphid parasitoid *Aphidius rhopalosiphi* De Stephani-Pérez and the more generalist *Ephedrus plagiator* Nees (Hymenoptera: Braconidae) were evaluated in the laboratory. The parasitoids were reared in *Sitobion avenae* (F.) (Homoptera: Aphididae), using plants grown in greenhouse conditions. *A. rhopalosiphi* showed longer developmental time on wheat cultivar T-1500 than on the two oat cultivars. This effect was accounted for by a significant increase in the time from oviposition to mummy formation, while the duration of the parasitoid pupal stage remained constant between treatments. No further effects were observed in other variables evaluating performance, such as adult longevity, adult body weight and secondary sex ratio. The generalist *E. plagiator* did not show significant differences in any of the analyzed variables between cultivars or cereal species. The levels of hydroxamic acids, secondary metabolites involved in plant resistance against cereal aphids, were measured in the different cultivars as they are known to affect the mean relative growth rate of cereal aphids. These levels correlated with the observed pattern in the developmental time of *A. rhopalosiphi*. The results are discussed in terms of tritrophic effects and the development of breeding programs trying to improve plant resistance to aphids.

Notes:

APHID CHEMICALS AFFECTING FEEDING BEHAVIOUR OF A LADYBIRD BEETLE

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A ladybird beetle species, *Harmonia axyridis*, feeds on a variety of aphid species during its larval and adult stages. Aphid extracts were found to contain chemical factors affecting the coccinellid feeding behavior.

A hexane rinse of an aphid, *Megoura crassicauda*, contained a complex mixture of chemicals which induced searching and biting responses of the adult beetle, in which (E)- β -farnesene and a series of triglycerides containing sorbic acid moiety were characterized as parts of such chemical factors. Several sugar components were also identified as feeding stimulants from the water soluble fraction of a whole body extract of *M. crassicauda*.

In contrast, *H. axyridis* strongly rejected a Rubiaceae-feeding aphid, *Acyrtosiphon nipponicus*. A potent deterrent against the beetle was isolated from the aphid and identified as paederoside, an iridoid glycoside originating from the aphid's host, *Paederia scandens*. Upon predatory attacks, the aphids quickly secreted a high concentration of paederoside as droplets from the cornicles, and thus successfully deterred feeding of *H. axyridis*.

Notes:

CHEMICAL MIMICRY OF APHID PARASITOIDS OF THE GENUS *LYSIPHLEBUS* (HYMENOPTERA, APHIDIIDAE)

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Species of the family Aphidiidae are solitary endoparasitoids of aphids. When searching for host aphids these wasps often face the problem that their host colony is associated with ants that collect the honeydew droplets excreted by the aphids. This trophobiotic relationship between aphids and ant confers some advantages for the aphids, among other things protection against predators and parasitoids. The majority of aphidiid wasps is recognized and killed by ants when encountered in the aphid colony. However, members of the genus *Lysiphlebus* are able to exploit this rich resource for reproduction. Ants do not molest *Lysiphlebus*, they either totally ignore the parasitoid or only tap it briefly with the antennae. Moreover, this characteristic ant behavior does not depend on the ant species. These field observations put forward the hypothesis that *Lysiphlebus* uses chemical mimicry to be invisible for ants.

Chemical analysis of the cuticle extracts of the aphidiid parasitoid and the respective host aphid revealed that *Lysiphlebus* imitates the cuticular hydrocarbon profile of its host. This strategy shall be demonstrated in detail for some *Lysiphlebus* species.

The majority of the Central European *Lysiphlebus* species is monophagous, specialized on only a single host aphid species. In these cases they mimic the hydrocarbon pattern of the respective host. In contrast, parasitizes aphids of even different genera. Chemical analysis showed that *L. fabarum* is able to adapt its chemical signature due to the host species from which it eclosed. How this adaptation can be achieved is still unknown.

Notes:

GLYCINE MAX INTRINSIC AND EXTRINSIC CHEMICAL DEFENSE AGAINST HERBIVORY

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Tritrophic interactions among plants, herbivores and natural enemies of the herbivore are a major focus in chemical ecology. Information regarding the absolute sources of volatiles in tritrophic interactions has remained scarce. We now report here that if the host plant provides a precursor, apparently catechol, in the food of *Pseudoplusia includens*, it then involuntarily releases a kairomone, guaiacol, in its feces, which cues the specialist parasitoid, *Microplitis demolitor*, so it finds its host *P. includens* larva. Further results indicated a positive correlation between the levels of a plant's intrinsic (direct) and extrinsic (indirect) chemical defenses against the herbivore. When the herbivore was fed artificial diet that lacked the precursor of guaiacol, none of the kairomone was detected in the feeding larva's feces. Our current understanding supports the interpretation that both types of chemical defense in *G. max* probably involve a common phenolic precursor, catechol. The presence of one or more attractive volatiles, e.g., guaiacol, in the feces of an oligophagous-polyphagous insect regardless of which plant species or cultivar it is eating, could provide host insect-specific cues to the searching specialist parasitoid. This situation should be evolutionarily advantageous for a host-specific parasitoid such as *M. molitor*.

Notes:

CHEMOECOLOGY IN PLANT-HERBIVORE-PARASITOID INTERACTIONS. *ARISTOLOCHIA ARCUATA* x *BATTUS POLYDAMAS* x *AREOSCELIS RUFA*

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Larvae of the aposematic butterfly *Battus polydamas* (Lepidoptera: Papilionidae: Troidini) feed on leaves of *Aristolochia* (Aristolochiaceae) and are parasitized by ichneumonid wasps *Areoscelis rufa*, that show a tight association with this butterfly species. Experiments on the attraction of *A. rufa* to *Aristolochia arcuata*, carried out in an olfactometer, showed that the wasps were attracted preferentially to intact leaves of larval host plant, Troidini larvae, and troidine larvae on leaves in relation to the controls (larvae of a generalist noctuid moth *Anticarsia gemmatalis* and leaves of *Psidium* sp.-Myrtaceae)(n=10 for each trial). In the first experiment, after finding the leaves, the wasp searches intensively for larvae; in the other two the wasp rapidly parasitizes the larva after finding it. Analysis of volatiles of the intact leaves and larvae (adsorbed in Porapak Q) showed a presence of a great variety of sesquiterpene hydrocarbons [M⁺ 204] in both. Preliminary experiments showed that the wasps were also attracted preferentially to dichloromothane extracts of intact leaves of *A. arcuata*, which also showed almost the same sesquiterpenes present in the Porapak Q extract. These results suggest that the wasps are initially attracted by the chemical environment of the host plant and then search for and find the larval host by visual or chemical cues.

Notes:

Symposium 7: Pheromones: isolation, mechanisms, uses

WHY IS PHEROMONE PERCEPTION SO SPECIFIC?

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Pheromone specificity in the Lepidoptera is encoded in protein components of the antennal sensillum lymph and dendritic membrane. Recent work on the molecular determinants of pheromone binding affinity of pheromone binding proteins (PBPs) of three genera are highlighted. Several new cDNA sequences for *Lymantria dispar* (Lymantriidae) and *Agrotis segetum* (Noctuidae) PBPs are described and their phylogenetic significance will be discussed. Bacterially-produced recombinant PBPs have allowed structural studies of PBP-pheromone complexes. First, the ligand binding site of Apol-3 from *Antheraea polyphemus* was identified using photoaffinity labeling. This protein has been produced in ^{13}C -, ^{15}N -labeled form for multidimensional NMR structural determination. Second, a novel binding assay was developed to measure PBP-ligand binding affinities. This assay has been used to compare Aper-1 and Aper-2, two recombinant PBPs from *Antheraea pernyi*. Our results support a model in which ligand specificity for chain length, double bond position, and terminal functionality are partially encoded in the PBPs. Third, the subtle amino acid sequence differences have allowed us to prepare PBP-specific antibodies, which can be employed to determine the location of PBPs specific for a given ligand in a single sensillum. Finally, we propose a model in which the final decoding is accomplished when the PBP-pheromone complex activates a G-protein-coupled seven-transmembrane domain receptor. In this model, the dendritic receptor protein contains recognition sites for both the presenting PBP and the presented pheromone.

Notes:

SEX PHEROMONE STUDIES IN THE DESERT LOCUST *SCHISTOCERCA GREGARIA* (FORSKAL) [ORTHOPTERA: ACRIDIDAE]

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Long-range attraction of male *Schistocerca gregaria* in the solitarious phase has been previously shown to be due to olfactory cues emitted by the females (Inayatullah *et al.* 1994. Environ. Entomol. 23:1544-1551). Volatile emissions of adult males and females of solitaria were compared using gas-chromatographic-electroantennographic detection (GC-EAD). The volatile emissions of 3-4-week-old females contained five EAG-active peaks which elicited higher EAG activity than those contained in the emissions of younger (2-3-week old) and older (4-5-week-old) females. Two of these peaks were detected in the volatile emissions of males. The three female specific EAG-activity peaks evoked higher EAG amplitudes in males than females. Retention time matches, GC-mass spectrometry and GC-EAD analyses of authentic compounds revealed the two common compounds as phenol and guaiacol, one of the female specific components as (E,Z)-2,6-nonadienal and the other two aliphatic unsaturated compounds with unconfirmed structures. The natural female-produced pheromone is currently being elucidated in behavioral assays with blends of the identified components. The potential application of the pheromone in the prognosis of outbreaks will be discussed.

Notes:

STUDY OF THE AGGREGATION PHEROMONE OF THE BANANA WEEVIL *COSMOPOLITES SORDIDUS* (GERMAR)

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The banana weevil *Cosmopolites sordidus* (Germar) is one of the main pest in most of banana plantations in the world. In order to isolate and identify the aggregation pheromone, volatile compounds emitted by stimulated insects were trapped on adsorbent polymers Porapak-Q and Supelpak-2. An air stream was drawn 10 minutes per hour during 15 days, into flasks with and without insects. Compounds were desorbed from the polymers with acetone and bioassays were made to confirm biological activity. Extracts were analyzed by gas chromatography - mass spectrometry (GC-MS). A methyl-branched nonenol was tentatively identified as one of the aggregation pheromone components.

Notes:

(Z,E)- α FARNESENE - A SEX/AGGREGATION PHEROMONE COMPONENT OF THE MALADERA MATRIDA BEETLE

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Field trapping experiments and laboratory olfactometer bioassays revealed that (Z,E)- α -farnesene is a component of a sex and/or aggregation pheromone in the *Maladera matruda* Argaman beetle (Coleoptera, Scarabaeidae). This beetle, recognized as a serious polyphagic pest, was first detected in Israel in 1983 and was classified as a new species. *M. maladera* beetles are highly attracted by live virgin females as well as by volatiles collected from the females in the presence of food (cut peanuts leaves). It has been also shown that (Z,E)- α -farnesene is an electroantennogram-active component of the *M. matruda* volatiles. In the study we again found that the best attractant in the field is live females with food; the second best category of baits included (at the same significance level, i.e. $p < 0.001$ Anova): synthetic (Z,E)- α -farnesene (in a mixture with other isomers of farnesene) with food; live females (without food); and eugenol, used as attractant for monitoring of the pest. All other baits were less attractive. The same order of activity was obtained in laboratory olfactometer studies.

Notes:

SCARAB BEETLE *ANOMALA JAPONICA* UTILIZES A MORE COMPLEX SEX PHEROMONE BLEND THAN A SIMILAR SPECIES, *A. CUPREA*

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Although some scarab beetles, namely *Anomala cuprea*, *A. octiescostata*, and *A. albopilosa sakishimana*, utilize the same sex pheromone blend (buiuilactone+japonilure), cross-attraction among these species has not been observed because the scarabs are geographically and/or seasonally isolated. We have now investigated the pheromonal communication of *A. japonica* ("yamatoaodogane" in Japanese), a similar species that shares the same habitat and has a common flight season to *A. cuprea*. The fact that *A. japonica* utilizes a distinct sex pheromone blend (buiuilactone, 2-E-nonenol, 2-E-nonenal, and methyl benzoate), having only the major component in common, suggests that pheromonal difference may be involved in speciation.

Notes:

CHEMICAL COMMUNICATION SYSTEM OF THE SUGARCANE WEEVIL, *METAMASIVUS HEMIPTERUS*

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In the tropical Americas the West Indian sugarcane weevil (WISW), *Metamasius hemipterus*, is a moderate pest of sugarcane and has recently been implicated as a vector of red ring disease in oil palm. In most palm plantations red ring disease is managed by removal of infected palms and mass trapping its major vector, *Rhynchophorus palmarum*. We have developed a pheromone-food trap which captures both red ring nematode vectors.

Coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses and coupled GC-mass spectrometry (MS) of volatiles produced by male and females West Indian sugarcane weevils (WISW) revealed eight male specific, EAD-active compounds: 4-methyl-5-nonanol, 2-methyl-4-heptanol, 2-methyl-4-octanol, 3-pentanol and the corresponding ketones. In field experiments in Florida, the quaternary blend of alcohols in combination with sugarcane was most attractive. Addition of the ketones or replacement of alcohols with ketones significantly reduced attraction.

Fields tests in Costa Rica examined the attraction of alcohols singly and in all binary, ternary and quaternary combinations. 4-Methyl-5-nonanol (1) was the major aggregation pheromone equally attracting male and female WISW. Stereoisomeric 1 and 4*S*,5*S*-1, the only isomer produced by WISW, were equally attractive. Addition of 4*S*,4*R*- or (±)-2-methyl-4-heptanon (2) to 4*S*,5*S*-1 slightly enhanced attraction. Sugarcane stalks in combination with 1 plus 2 (8:1 ratio) was highly synergistic, whereas EAD-active sugarcane volatiles ethyl acetate, ethyl propionate or ethyl butyrate only moderately increased attraction of the pheromone lure.

Food-baited traps containing aggregation pheromones for both *M. hemipterus* and *R. palmarum* capture both species as efficiently as traps in which the pheromones of these species are presented separately.

Notes:

THE AGGREGATION PHEROMONE OF THE COCONUT RHINOCEROS BEETLE, *ORYCTES RHINOCEROS*

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The rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) is an important pest of coconut and oil palms in South East Asia. Adult beetles burrow into the growing point of the palm and feed on unopened fronds, causing reduction in photosynthetic area which decreases and delays fruit production. Prolonged attacks can kill mature palms and young palms may be killed if the growing point is destroyed. Wounds produced by the beetle provide entry points for lethal budrot and palm weevils. We have identified a male-produced aggregation pheromone which effectively lures rhinoceros beetles to traps.

Walking olfactometer bioassays were used to determine that male *O. rhinoceros* beetles produce volatiles that are attractive to both sexes. Female *O. rhinoceros* produce volatiles that are attractive to males but unattractive to females. Gas chromatographic-electroantennal detection reveals three male-produced volatiles elicited significant antennal response in both sexes. These were identified as ethyl 4-methyloctanoate, ethyl 4-methylheptanoate and methyloctanoic acid. Only ethyl 4-methyloctanoate was attractive in olfactometer bioassays and was attractive to both sexes. In field experiments only the 4-isomer was biologically active. Tests with buried bucket or ground level vanned bucket traps revealed that the latter were significantly more efficient. Vanned bucket traps from which ethyl 4-methyloctanoate was released were significantly more attractive than those baited with ethyl 4-methylheptanoate, or methyloctanoic acid or ethyl chrysanthemumate. Addition of ethyl 4-methylheptanoate or methyloctanoic acid to ethyl-4-methyloctanoate did not increase attractiveness. In ground level vanned bucket traps but not in pitfall traps decaying empty oil palm fruit bunches synergize attraction to ethyl 4-methyloctanoate.

Initial field tests indicate that pheromone-baited vanned-bucketed traps at densities of less than 1 per hectare can reduce populations of *O. rhinoceros* and reduce frond damage on young oil palm.

Notes:

RECEPTIVITY OF MALES FROM TWO HOST RACES OF THE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* TO DIFFERENT SEX PHEROMONE LURES

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It was recently reported that two distinct host races of the fall armyworm exist, one feeding on gramineae and the other on corn. However, while these races are frequently sympatric there appears to be very little interbreeding (<1%) in the field. Studies on the behavior of virgin females showed that during the first two days the calling windows are temporally separated. On subsequent nights, the calling periods overlap although the levels of pheromone from gland extracts differ significantly between the two races. Work was therefore undertaken in a wind tunnel to determine if there were inter-strain differences in (i) the diel periodicity of male receptivity and (ii) the response of males to a range of concentrations of the two and four component pheromone blends that have been recommended for monitoring field populations of the fall armyworm.

The highest level of response for each host race coincided with the calling window of their respective females. However, the most striking feature of the study was the extremely poor response of corn strain males, regardless of their age and the pheromone lure used. The importance of these observations with respect to reproductive isolation in the two races, as well as the proper identification of the sex pheromone and the use of pheromone traps for monitoring this pest species, will be discussed.

Notes:

GC-EAD ANALYSIS AND CHEMICAL ECOLOGY OF THE WHITE PINE WEEVIL *PISSODES STROBI*

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White pine (*Pinus strobus*) is one of the most valuable saw timber species in the US; the potential would be greater, however, were it not for the damage caused by the white pine weevil, *Pissodes strobi* (Peck). Pines attacked by this species are reduced in both quantity and quality of the timber produced, and studies show that an average of 35-40 % of the trees in open grown plantations have been damaged. The chemical ecology of *P. strobi* (and sibling species, *P. nemorensis*) have been the subject of research for many years, yet there remains many unanswered questions concerning the role of secondary attractants in this species. Our interest in this species has led us to modify the traditional GC-EAD technique to work effectively on small insects such as bark beetles (e.g. *Ips pini*) and weevils (e.g. *P. strobi* and *P. nemorensis*). Our modification allows us to analyze chemical component derived either from aeration or hindgut extracts from *P. strobi*. Results show response to both host and weevil produced compounds; we are currently identifying pheromone components associated with this species. In addition, we are investigating the chemical relationship between the two sibling species, *P. strobi* and *P. nemorensis*.

Notes:

SEX PHEROMONES IN MARINE POLYCHAETES: HETEROSPECIFICITY OF CHEMICAL SIGNALS

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Most nereid polychaetes undergo a metamorphosis to ripe heteronereis stages and reproduce in the free water, performing a nuptial dance. Epidemic swarming of nereids is timed by environmental and endocrine control of maturation via spawning hormones, to occur simultaneously in a given population. The reproductive behaviour and the release of gametes into the sea water is controlled by sex pheromones which are located in the coelomic fluid of gravid specimens. Behavioural and electrophysiological assays with a number of nereid species such as *Platynereis dumerilii*, *Nereis succinea*, *N. virens* and *N. japonica* confirm that heterospecific activity of coelomic fluid occurs. In both sexes, the release of gametes is elicited when individuals are exposed to coelomic fluid of another species.

This heterospecific activity occurs due to similar or identical chemical signal molecules extractable from the coelomic fluid. HPLC analysis and biological assays show that all investigated species contain a 'cocktail of substances' of which they use perhaps only one or two species specific signals for the control of their reproduction, whilst the signals of other species may also be present and in some case in very high concentrations. Although all active extracts contain a biologically active tri-peptide glutathione, this substance is not the natural cue. Chemical characterisation of the active substances are under way and present results suggest relatively diverse molecules that are related to gamete maturation. Whilst heterospecific activity of signals may be advantageous for some coral species reproducing simultaneously *e.g.* at the Great Barrier Reef (see Babcock *et al.*, 1992), monotelic species, such as the nereids, could face severe disadvantages including waste of shedded gametes in response to false signals from other species. Heterospecific signals could also disturb other nereid species reproducing in the same area.

Present data show that other factors prior to emission of the 'gamete release' inducing pheromones must exist to ensure species recognition and isolation. This is achieved by environmental factors such as lunar periodicity, temperature, different locations, diurnal rhythmicity and reproductive behaviour. Other sex pheromones, such as the volatile sex pheromone 5-methyl-3-heptanone, also play a significant role triggering the nuptial dance behaviour prior to the release of gametes in *Platynereis dumerilii* and *Nereis succinea*. In these species, the use of species specific concentrations of the same pheromone prevents direct contact during reproduction.

Notes:



Symposium 8: General subjects in chemical ecology

THE ROLE OF SECONDARY METABOLITES AND GENE PRODUCTS IN THE HYPERSENSITIVE RESPONSE DEVELOPED BY HIGHER PLANTS UPON FUNGAL ATTACK.

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The hypersensitive response (HR) developed by plants within an incompatible interaction, involves the biosynthesis of phytoalexins as well as of several pathogenesis related proteins (PR). Both phytoalexins and PR proteins are *de novo* synthesized as part of the response.

The HR of *Citrus limon* seedlings, upon inoculation with *Alternaria alternata* is triggered by oligosaccharides released through the action of fungal endopolygalacturonase on the plant cell wall, signal that is recognized on the plasma membrane and that is initially transduced through calcium ions from the cell wall. These enter the plant cell through calcium channels, as has been demonstrated with calcium channel blockers.

The *de novo* synthesis of Phenylalanine ammonia-lyase (PAL) and the activation of the phenylpropanoid metabolism results in the synthesis of umbelliferone and scoparone, secondary metabolites that inhibit fungal growth. On the other hand, and at the same time period that these phytoalexins are synthesized, the activity of new isoenzymes of chitinases and of β -1,3-glucanases, are detected. These PR proteins hydrolyze the components of the fungal cell wall.

Then, the convergence of multiple defense mechanisms appears to be important in order to allow fungal control, preventing the development of disease, where both secondary metabolites such as phytoalexins and gene products as PR proteins play essential defense roles.

Supported by FONDECYT (157/88, 91/886 and 1940441)

Notes:

PAL ↑

* induced within 4 hours

No idea about N

Good
Talk

THE CHEMICAL BASIS OF THE RESISTANCE OF CEREAL SEEDS TO PATHOGENIC FUNGI

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Studying the genetic resistance of seeds from *Hordeum vulgare* and *Triticum aestivum* to pathogenic fungi like *Aspergillus niger*, *Penicillium* sp.; *Helminthosporium sativum* and *Helminthosporium theres* we found that the fatty acid and the 5-(n)-alkyl resorcinols fractions of the epicuticular waxes of the seeds were responsible for the observed behaviour. By designing a novel bioassay where the extreme lipophilicity of these compounds was taken into account, the antifungal properties of both fractions were evaluated qualitatively and quantitatively. The MICs ranged 2-10 $\mu\text{g}/\text{cm}^2$ in the alkylresorcinol case for the fungi tested. With these values in hand, the behaviour of the different cultivars against these fungi could be predicted by measuring the amount of resorcinols by TLC-Scanning densitometry. The ranking of cultivars thus established correlates very well with the field behaviour of each cultivar providing a very useful and quick method for predicting the behaviour against fungi of new varieties in development.

Notes:

VITAMINE E DERIVATIVES IN THE DEFENSIVE SECRETION OF PUPAE OF *EPILACHNA BOREALIS* (COLEOPTERA: COCCINELLIDAE)

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The pupae of insects, being sedentary, are particularly prone to predation. They can therefore be expected to possess chemical or other defenses to deter predators. Pupae of certain coccinellid beetles (genus *Epilachna*) are covered with secretory hairs, each beset with a minute, oily droplet. Bioassays with predatory ants (*Crematogaster* spp.) revealed the pupal secretion of the squash beetle (*E. borealis*) to be a potent contact deterrent. Subsequent chemical analyses showed the droplets to consist primarily of tocopheryl acetates, thus establishing *E. borealis* as the first known natural source of these acetates of vitamin E. Tocopheryl acetate, however, does not account for the secretion's defensive action: pure α -tocopheryl acetate failed to deter ants. We thus suspect that the tocopheryl acetates serve as a vehicle for an active component(s) that we are currently attempting to characterize.

Notes:

Cmgds are prob. cucurbitacins

Ruled out are the azamicrobicides

The tocopheryls
vehicles or antioxidants
or odor camouflages
so they cannot be
recognized.

Possible
Seminar
Darker
Visits Archbold
in March

THE MULTIFUNCTIONAL ROLE OF CANTHARIDIN

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* For the palaeartic pyrochroid *Schizotus pectinicornis* L. (Coleoptera: Pyrochroidae) the multifunctional role of the terpenoid cantharidin will be described:

1. Aggregation pheromone: Both sexes of *Sch. pectinicornis* are canthariphilous and can be attracted in the field with cantharidin baits. Cantharidin contents of these individuals were determined by means of quantitative gas chromatography.
2. Paternal investment: Feeding and copulation experiments with [$^2\text{H}_2$]cantharidin indicate that males transfer cantharidin to females at copulation. Thus, males of *Sch. pectinicornis* invest in the protection of their offspring by cantharidin gifts to the mate.
3. Sex pheromone: Cantharidin plays an important role in mating behaviour of *Sch. pectinicornis*. Females select those males for copulation which have a high cantharidin content.
4. Feeding deterrent: To ants of the genus *Myrmica* which live sympatric with developmental stages of *Sch. pectinicornis* under the bark of dead trees cantharidin serves as feeding deterrent.

Notes:

Aggregation
♀ choice in mating
Protection against predaceous ants.

MATERNAL PEDERIN TRANSFER IN *PAEDERUS* ROVE BEETLES AND ITS ECOLOGICAL SIGNIFICANCE

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Rove beetles of the genus *Paederus* (Coleoptera: Staphylinidae) have a well-known toxin in their hemolymph, which is called pederin. The complex chemical structure of this amide has been elucidated in the past but its significance for *Paederus* remained obscure. In order to investigate this open question, the pederin content of individual specimens representing the whole life cycle of *Paederus riparius* was quantified. Most females are obviously able to biosynthesize pederin as they accumulate this substance and transfer small amounts into their eggs. Some females, however, do not transfer pederin into the eggs and likewise do not possess the amide after the egg laying period. If such female is fed with pederin, she can also endow her eggs with the substance until depletion of the resource. So, it is a certain biosynthetic capability that is lacking in these females.

Hatching larvae store the pederin load supplied by their mother. As they do not increase their pederin content, the difference between larvae with and without pederin is preserved until imaginal eclosion. After eclosion, an increase of pederin could be shown in females, while in males the amount of the amide found per specimen never extends the quantity transferred maternally.

The polymorphism of females enables to test the reaction of arthropod predators on offspring with and without pederin. Among the potential predators collected from *P. riparius* sites, insects generally do not respond to pederin present in the prey. *Paederus* larvae might often escape by running away from these predators because they are very agile. In case of sudden attacks by (wolf) spiders, larvae never have any chance to escape. The spiders, however, are deterred by prey that possesses pederin. They turn away from already captured larvae and show cleansing behavior. *P. riparius* larvae containing pederin survive the attacks of spiders without damage, whereas larvae without pederin are often killed and eaten. Larval survival thus depends on chemical defense, which is provided by most females.

Notes:

THE BEHAVIORAL RESPONSES OF *Aedes aegypti* (L.) (DIPTERA: CULICIDAE) TO CARBON DIOXIDE PLUMES

Alvaro E. Eiras¹ and Paul C. Jepson², ¹Setor de Semioquímicos, CCTA, Universidade Estadual do Norte Fluminense (UENF), Campos, RJ, Brazil; ²Department of Entomology, Oregon State University, Oregon, USA

The behavioral responses of female *A. aegypti* to constant and pulsed carbon dioxide stimuli of varying concentration, were studied in a wind tunnel bioassay. Activation (take-off) was observed at threshold increases in concentration over ambient of 0.01 - 0.02%. This and the subsequent trend in take-off with increasing carbon dioxide concentration were consistent with the known threshold and pattern of receptor response. Mosquitoes exhibited straight, upwind flights in response to low carbon dioxide concentrations, whereas "zig-zag" upwind flights occurred in response to high concentrations. They responded similarly to continuous and intermittent carbon dioxide stimulation, contradicting previous findings.

Notes:

CHEMICAL ECOLOGY OF THE NEW WORLD SANDFLY *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE)

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The sandfly *Lutzomyia longipalpis* is the the only proven vector of visceral leishmaniasis in South and Central America. Recent work has shown that inter and intraspecies interactions are modulated by a number of chemical cues.

Male produced sex pheromones released from abdominal glands attract females for mating. These compounds have recently been identified as novel homosesquiterpenes 3-methyl- α -himachalene and 9-methylgermacrene-B in two members of the species complex. A diterpene, which has been shown to be a cembrene, is produced by a third member of the species complex. Attraction of females to males is synergised by the presence of host odours which act as attractants for both males and females. Different members of the complex show differing degrees of attraction/repellancy to human odour which in turn can be more or less attractive/repellant depending on the individual from which the odour is derived. Differences in attractancy/repellancy may be important in determining risk factors for leishmania transmisson.

Females also place an oviposition pheromone on newly oviposited eggs which in combination with apneumones from decaying plant material attracts other gravid females. Larva and pupae are vulnerable to predation and evidence suggests that they may protect themselves by a combination of mechanical (behavioural) and chemical means.

Notes:

1. Sex pheromones - sesquiterpenes + diterpene
2. Oviposition attractants
3. Host attractants (i.e. human)
4. Defensive strategy of immature stages

CHEMICAL DEFENSES OF BRAZILIAN OCTOCORALS

Rosângela de A. Epifanio¹, William Fenical² and Lenize F. Maia³.¹ Departamento de Química Orgânica, Instituto de Química, Universidade Federal Fluminense, 24020-150, Niterói, R.J. Brasil; ²Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA, 92093-0228, USA; ³Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, R.J, Brasil

The antipredatory chemical defenses of Octocorals collected in Rio de Janeiro, Brazil, were investigated. The crude extracts of the Telestacea *Carijoa riisei* and the Gorgonacea *Lophogorgia punicea*, *Lophogorgia violacea*, *Heterogorgia uatumani* and *Phyllogorgia dilatada* were incorporated into food pellets and tested in the field. All the extracts showed statistically significant feeding avoidance against generalist fishes.

Our studies comprising isolation, structure elucidation and bioassays, allowed us to identify the active metabolites of *Lophogorgia violacea* and *Heterogorgia uatumani*. Although several correlated diterpenes were isolated from *L. violacea*, only lophotoxin showed potent feeding deterrent activity. On the other hand, the chemical defense against predators of *H. uatumani* is due to the presence of a new tetracyclic sesquiterpene and a known eunicelane diterpene.

Notes:

SEMIOCHEMICALS MEDIATING OVIPOSITION-AGGREGATION IN THE GREGARIOUS DESERT LOCUST, *SCHISTOCERCA GREGARIA*

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A chemical signal, originating from the froth of egg pods attracts gravid female *Schistocerca gregaria* (Orthoptera:Acrididae) to common egg laying sites. Behavioural experiments indicated that females preferred to oviposit in moist sand contaminated with froth (60% egg laying vs. 34% in sterilized sand). Extracts and volatiles collected from froth were also attractive to gravid females. In fact, the latter were the most attractive with 80% egg laying occurring in sand contaminated with froth volatiles. Results with froth extracts obtained by sequential extraction with solvents of increasing polarity suggest that both non-polar and polar compounds are involved in the attraction of gravid females. Electroantennogram recordings with extracts and volatiles collected from froth confirmed the presence of olfactory receptors on the antennae that are responsive to compounds in the extracts and the volatile collections. Two electrophysiologically active compounds were identified using GC MS as acetophenone and veratrole. Both the compounds in behavioural bioassay attract gravid females, however no synergistic effect was observed.

Notes:

THE ECOLOGICAL ROLE OF *CLUSIA* FLORAL RESINS AND THEIR DETAILED CHEMICAL COMPOSITION

Cecília M.A. de Oliveira², André L. M. Porto¹, Volker Bittrich³ and Anita J. Marsaioli¹. ¹Instituto de Química, UNICAMP, CP 6154, Campinas 13081-970, SP, Brazil, ²Instituto de Química e Geociências-UFG, Goiás; ³Instituto de Biologia, UNICAMP, Campinas, SP Brazil

Guttiferae, a family of mainly tropical plants is represented in Brazil by 18 genera and about 200 species most of which are woody. The plants of the family are generally characterized by the presence of latex in most of their tissues. Several *Clusia* spp. offer floral resins as a reward for pollinators¹. However, little is known about the detailed chemical composition of *Clusia* floral resins and oils and their role in the pollination process. The same is true for the latex' chemistry. From preliminary investigations on the chemistry of floral resins, the presence of triterpenes /1/ and or poliprenylated benzophenones² was suggested, but the characterization had insufficient information for a structural determination of the resin composition. In spite of the absence of more detailed structural data, ecologists have raised several questions about *Clusia* floral resins and their use by bees¹. These resins, in contrast to other (non-floral) resins (e.g., *Pinus*, which solidifies rapidly by oxidation and polymerization) stay plastic for several weeks after secretion.

This paper presents the detailed chemical composition of seven *Clusia* floral resins and lateces reporting the structure of novel polyprenylated benzophenones. These *Clusia* are cultivated in "Fazenda Santa Elisa", Instituto Agronômico, Campinas, SP, Brazil. The connection between pollinating insects and the resins was also chemically investigated.

1. W.S. Armbruster, The Role of Resin in Angiosperm Pollination: Ecological and Chemical Considerations. Amer. J. Bot. 1994, 1149-1160.
2. S.A.T. Barberan, F.E. Ferreres and S.T. Lorenti, *Phytochemistry* 1993, 34, 191-196.

Notes:

NEOTROPICAL BRACKEN FERN: ARE ESTABLISHMENT STRATEGIES DEPENDENT UPON PLANT VARIETY?

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Bracken fern is one of the five most successful plants in the world. The two varieties, *caudatum* and *arachnoideum* found in the neotropics, are also dominant components of plant communities of mountain savannahs and the edges of mesophile forests, specially in the early and middle successional stages. A detailed study of these two varieties was undertaken based on the assumption that, if sufficiently well understood, bracken fern would be an appropriate model of study of the various strategies that prevalent plants possess, including their chemical arsenal, to achieve such degree of success and how these strategies are integrated to reduce the costs of competition and defense. With this view, the comparative phenology expressed as rate of growth, density, and aerial and underground biomass recorded for these plants in the northern Andes, gave widely differing values for the two varieties of tropical bracken. Other differences including altitudinal distribution density and chemical composition of selected defensive compounds -class and amounts- were also found. These variations suggest profound contrasts in encroachment strategies of the two varieties that may be even larger than the expected differences between related plant species.

Notes:

One of 5 most successful plants in world,
None in Chile.

- genetic diversity
- multiple establishment strategies
- 2^o chem. ϕ , terpenes, coumarins (1 variety only)

Too Diff-

12 month growing season
root mass 1.2 vs 7.2
(Scotland)

* Castillo et al - (Phytochem - in press)

Glycosides of *caudatum*

Ptaguiloside

? >>
' ')

* strong cyanogen - found in milk
major problems

Diff in cyanogenesis

Symposium 9: Nitrogen in plant chemical defense

CHEMICAL DEFENSE OF ALKALOID PRODUCING PLANTS

Michael Wink, Universität Heidelberg, Inst. f. Pharm. Biologie, INF 364, D-69120 Heidelberg, Germany

It has been estimated that about 20% of all higher plants produce alkaloids. Because of their substantial biological activities it is quite clear that many alkaloids function as chemical defence compounds for the plants producing them. Alkaloids are mainly directed against plant-feeding arthropods and vertebrates and to a lesser extent against microorganisms and competing plants (Wink, 1993).

In order to fulfil this function the biosynthesis, transport and storage of alkaloids must be optimized in plants, so that alkaloids are present at the right place, time and in a concentration that is high enough to affect herbivores and microorganisms. Examples will illustrate the situation in quinolizidine and pyrrolizidine alkaloid producing plants.

On the other side, the structure of alkaloids needs to have the right shape to interfere with molecular targets in animals or microorganisms. We assume that this has been achieved through a process of "evolutionary molecular modelling". Many alkaloids are not directed against a single target, but usually to several although with different affinities. Alkaloid mixtures which are typical for plants, are often composed in a way that many targets are affected concomitantly. We interpret this as a means to increase toxicity and to prevent a quick evolution of resistance. Examples will be given for quinolizidine, pyrrolizidine and tropane alkaloids.

Wink, M. (1993) Allelochemical properties of the raison d'être of alkaloids. In *The Alkaloids*, Vol. 43, 1-118.

Notes:

150 QA's
 molec mechanisms -
 Receptors of neurotransmitters
 Acetylcholine
 Na⁺K channels
 Protein biosynth
 membrane stability
 mutagenicity

Confining
 alk must → in vitro effect

{ Nicotinic acetylcholine reecept
 Muscarinic acetyl reecept

Both alks aa Fluehck daily
 Wounding → ↑ alks (lucines)

Rabbits good selectors for
 lucine alks
 aphids - too

His - bulky aa in plants
 single point mutation →

ATPase,
 resistance

Erythrina
 low level His
 high level His

→ Erythrina - high His content -

ACTIVITY OF PYRROLIZIDINE ALKALOIDS IN CHEMICAL DEFENSE AGAINST THE ORB-WEAVING SPIDER *NEPHILA CLAVIPES*. I. SENEACIONINE FREE BASE AND N-OXIDE

N.Chemin and J. R. Trigo, Laboratorio de Ecologia Quimica, Departamento do Zoologia, Instituto de Biologia, UNICAMP, CP 6109, 13083-970 Campinas, SP, Brazil

Some Lepidoptera are chemically protected against vertebrate and invertebrate predators by pyrrolizidine alkaloids (PAs) in their tissues. The most studied PA/insect herbivore/predator system involves the liberation of PA-protected arctiid moths and ithomiine and danaine butterflies by the orb-weaving spider *Nephila clavipes*. Although these results show the effectiveness of PAs in the chemical protection of insects, little is known about PA dosage and structure in relation to activity against *Nephila*. The activity of the macrocyclic PA diester senecionine (found in some PA-insects that feed as larvae on *Senecio* or *Crotalaria*), applied as free base and N-oxide on a palatable prey, was measured against *Nephila* predation. Different amounts of the alkaloid dissolved in 10 μ l MEOH were applied topically on freshly killed bees (*Apis mellifera*). A bee was offered to a *Nephila* and the response of the spider (preyed or liberated) was recorded. When a given spider ate a bee treated with alkaloid, the trial was concluded. If the spider liberated the bee, another bee treated with 10 μ l of pure MEOH was immediately offered, and the experiment was considered valid if the spider ate it. Each dose level of alkaloid was tested ten times. In the interval of 1.46×10^{-4} to 9.35×10^{-3} μ moles/mg (of bee dry weight) of free base and 1.46×10^{-4} to 4.67×10^{-3} of N-oxide the response of the spider was variable. At very low amounts the PAs were ineffective, and at 1.87×10^{-2} μ moles/mg of free base and 9.35×10^{-3} of N-oxides the alkaloid was totally effective against *Nephila* predation. The senecionine free base (Liberation Dosage₅₀ = 1.48×10^{-3} μ moles/mg) had lower activity than the N-oxide form (LD₅₀ = 0.045×10^{-3}). Preliminary trials with retronecine free base (necine base from senecionine) showed no activity even at high dosage. These results suggest that N-oxidation and biosynthesis of insect-PAs from necine bases by specialist Lepidoptera are metabolic processes that could optimize the use of PAs as protective compounds.

Notes:

ALKALOIDS AND ANTHRACNOSE IN *LUPINUS ALBUS*

D. von Baer¹, E. von Baer², U. Hashagen², P. Marivil¹ and J. de la Fuente¹, ¹ Facultad de Farmacia, Universidad de Concepción, Casilla 237, Concepción, Chile, ² Campex Semillas Baer, Casilla 87, Temuco, Chile

Anthracnose is the most serious disease affecting *L. albus* in France, Chile and Brazil. It is caused by the fungus *Colletotrichum gloeosporioides* (*Cg*). Some genotypes are infected less than others¹. A high quinolizidine alkaloid (QA) level in *L. albus* seed, which contains $36,2 \pm 2,6$ % of protein, limits direct utilization in food and feed, due to bitter taste and toxicity of QA, so that as low as possible alkaloid levels in seed are desirable. This is achieved in sweet lupins. On the other hand, in the last decade Wink found that QA protect plants against certain fungi and bacteria².

The aim of our work in this context was to study if there is a relation between the resistance against anthracnose and the QA profile of lupin plants in successive development steps of different cultivars of *L. albus*, which is the lupin species most widely grown in Chile. Special emphasis was made on the ester alkaloids (EA). The EA concentration, mainly derived from 13-hydroxylupanine, is very low in ripe seeds, but shows a significant increase during germination. For one-month old plantlets of a more anthracnose resistant cultivar, EA represent up to 72 % of total QA. In plants of an anthracnose resistant breeding line, after losing their cotyledons up to 100 % of their alkaloids were found as esters. *In vitro* experiments with isolated *Cg* showed that the QA lupanine inhibit the growth of the fungi up to 60 %, whereas the QA sparteine had no effect. The amounts of EA isolated by us so far have not been enough yet to test their effect on *Cg in vitro*, which is necessary to prove their protecting role against anthracnose in *L. albus*, but at least we know that during plant growth it is possible to find significant differences in the EA levels of certain *L. albus* cultivars more resistant to anthracnose. Against another fungi (*Erisiphe graminis*), an EA (tigloyloxylupanine) was 12 times more active than the QA lupanine³, which at least *in vitro* inhibits the growth of *Cg*.

Financial support: FONDECYT (Chile), Project 1930518

¹J. Gondran, R. Bournoville, C. Duthion, Identification of Diseases, Pests and Physical Constraints in White Lupin, INRA Editions, Versailles, (1994).

²M. Wink, Methods in Plant Biochemistry, 8, 197 - 239 (1993)

³C. Wippich, M. Wink, Experientia 41, 1477 (1985)

Notes:

ROLE OF CAFFEINE IN THE RESISTANCE OF TEA CLONES TO THE SHOT HOLE BORER, XYLEBORUS FORNICATUS

N.K.B. Adikaram, N. Savitri Kumar, Vijaya Kumar and Priyadarshine Hewavitharanage, Departments of Botany and Chemistry, University of Peradeniya, Peradeniya, Sri Lanka

Caffeine which is an important constituent of tea, appears to play a role in the resistance of tea clones to shot-hole borer beetle of tea, *Xyleborus fornicatus*. The beetle carries with it the spores of the Ambrosia fungus, *Monacosporium ambrosium*. Caffeine was found to have an inhibitory effect on the growth of the fungus.

The caffeine content of infected tea stems was higher than that of healthy stems. The highest caffeine concentration was found in infected stems of the resistant clone and the lowest in its healthy stems. The difference in caffeine content between healthy and infected stems of the susceptible clone was found to be much less than in the more resistant clone.

The effect of caffeine on the growth of the adult shot-hole borer beetles was tested in the laboratory by incorporating caffeine into the growth medium. No fungal growth was observed in tubes containing 5000 ppm of caffeine, little in those containing 1000-2000 ppm and normal growth in those containing 500, 50 ppm. Galleries were constructed but not eggs were laid in all the tubes containing caffeine, although beetles remained alive for 30-60 days.

Caffeine in the fairly low concentration found in the susceptible clone, but not at high concentration, appears to attract the shot-hole borer beetle. Attack by the beetle seems to promote increased production of caffeine as part of the defence mechanism of the tea stem. The increase in caffeine content in the resistant clone appears to take it to a level toxic to the Ambrosia fungus, thus reducing colonisation by the fungus and impairing the survival of the beetle. However, this increase in the susceptible clone seems to be insufficient to inhibit the growth of the fungus.

Notes:

THE ECOLOGICAL ACTIVITY OF ALKALOIDS IN NATURAL SYSTEMS

K. S. Brown, Jr. and J. R. Trigo, Laboratorio de Ecologia Quimica, Departamento de Zoologia, Instituto de Biologia, UNICAMP, CP 6109, 13083-970 - Campinas, SP, Brazil

A review with 583 references, describing the known ecological activities of alkaloids, is being published in volume 47 of the series "The Alkaloids," expanding upon a previous review by Wink in volume 43. While most natural alkaloids have been subjected to pharmacological testing, surprisingly few (the review mentions less than 300 structures) have been examined for their activity in natural systems, in which their accumulation has been selected by pressures from the environment, often but not necessarily biotic. The few more complete experimental tests that have been done with alkaloid-synthesizing organisms and alkaloid receivers associated with them in nature, have revealed a very wide range of chemical mediation, some suggesting promising channels for new investigation of pharmacological action. In interactions within (pheromones) or among populations and species, alkaloidal infochemicals have been shown to have protective (155 structures), repellent (23), inhibitory (46), deterrent (36), toxic (27), attractant (27), and stimulant (33) activity, and to be excreted (10 structures), tolerated (8), sequestered (94), transferred (9), metabolized or transformed (17) by receivers, usually conditioned by strong natural selection acting on a variety of enzymes, structures, and behaviors on all sides. Most examples involve alkaloids that are easy to recognize and assay at different points in the system, especially pyrrolizidines, quinolizidines, nicotine, simple indoles, diterpenes, and hydroxamic acids; many have multiple activities. Especially significant are studies of post-attack induction and the great qualitative and quantitative, spatial and temporal variation of alkaloids in single individuals, populations or species. These data reveal a pervasive action of these diversified compounds in the structuring of soil, terrestrial, aerial and aquatic communities, and help to explain the difficulty in maintaining alkaloid production in organisms removed from environmental pressure.

Notes:elephants
frogs
ants

fish

Fungal endophytes on aphids

Review

Symposium 10: Chemical ecology of vertebrates

THE ROLE OF CHEMICAL ECOLOGY IN VERTEBRATE BIODIVERSITY CONSERVATION

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

This review of some vertebrate interactions by chemicals deals with ecological processes and applications that are important in conservation of biodiversity. The talk focuses on feeding behavior, habitat imprinting and homing, and social odors.

Plant-herbivore interactions: *Seed conditioning* by reptilian, avian and mammalian digestive tracts may be essential for germination of various plants, some rare, ranging from tomatoes to island trees. *Secondary plant compounds* may cause high mortality in mammalian herbivores under intense competition or when fenced-in vegetation becomes impoverished, as examples from Africa and North America show. *Introduced plants and herbivorous mammals* often suffer heavy losses because they lack the adaptations that provide for a balance in their herbivore-plant relationships. Other *feeding behaviors*: Feeding stimuli are being sought that would help in trapping the destructive brown snakes on Guam. In freshwater systems, change in pH can alter feeding responses by fish.

Homing and *chemical habitat imprinting*: The best example are the lessons from the attempt to create a new breeding ground in Texas for the Kemp's ridley sea turtle by chemical imprinting. *Social odors*: Pheromones for trapping fauna-destroying predators, as is being tried in the brown snake, is more promising in reptiles than in mammals.

These examples will be reviewed critically. The talk covers encouraging and discouraging experiences and points out further research opportunities.

Notes:

CHEMICAL AND DIGESTIVE ECOLOGY OF FORAGING IN VERTEBRATES

Francisco Bozinovic, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

How animals cope with food chemistry has traditionally been a problem in chemical ecology. However, recently it has become evident that organismal structural and physiological limitations may exert impact before the animals are confronted with such food resources. The idea that ultimate physiological limits to energy budget exists underlies most theories of life history, optimal resource allocation principles, and in particular optimal foraging and digestion theory.

I illustrate here topics related to digestion that have special relevance for the chemical ecologist. First, the utilization efficiencies and foraging behavior of small mammals confronted to different dietary chemistry. Second, I consider the major chemical features of food models that determine or affect the efficiency with which small herbivorous rodent utilizes food. Third, since utilization efficiency is also affected by properties of the animal such as digestive efficiency, their mode of action and their interrelations are exemplified and discussed. Finally, I consider how digestion rates might limit energy intake and hence affect the interaction between animals and their food resources. These approaches about digestion operating as a constraint in animal behavioral and population ecology, may represent a vein of research in chemical aspects of ecology.

Funded by FONDECYT 1950394

Notes:

HUMAN ODORS. A BASIS FOR HUMAN SEXUAL SELECTION AND HOST RECOGNITION BY HEMATOPHAGOUS ARTHROPODS: A CHEMICAL-ECOLOGICAL APPROACH

Klaus Jaffé and Asdrúbal Briceño, Universidad Simón Bolívar, Laboratorio de Comportamiento, Apdo. 89000, Caracas 1080-A, Venezuela

Odours are an important source for kin-recognition and sexual selection in a variety of vertebrates. Humans recognize conspecific individuals by their odour. Some of these odours may be involved in human kinship and sexual behaviour, playing a role as aphrodisiac and as kin-recognition signals. On the other hand, human odours differ in their power to attract arthropod parasites and hematophagous insect vectors. Individual variability in attractiveness to mosquitoes for example has been well documented. The present study attempts to characterize the chemical components of human axillary odours, analyzing the variability of the relative proportions of various volatile substances from axillary ethanol washings. Forty individuals were sampled repeatedly and each sample was analyzed with Gas Chromatography and GC-Mass Spectrometry. A multifactorial analysis of the GC spectra suggest the existence of species- sex- family- and individual-specific characteristics of the chemical blends of human axillary odour with stable reproductive pair formation and with susceptibility to attack by hematophagous diptera are presently studied.

Notes:

IDENTIFICATION AND BIOACTIVITY OF AN ESTROUS PHEROMONE OF ASIAN ELEPHANTS: A SURPRISE, PERHAPS A TREND

L.E.L. Rasmussen, Terry D. Lee and G. Doyle Daves, Oregon Graduate Institute, Beckman Research Institute of the City of Hope and Rensselaer Polytechnic Institute, USA

In the Asian elephant, *Elephas maximus*, discrete chemosensory responses are explicitly linked to subsequent reproductive behavior. In captivity the elephant is easy to observe and exhibits an obvious mode of transferring the chemical signal to the vomeronasal organ ductal openings in the mouth by the trunk (*i.e.*, the flehmen). An unusually high frequency of non-habituating flehmen responses by bull elephants to preovulatory urine from females is followed by definitive pre-mating behavior. The presence of a urinary sex pheromone is clearly indicated. After 15 years of effort we have succeeded in purifying and identifying the active chemical component. A series of fractionation steps (Rasmussen *et al.*, 1993), including recent modifications of a final reverse phase high performance liquid chromatographic separation, yielded a highly active fraction with a characteristic odor. First, by capillary gas chromatography, and subsequently by capillary gas chromatographic/mass spectrometric analysis, only a single component was demonstrated. Analyses by mass and nuclear magnetic spectrometries of the active fraction clearly established the structure. The spectrometric data allows the possibility of two positional isomers relevant to the position of the lone double bond. We are determining cis or trans isomerization by the appropriate standards, using capillary GC and proton NMR and ¹³C NMR. Bioassays of one readily available, commercial isomer, using four different Asian male elephants have demonstrated a robust chemical signal. The active compound is not a steroid. Instead the elephant sex pheromone is an acetate of moderate volatility that a variety of female insects release as their sex pheromones. Our startling identification of the sex pheromone of a higher mammal, the Asian elephant, has the potential to change thinking about mammalian chemical signals.

Supported by RO1 HD19219

Notes:

Poster presentations

1. APHID SETTLING BEHAVIOR MODIFYING TERPENES: EFFECT ON *MYZUS PERSICAE* (HOMOPTERA: APHIDIDAE)

Carmen Gutiérrez¹, Alberto Ferreres¹, M^a Sol Vieira¹, Matías Reina² and Azucena González-Coloma^{1,2}, ¹CCMA, CSIC, Serrano 115-dpdo., 28006 Madrid, Spain; ²IPNAC, CSIC, Ave. Astrofísico F. Sánchez 2, 38206 La Laguna, Tenerife, Spain

We have improved a leaf-disk bioassay to test aphid settling-behavior modifying effects of natural compounds on the species *Myzus persicae* using *Capsicum annum* leaf disks. Several commercially available terpenes (the monoterpenes geraniol, citronellol, and farnesol; and the sesquiterpene caryophyllene oxide) and the natural sesquiterpene 2,10-bisaboladien-1-one, isolated from *Senecio palmensis* as an antifeedant against *Leptinotarsa decemlineata* (Colorado potato beetle), have been tested.

Our results showed that all these compounds altered the aphid settling behavior at the highest level (100 µg/disk) tested in choice assays. Dose-response experiments showed that among the monoterpenes the most active ones were geraniol and farnesol; being the bisabolene sesquiterpene as active as its precursor farnesol. Long-term no-choice experiments (4-6 days) run with both farnesol and the bisabolene showed that both compounds reduced aphid fecundity at a single dose of 60 µg/disk.

We conclude that the monoterpenes geraniol, citronellol and farnesol plus the sesquiterpene bisabolene alter the settling behavior of *M. persicae*. The bisabolene sesquiterpene and its precursor farnesol had negative effects on offspring production, while the other sesquiterpene tested, caryophyllene oxide, did not show any significant effect. Structure-activity relationships among these compounds will be discussed.

Notes:

2. CHARACTERIZATION OF THE RESISTANCE OF MELON LINES TO *APHIS GOSSYPHII* (HOMOPTERA: APHIDIDAE) AND CUCUMBER MOSAIC VIRUS (CMV)

Fereres, A., Martin, B., Duque de Cela, M. and J.L. Alvarez*, Consejo Superior de Investigaciones Científicas, Centro de Ciencias Medioambientales, C/Serrano 115 dpdo. 28006 Madrid, Spain; *SLUIS Y GROOT Semillas S.A., Carretera de Málaga km 87.2 Apartado 57. 04700 El Ejido (Almeria), Spain

The plant resistance mechanisms of melon lines previously selected under field conditions based on resistance to *Aphis gossypii* and/or cucumber mosaic virus (CMV) was investigated. All melon lines were tested for the presence of the virus aphid transmission resistance ('Vat') gene. This gene confers antixenosis, antibiosis and virus transmission resistance to *A. gossypii*. Also, the same lines were screened for resistance to CMV using mechanical and vector (*Aphis gossypii* and *Myzus persicae*) inoculation. Five out of 8 lines showed some degree of antixenosis and antibiosis type of resistance to *A. gossypii* when compared to a susceptible control (Doublon).

However, there was one of these resistant lines that was susceptible to CMV infection when *A. gossypii* was used as vector. Therefore, we concluded that this line was not carrying the 'Vat' gene, although it was a very poor host for *A. gossypii*. Our results indicated that for the purpose of the antixenosis test, the criterion proposed in the past by Pitrat & Lecoq (1980) to differentiate between susceptible and resistant germplasm may be improved by extending the time of exposure of aphids to the test plants to 72 hours. Also, we found that two of the four lines carrying the 'Vat' gene, were not only resistant to CMV when *A. gossypii* was used as a vector, but also resistant to mechanical inoculation. Further characterization of the resistance mechanisms of melon lines to virus transmission is under progress.

Pitrat, M.Y and LeCoq, H., 1980. Inheritance of resistance to cucumber mosaic virus transmission by *Aphis gossypii* in *Cucumis melo*. *Phytopatology* 70, 958-961.

Notes:

3. USING APHID SEX PHEROMONES TO MANIPULATE PARASITIC WASP POPULATIONS

Wilf Powell, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, UK

The sex pheromones of a range of important aphid pest species have been identified in recent years. The main volatile component of these pheromones can be chemically synthesized, and we have shown that these synthetic pheromones are highly attractive to female parasitic wasps which use them as a host location cue.

At Rothamsted an aphid control strategy is being developed based on the use of aphid sex pheromones to concentrate parasitic wasp populations in reservoir vegetation strips around crops at critical times of the year. These reservoir strips could be used to provide a refuge when hazardous chemical applications are required on the crop. They also can be managed to provide an overwintering site for parasitic wasp populations which will emerge alongside the crop in Spring, ensuring synchrony with colonising aphid populations. Such host/parasitoid synchrony at the beginning of a pest infestation is essential for efficient biological control of aphids.

The poster will outline the proposed strategy and present laboratory and field data to demonstrate the responses of parasitoids to the synthetic aphid sex pheromones.

Notes:

4. THE EVOLUTION OF HOST-DERIVED DEFENSE IN *OREINA* LEAF-BEETLES

Pasteels, J.M.¹, Hsiao, T.S.², Dobler, S.³, Rowell-Rahier, M.⁴, ¹Université libre de Bruxelles, Belgium, ²Utah State University, Logan, USA, ³University of Colorado, Boulder, USA, ⁴Université de Neuchâtel, Switzerland

Three different chemical defensive strategies are observed in *Oreina* leaf beetles: autogenous production of cardenolides in species feeding exclusively on Apiaceae or Cardueae (Asteraceae), sequestration of PAs in *O. cacaliae* feeding exclusively on Senecioneae (Asteraceae), the production of both autogenous cardenolides and sequestered PAs in species feeding exclusively on Senecioneae or on both Senecioneae and Cardueae, or still on both Senecioneae and Apiaceae.

Phylogenies based on allozymes and 12S and 16S mtDNA were constructed to investigate the historical evolution of host-plant affiliation and chemical defense. Despite some conflicting results in the branching patterns obtained by the two methods, phylogenies indicate that sequestration is apomorphic to autogenous defense. They also suggest that *Oreina* species were originally associated with Asteraceae, with an inclusion of Apiaceae in the diet of one oligophagous species and an independant switch to Apiaceae in a derived clade. Sequestration of PAs could have either originated at the base of the genus and lost twice or evolved two or three times independently.

Notes:

5. FLORAL SCENTS IN *PASSIFLORA* SPECIES POLLINATION

I.G. Varassim and J. R. Trigo, Laboratorio de Ecologia Quimica, Departamento de Zoologia, Instituto de Biologia, UNICAMP, CP 6109, 13083-970 - Campinas, SP, Brazil

In zoophylic pollination, morphological cues, such as shape, and chemical cues, as pigments and scents can act attracting or guiding pollinators. Recent studies show that foraging choice in bumblebees is associated with the variation of scent profiles, in spite of colour diversity. The use of scent profiles as a taxonomic character is related with its potential role in plant speciation, since floral fragrances can function as an isolating mechanism. Report on *Passiflora* pollination has shown groups of animals not always taxonomically or phylogenetically related. This investigation dealt with pollination ecology of three species of *Passiflora* (Passifloraceae) occurring at Southeastern Brazil. The composition of the species scent profile was determined by extraction in CH_2Cl_2 . The samples were gently concentrated prior to analysis by gas chromatography-mass spectrometry (GC-MS). The sample of *P. alata* presented 232 compounds and is pollinated by large bees, *P. galbana*, a chiropterophilous species, showed 243 compounds and *P. speciosa* presented 87 compounds and is pollinated by a hummingbird. The greater abundance of compounds in the two former species of *Passiflora* is probably due to olfactory orientation by bees and bats, since birds have an accurate visual sense. The major compounds, identified by comparison with data from Wiley Mass Spectra Library, are citronelol, geraniol, benzenemethenol, benzene-1-4 dimethoxy, 2,6-octadienoic acid and 1,13-tetradecadiene in *P. alata*; dodecane, two unidentified alkanes and an unknown compound in *P. galbana*, and an unidentified alkane and benzene 1-ethyl, 4-methyl in *P. speciosa*.

Notes:

6. GEOMETRY OPTIMIZATION AND CONFORMATIONAL ANALYSIS OF PYRROLIZIDINE ALKALOIDS BY MOLECULAR MECHANICS. A CHEMOECOLOGY APPROACH

M. Giordan¹ and J. R. Trigo², ¹Instituto de Química, UNICAMP; ²Laboratório de Ecologia Química, Departamento de Zoologia, IB, UNICAMP, Campinas, SP, Brazil

Pyrrrolizidine alkaloids (PAs) are important chemical mediators of interactions between organisms, protecting plants that make them, as well as the insects that sequester them, against natural enemies; they also are utilized by some Lepidoptera as pheromone precursors. Stereochemical inversion in ingested monoester PAs from 7*S*-OH to 7*R*-OH by arctiid moths and ithomiine butterflies is known. Theoretical studies on PA structures help to understand their conformations, that could be related to the action of oxyreductive enzymes responsible for the inversion. In preliminary studies the MM3 force field was applied in the optimization of the geometry of PA free base (4 macrocyclic diesters, 5 monoesters, 1 open diester and 2 necine bases). The results of the bond length, bond and torsional angles were compared with experimental geometries obtained from x-ray diffraction. The comparison showed good agreement for bond lengths and angles with mean deviations between 0.015 and 0.013 Å, and 1.3 and 2.7°, respectively. The conformational analysis was based on the behaviour of the dihedral angles, which were shared in two groups: the necine bases and the necic acids. With this approach we observed the interactions between 7 and 9-OH groups belonging to the ring systems (for necine bases), interactions between the 7*S*-OH groups of the ring and the N lone electron pair (for *endo* heliotridine, a necine base), and interaction between 7-OH and OH in the acid part of open monomers. These results suggest that the convex face of the pyrrolizidine is more hindered in 7*S* than 7*R* PAs for the approach of probable enzymes responsible for the accumulation of PA in the integument, leading to oxidation and asymmetric reduction of 7*S*-OH to the less hindered 7*R*-OH.

Notes:

7. ALKALOID PROFILE DURING THE DEVELOPMENT OF SWEET VARIETIES OF *LUPINUS ALBUS* PLANTS

P. Marivil¹, J. de la Fuente¹, D. von Baer¹, U. Hashagen² and E. von Baer², ¹Facultad de Farmacia, Universidad de Concepción, Casilla 237, Concepción, Chile; ²Campex Semillas Baer, Casilla 87, Temuco, Chile

The aim to study the variation of the alkaloid profile at different development stages of sweet varieties of *L. albus* is discussed in another paper submitted to this Congress¹. In this paper, it is discussed the evolution of the alkaloid profiles of four sweet varieties of *L. albus* (Multolupa, Victoria, Bío-Bío, Katy) sown in the seasons 1993/94 and 1994/95 at the Experimental Station of CAMPEX in Cajón, IXth Region (Chile). Samples of plant material were taken at the following developmental stages: plantelet (PL), rosette (RS), plants after loosing their cotyledons (P-C) (only season 1994/95), leaves at the beginning (L 1) and at the end of flowering (L 2). Samples were stored at -24 °C up to analysis, for which they were extracted with 0.1 N HCl, purified through an Extrelut-20 column and analyzed by capillary GC with an NPD detector². Identification of alkaloids was made with their retention index and by GC-MS.

In both seasons, important differences were observed between the studied varieties but all showed a successive decrease of total alkaloids (TA) from PL to P-C step at which the TA reach their minimum, whereas at the following step (L 1), only two weeks later, it was observed a significant increase of TA, especially of non-ester alkaloids (NEA). During this period, the plants of some varieties were affected by a severe anthracnose attack. In both seasons, ester alkaloids (EA) were found at highest concentrations in PL, showing the more anthracnose resistant varieties a greater proportion of EA. It is interesting to point out that just at the step in which the new small plant is more vulnerable to the attack of diseases, it was found the highest proportion of EA. This could indicate a possible role of EA as a natural protection of lupins against anthracnose.

Financial support: FONDECYT (Chile), Project 1930518

¹ D. von Baer, E. von Baer, U. Hashagen, P. Marivil, J. de la Fuente, Alkaloids and Anthracnose in *Lupinus albus*, XIIth Annual Meeting Soc. Chemical Ecology, Termas El Corazón (1995).

² D. von Baer, J. de la Fuente, V Congreso Latinoamericano de Cromatografía, p.12 (1994).

Notes:

8. BREEDING CEREALS FOR INCREASED SELF-DEFENCE AGAINST APHIDS THROUGH INCREASES IN THEIR HYDROXAMIC ACID CONTENT

Erik von Baer¹, Uta Hashagen¹ and Hermann M. Niemeyer², ¹CAMPEX Semillas Baer, Casilla 87, Temuco, Chile; ²Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Aphids are a problem of varying intensity in the south of Chile, where most of the cereals produced in our country are sown. Transmission by aphids of barley yellow dwarf virus increases losses, and affects wheat, barley, triticale and oats to variable degrees. It has been shown in the laboratory that aphids multiply and feed to different extents on seedlings of different wheat cultivars depending on their hydroxamic acid (Hx) content.

Varieties of wheat, rye and triticale selected under conditions prevailing in Southern Chile showed differences in Hx content. Hx concentrations determined in the laboratory in seedlings in decimal growth stage Z11 correlated negatively with the mean score of visual symptoms (1-no symptoms to 9-high symptoms) determined in seedlings in growth stage Z36 from field trials carried out in natural conditions (without pest control chemicals).

Species	Variety (Baer)	Yield (t/ha)	Mean score of symptoms	Hydroxamic acids (mmol/Kg fr. wt)	
				DIBOA	DIMBOA
Wheat	Taita	8.5	1-2	nd	3.3
	Paleta	7.3	2	nd	2.2
	Colono	6.2	2	nd	2.0
	Otto	5.5	4	nd	2.0
	Amigo	4.8	3	nd	1.6
Triticale	Cacique	3.8	2	2.0	4.2
Rye	Forrajero	3.5	1	13.0	nd

The Hx concentrations determined are near the mean of the range found in a worldwide screen of wheats, and suggest the potential in these cultivars for decreasing aphid infestation by increasing Hx levels through breeding.

Notes:

9. VOLATILE COMPONENTS FROM THE SALIVARY GLANDS OF CALLING MALES OF THE SOUTH AMERICAN FRUIT FLY *ANASTREPHA FRATERCULUS* (WIED.): PARTIAL IDENTIFICATION AND BEHAVIOURAL ACTIVITY

Ivanildo S. de Lima,^{1*} Philip E. Howse,¹ and Ian D. R. Stevens². ¹Department of Biology, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX U.K., ²Department of Chemistry, University of Southampton, Building 27, Southampton, SO16 7PX, U.K. *Present address: Departamento de Química, Universidade Federal de Alagoas, BR 101 Norte Km 14, Tab. do Martins, CEP 57.072-970, Maceió-AL, Brasil

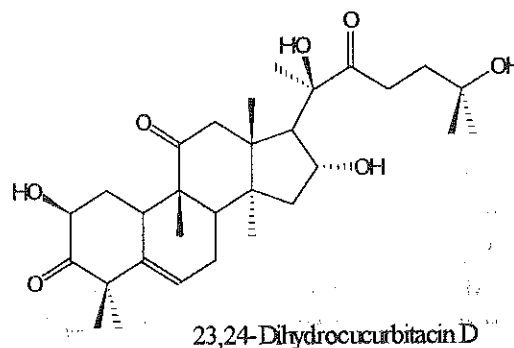
Volatile compounds produced by males have previously been reported from *Anastrepha ludens* and *A. suspensa*. Both species produce and release a similar range of compounds, but in different proportions. The dimorphic salivary glands of virgin calling male *A. fraterculus*, showed behavioural activity when bioassayed in the laboratory with virgin mature females flies, but immature females were not attracted. We report for the first time that two isomers of the sesquiterpene hydrocarbon α -farnesene, several pyrazines and one isoprenoid lactone (*E,E*)-suspensolide, are present in the salivary glands of virgin male *A. fraterculus*. Two of these compounds have been reported from male *A. ludens* and *A. suspensa*. We also report the presence of unidentified compounds from male salivary glands of South American fruit fly.

Notes:

10. ECOLOGICAL ASSOCIATION OF NEW AND OLD WORLD CHRYSOMELID LEAF BEETLES WITH CUCURBITACINS

Ritsuo Nishida and Masaru Yokoyama*, Pesticide Research Institute, Kyoto University, Kyoto 606-01, Japan;
*National Research Center for Rice and Beans, P.O. Box 179, Goiás 74000, Brazil

A number of chrysomelid leaf beetles in the tribe Luperini show a strong affinity to cucurbit plants. Two South American polyphagous leaf beetles, *Diabrotica speciosa* and *Cerotoma arcuata*, selectively accumulated the bitter tasting compound 23,24-dihydrocucurbitacin D in their body, pharmacophagously feeding upon the bitter cucurbit plant tissues. Similarly, several Asian Cucurbitaceae-feeding specialists in the genus *Aulacophora* were found to sequester the same compound in substantial quantities. Cucurbitacin analogs were shown to deter feeding by a bird predator, indicating an allomonal role for these compounds in cucurbitacin associated chrysomelid leaf beetles both of New and Old Worlds. The strong affinity to cucurbitacins, selective sequestration of the analogs and consequent protection from predators suggested an ecological adaptation mechanism developed in common among these two geographically isolated subtribes in the Luperini.



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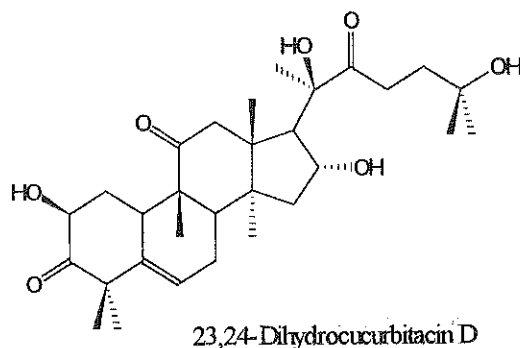
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Notes:

11. VOLATILES EMITTED BY LARVAE OF THE EUROPEAN APPLE SAWFLY AND BY THE FRUIT THEY INFEST: POTENTIAL SIGNIFICANCE FOR FORAGING NATURAL ENEMIES

Jean-Luc Boevé and Ted C.J. Turlings, Institute for Plant Sciences/Applied Entomology, ETH, 8092 Zurich, Switzerland

Larvae of the European apple sawfly *Hoplocampa testudinea* feed within apple fruitlets. Insect and fruit volatiles were analyzed by GLC and GC-MS. Disturbed sawfly larvae evert ventral glands, thereby emitting a series of mainly aliphatic compounds. One of these compounds was *E,Z,Z-2,4,7*-decatrienal, which, as far as we know, has not been reported from insects previously. Frass of larvae included mainly butanoic compounds as volatiles. Healthy apples were found to emit relatively small amounts of terpenoids (among which *E-2(3)*-epoxy-*2,6*-dimethyl-*6,8*-nonadiene is probably new for apples) and a few aromatic and short aliphatic compounds. Sawfly-infested apples emitted significantly larger amounts of *E,E-α*-farnesene, β -ocimene and a terpenoid that remains unidentified. This increase was primarily observed with picked apples and could be confirmed in the field with single apples left on the tree. The insect volatiles might constitute a defence against potential predators such as ants, although a rather symbiotic relationship seems to be prevalent in the field between the sawfly larvae and the ant *Lasius niger*. The apple volatiles that increased under infestation might attract the specialized ichneumonid parasitoid *Lathrolestes ensator* during its search for the sawfly larvae.

Notes:

12. DEVELOPMENTAL EFFECTS OF AN ACQUIRED ALKALOID AND ITS ADAPTIVE CONSEQUENCES IN A MOTH (*UTETHEISA ORNATRIX*).

Marta L. del Campo Couratier¹, Scott R. Smedley² and Thomas Eisner². ¹Department of Entomology, Comstock Hall, ²Section of Neurobiology and Behavior, Mudd Hall, Cornell University, Ithaca, New York, USA 14853

The arctiid moth *Utetheisa ornatrix* as a larva feeds on leguminous plants (*Crotalaria* spp.) containing pyrrolizidine alkaloids. Larval sequestration of these compounds affords the moth with protection from predation. Using a sibling-paired design experiment, we examined the effect of one such alkaloid, monocrotaline (MC), on several other fitness parameters, including duration of development (egg to adult) and body mass. Larvae were reared on either a 0.5% MC semisynthetic diet, or a diet devoid of MC. We found that MC significantly accelerated development (almost 10% in males and 5% in females), but did not directly affect body mass. *Utetheisa* that developed more rapidly, however, were significantly heavier than those that developed more slowly, indicating an indirect effect of MC on body mass. Several adaptive benefits of these effects are apparent. Earlier emergence and greater body mass can increase a male's likelihood of successfully siring offspring. Furthermore, female mass correlates positively with fecundity and longevity. For both sexes, accelerated development may reduce the probability of predation and parasitism, particularly for the relatively sedentary larvae and pupae.

Notes:

13. FOOD SEARCHING BEHAVIOR OF THE PLEASING FUNGUS BEETLE, *DACNE PICTA* (CROTCH)

¹Masahiko Tokoro, ²Takashi Sato, ¹Kiyoshi Nakamura and ¹Tadakazu Nakashima, ¹Forestry & Forest Products Res. Inst., Tsukuba, Ibaraki 305, Japan; ²Chiba University, Matsudo, Chiba 271, Japan

Dacne picta attack shiitake mushrooms (*Lentinus edodes*) throughout their larval and adult stages. A number of beetles were captured using water pan traps baked with fresh shiitake mushrooms during a field test. The behavioral responses of the beetles to shiitake fruit bodies and to extracts of fruit bodies were observed in laboratory tests using a linear track olfactometer. In addition, main odor component of the mushroom, 1-octen-3-ol, was also tested. Females of the beetles were significantly attracted by 1-octen-3-ol at 10^{-3} g but responses of the males for the same component were not clear. Significantly more female and male beetles attracted to fruit bodies of fresh mushrooms and their hexane extracts than to the control. The beetles showed significant responses for the active substances within a limited range of quantity.

Notes:

14. "HEAD SPACE" COMPOSITION AND SURFACE TOPOGRAPHY OF TWO VARIETIES OF *LYCOPERSICON ESCULENTUM*: CORRELATION WITH THE OVIPOSITION PREFERENCES OF THE FRUITMOTH, *SCROBIPALPULOIDES ABSOLUTA* MEYRICK (GELECHIIDAE)

Ximena Arias¹, Alejandro Urzúa¹ and Patricia Estay², ¹Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 307, Santiago, Chile; ²Centro Regional de Investigación La Platina, Casilla 439-3, Santiago, Chile

Tomato, *Lycopersicon esculentum* Mill., is the most important vegetable crop grown in Chile with the total surface of around 12.000 ha. Because of its perennial occurrence, the level of damage that it causes in fruit and leaves and the fact that it has developed an increasing resistance to insecticides, the tomato cultivars are seriously affected by the tomato fruitmoth *Scrobipalpuloides absoluta* Meyrick. As part of an integrated model to attack of the problem, the relation between the "head space" composition and surface topography of two varieties and oviposition preferences of *S. absoluta* Meyrick is presented.

GLC-EM analysis of the "head space" of CAL-ACE VF. and ROMA VF. shows the same following compounds: α -pinene, β -myrcene, β -phellandrene, 1-methyl-4-(1-methylethyl)-benzene, caryophyllene, copaene, 2-carene. In addition in the "head space" of CAL-ACE VF. α -caryophyllene and methyl salicylate and in the "head space" of ROMA VF. 1-methyl-3-(1-methylethyl)-benzene and nicotine were respectively identified.

The surface of the folioles of both varieties shows by electronic microscopy similar glandular trichomes. The only difference observed was in the folioles of the apical and basal leaves of each variety.

Despite of differences between the composition of the "head space" and surface of the two varieties no differences in the oviposition of *Scrobipalpuloides absoluta* Meyrick were observed.

Acknowledgement. Research is financially supported by DICYT, Universidad de Santiago de Chile, the authors are gratefully acknowledge.

Notes:

15. COMPARISON OF ALLELOPATHIC POTENTIAL OF FOUR FORESTRY SOILS

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The allelopathic potential of four woodlands holding *Quercus robur* (autoctonous in the studied place), *Eucalyptus globulus*, *Acacia melanoxylon* and *Pinus pinaster* (alloctonous species) like main species, was analyzed. Thus, the capacity of the four soils to modify the pattern of different physiological processes of *Lactuca sativa* was analyzed by means of growth and germination bioassays. The different effect on microorganisms of the soil from each woodland was analyzed too. The amount of phenolic compounds in the soil solutions of the four woodlands was analyzed by HPLC.

The phenolic profile of the four soils was different and the relationship between these compounds and the different allelopathic potential shown in the bioassays is discussed.

Notes:

16. ALLELOPATHIC EFFECTS IN *CAPSICUM ANNUUM* L. SOME CONSIDERATIONS ABOUT PRACTICAL USES OF ALLELOPATHY

Reigosa, M.J.¹, González, L.¹, Sánchez, A.M.¹, Souto, X.², ¹Facultade de Ciencias de Vigo, Dep. Recursos Naturais e Medio Ambiente, Universidade de Vigo, Aptdo. 874, 36200, Vigo, Spain, ²Escola Universitaria de Enxeñeiros Técnicos Forestais. Dep. Recursos Naturais e Medio Ambiente, Universidade de Vigo

Low molecular weight phenolic compounds in leaves and roots of nine commercial *Capsicum annuum* accessions in four phenological phases (acclimatization, blossom, fruitification, senescence) were studied. Also, the amounts of those compounds in the soil where the pepper accessions grew were recorded along plant parts decomposition process.

Allelopathic effects of decomposing plant parts in soils, artificial leachates and root exudates were tested bioassaying on *Lactuca sativa* var. Great Lakes.

Some of the identified low molecular weight phenolic compounds found in plant parts and soils were tested on six weeds (*Cirsium* sp., *Amaranthus retroflexus*, *Chenopodium album*, *Solanum nigrum*, *Plantago lanceolata* and *Rumex crispus*) and on eleven microorganisms groups (Fungi, Bacteria, Free living dinitrogen fixers, Denitrifying bacteria, *Nitrosomas*, *Nitrobacter*, Ammonifiers, Cellulose Hydrolyzers, Algae, Proteolytic microorganisms and Starch hydrolyzers). Also, the effects of microorganisms on the amount of phenolic compounds in soils was assessed.

Some considerations about possible practical uses of allelopathy are done.

Notes:

17. BIOCHEMICAL ACTIONS OF VOLATILE ALLELOCHEMICALS FROM *TAGETES MINUTA* L.

J.A. Zygadlo, A.L. Lamarque, D.M. Maestri and N.R. Grosso, Cátedra de Química Orgánica, Facultad de Ciencias Exactas, Físicas y Naturales. Universidad Nacional de Córdoba. Avda. Vélez Sarsfield 299, 5000 Córdoba, Argentina

Tagetes minuta L. is of wide occurrence in Argentina. Chemical analysis of the aerial parts provided copious amounts of C8 ketones. Although no single structural features of the monoterpenes appear to be a critical factor in inhibiting germination, several of the most phytotoxic compounds are ketones. The objective of this study was to describe the biochemical changes that tagetone from *T. minuta* might cause on the growth of *Panicum miliacium* L. seeds. Although root and shoot growth was severely inhibited by tagetone, there were few significant effects on fatty acid composition and soluble protein patterns. In the sterol composition, an increase was measured in stigmaterol content. Peroxidase activity, polyamine and inorganic phosphorus contents were significantly different from nontreated seeds.

Notes:

18. PRESENCE OF ALLELOPATHIC ACTIVITY IN CALLUS OF *DATURA STRAMONIUM*

Carolina San Martín, Claudia Pulgar, Francisca Massardo and Gustavo E. Zúñiga. Laboratorio de Fisiología Vegetal, Departamento de Ciencias Biológicas, Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 307, Correo 2, Santiago, Chile

Plant tissue cultures are regarded as a potential source for the production of natural products. For more than two decades callus cultures from many plants have been carried out to establish suitable conditions for the possible production of pharmaceutical compounds and/or secondary metabolites with allelopathic properties.

Different studies have shown that *Datura stramonium* is a weed with allelopathic properties on seeds germination, which are caused by alkaloids, like scopolamine and hyocyamine. This compounds has been found in whole plant, specially in the seeds.

We have studied the allelopathic effects of ethanolic extracts from callus of *D. stramonium* on wheat seeds.

Callus were induced from petioles and leaves of seedlings in MS culture medium. The results obtained shown that germination and early growth of wheat were inhibited and retarded by the extracts. This could lead us to propose that callus tissue has inhibitor properties like seeds extracts. However, further studies are needed to determine if the same alkaloids founds in seeds, as has been reported in the literature, are the compounds responsible of the inhibition found in callus extracts of *D. stramonium*.

Supported by DICYT, Universidad de Santiago de Chile.

Notes:

19. ALLELOPATHIC EFFECTS AND BIOLOGICAL ACTIVITY OF CONSTITUENTS FROM *VERNONIA* SPECIES (COMPOSITAE)

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From the large genus *Vernonia* many 13-O-acyl sesquiterpene lactones of the glaucolide type have been isolated. These lactones showed molluscicidal and insect feeding deterrent activities.

We assayed crude extracts, fractions and pure compounds isolated from roots and aerial parts of *Vernonia scorpioides* and from aerial parts of *Vernonia pinguis*. The biological activity was tested on lepidopterous, mollusks and microorganisms. Effects on the germination of seeds of mono and dicotyledonous were also assayed.

Strong effects were observed when the sesquiterpene lactones isolated from *V. pinguis* were incorporated to the diets of *Biomphalaria peregrina* and *Aplexa marmorata* (Mollusca, Gasteropoda).

Extracts of *V. scorpioides* were detrimental to certain stages in the life cycle of *Sitotroga cerealella* (Lepidoptera, Gelechiidae).

Notes:

20. WHEAT ROOTLET GROWTH INHIBITION TEST OF AQUEOUS EXTRACTS OF SOUTH-AMERICAN PLANTS

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Phytotoxicity of aqueous extracts was tested by the wheat rootlet growth inhibition assay. Sixty two plant species belonging to tropical and temperate plant communities were studied. Thirty of these were collected in the Amazonian rainforest of Madre de Dios, Peru, while the remaining 32 were obtained from the central and southern provinces of Argentina. Differences in the inhibition and stimulation of the growth of wheat rootlets were observed among both communities. Eight plant species belonging to the neotropical community (26.7% of the total sample) showed considerable allelopathic activity, while 7 plant species (23.3% of the total sample) stimulated growth of rootlets. On the other hand, 23 plant species belonging to the temperate regions of Arhentina (71.9%) showed growth inhibition activity, while no plants in this sample stimulated the rootlets growth.

These results suggest a significant difference in the proportion of allelopathic inhibition activity in the wheat rootlet growth inhibition among the two plant communities ($p < 0.0001$). Significant differences in the proportion of plant species that stimulate the growth of the rootlets of wheat have also been observed among both communities ($p < 0.002$).

Notes:

21. EFFECTS OF ACETOPHENONES ON RED CLOVER (*TRIFOLIUM PRATENSE* L.) GROWTH

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The search for natural plant growth regulators as an alternative to the use of synthetic herbicides has taken off in the last few decades. Interference, which includes competitive and allelopathic effects and which is defined as the sum of all prejudicial effects that one plant exercises on another, is an indicator to guide research work in the search for bioregulators. In this respect, in 1977 Harborne put forward the hypothesis that acetophenone derivatives present in species of the Compositae were responsible for phytotoxicity, based on work carried out with 3-acetyl-6-methoxybenzaldehyde which is a compound isolated from *Encelia farinosa*.

Preliminary work carried out with an ethanolic extract of the creeping species *Baccharis magellanica* Pers. in which the presence of acetophenone derivatives was detected, allowed inhibitory effects of *p*-hydroxyacetophenone on the germination of clover seeds to be determined. Another effect observed was the selective inhibition the germination of seeds from traditional crops used in agriculture of Southern Chile and also from weeds frequently found growing among these crops.

This study reports on the results obtained in experiments carried out in a growth chamber with clover seedlings pre-germinated in sand using a nutritive solution with six different concentrations of *p*-hydroxyacetophenone (0, 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} M). The results showed significant statistical differences in the development of both roots and shoots of red clover at all levels studied.

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Notes:

22. THE APPLICATION OF AGGREGATION PHEROMONE OF THE *RHYNCHOPHORUS PALMARUM*

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The weevil *Rhynchophorus palmarum* is considered to be the most important pest of coconut plantation. This insect is a vector of the nematode *Rhadinaphelendus cocophilus* which causes the red-ring disease. In Brasil around 95% of the coconut plantations is found in the northeast region where the disease is widespread. The losses are high because it is necessary to burn the sick coconut tree. The traditional method of control of the pest is with the use of traps with plant tissue of sugarcane but the results are not satisfactory. The use of pheromone seems a good option for that plague. For that the aggregation pheromone rhynchophorol in the racemic form was synthesized by the Grignard reaction in good yield. The field application showed high activity when applied together with sugarcane and sugarcane syrup. The use of oil (mineral and coconut oil) did not have any additional effect.

EMBRAPA/Banco do Nordeste do Brasil

Notes:

23. MALE SEX PHEROMONE OF THE CRYPTOMERIA TWIG BORER, *ANAGLYPTUS SUBFASCIATUS*

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Male-released sex pheromone constituents of the Cryptomeria twig borer, *Anaglyptus subfasciatus* (Insecta: Coleoptera: Cerambycidae) are identified by GC-MS and GC-FTIR as a mixture of 3-hydroxy-2-hexanone and 3-hydroxy-2-octanone. These two compounds undergo thermal isomerization during GC analyses to give the corresponding 2-hydroxy-3-alkanones. Comparison of the retention times of the natural products with those of synthesized enantiomerically pure compounds revealed that both semiochemicals have (R)-stereochemistry. The absolute configuration were confirmed by comparisons of the methoxy (trifluoromethyl) phenylacetic acid esters (Mosher esters) of insect-derived and synthetic samples. Behavioral assays in the laboratory and trap captures in the field suggested that the mixture of (R)-stereoisomer of 3-hydroxy-2-hexanone and 3-hydroxy-2-octanone was a sexual attractant for *A. subfasciatus*.

Notes:

24. DROSOPHILA SEX PHEROMONES: STRUCTURE AND APPLICATION

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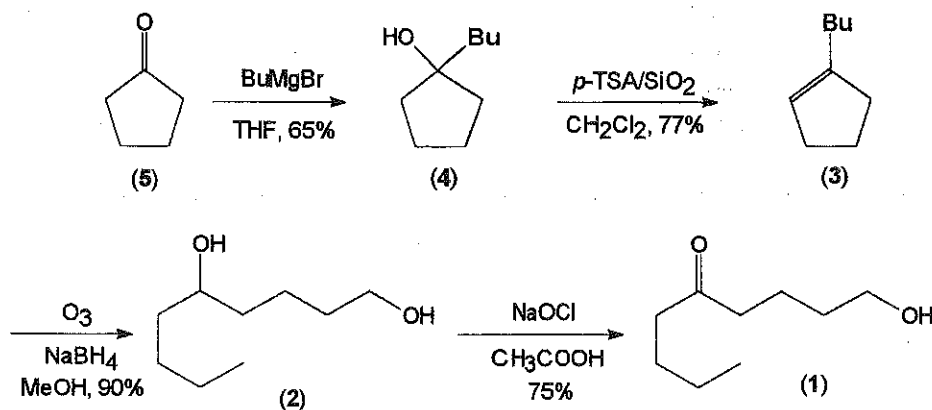
Drosophila isofemale strains have been established and are widely kept at many laboratories for the purpose of genetic studies. In our laboratory, the chemical structure of sex pheromone from cosmopolitan species, *D. ananassae* (HW strain) has been elucidated and that of localized species, *D. pallidosa* (NAN4 strain) which is closely related to *D. ananassae*, also. A qualitative and quantitative analysis method for the sex pheromones present in single fly was established by using on-column gas-liquid chromatograph. Males and females of two species (strains) can court actively under laboratory condition and F1 fertile-hybrids are obtainable. In the poster, chemical structures and application of single-fly analysis will be discussed.

Notes:

25. AN EXPEDITIOUS SYNTHESIS OF 1-HYDROXY-5-NONANONE: A VOLATILE EMISSION OF *BACTROCERA CACUMINATUS*

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1-Hydroxy-5-nonanone (1) was identified by Kitching¹ and co-workers as a component of the rectal glandular extract and volatile emission of male *Bactrocera cacuminatus*, a fruit-fly species located in Australia. In this work, a straightforward synthesis of (1) is described as shown below.



Addition of magnesium n-butyl bromide to cyclopentanone (5) afforded the alcohol (4). Endocyclic elimination of (4) with TSA/SiO₂ resulted in the olefin (3) which was submitted to reductive ozonolysis to give the diol (2). The hydroxy-ketone (1) was obtained by selective oxidation of the secondary hydroxyl group from the diol (2) with NaOCl in acetic acid.

¹Krohn, S., Fletcher, M.T., Kitching, W., Drew, R.A.I., Moore, C.J. and Francke, W. *J. Chem. Ecol.* 17, 485 (1991)

CNPq/FAPESP/IFS

Notes:

26. (Z,E)- α -FARNESENE - AN ELECTROANTENNOGRAM ACTIVE COMPONENT OF THE MALADERA MATRIDA VOLATILES

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It has been shown that virgin females of *Maladera matrida* Argaman (Coleoptera, Scarabaeidae) and their volatiles both with food (cut peanuts leaves) were efficient attractants for *M. matrida* males and females in field trapping experiments and laboratory olfactometer bioassays. GC-EAD experiments using male antennae and GC-MS experiments revealed that (Z,E)- α -farnesene is the active component of the *M. matrida* female volatiles. The identification and quantitative electrophysiological responses (EAG) of synthetic (Z,E)- α -farnesene were obtained with male and female antennae. It was also shown that (Z,E)- α -farnesene is not a component of the plant volatiles that serve as synergistic components of the mixture of attractants or of the source of food for *M. matrida*.

Notes:

27. EFFECT OF TEMPERATURE ON THE RELATIVE PERFORMANCE OF LIGHT AND PHEROMONE TRAPS TO CAPTURE HEMLOCK LOOPER ADULTS

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This study examined the influence of nightly temperature on the relative number of male and female hemlock looper, *Lambdina fuscicornis fuscicornis* caught in light (L), pheromone (P) and combined pheromone/light (PL) traps in Quebec in 1991 and 1992. Given that only males were captured in pheromone traps the data for each sex were analysed independently. Furthermore, to facilitate analyses the adult flight period was divided into warm and cool nights, when the nightly mean temperatures were >11 and <11 °C for both years, respectively.

PL traps caught more males than P or L traps alone under both temperature conditions. However, while the relative performance of PL and P traps was similar on warm and cool nights, the difference between PL and L traps were more pronounced under cool temperatures. The considerably lower number of females captured each night suggests the female flight activity is less than that of males. Furthermore, females were only caught on warm nights, at equal frequency in both L and LP traps which is not unexpected for as females do not respond to the sex pheromone the two trap types are effectively identical.

The periodicity of captures (at hourly intervals throughout the scotophase) in the three different traps was carried out in 1992. The pattern of male captures offers a possible explanation for the different performances of the L and P traps, relative to the PL traps. On warm nights, the peak capture in L traps occurred several hours earlier than in P or PL traps while on cool nights, they generally coincided in the hours immediately after sunset. Thus under cool conditions intertrap competition for males was higher and the greater active space of P and PL traps resulted in a better performance than light traps alone. The absence of female captures on cool nights is probably due to the earlier onset of calling than on warm nights. Calling behaviour is expressed by sedentary individuals and on cool nights by the time calling has terminated ambient temperatures have declined to a level when female flight activity would be limited.

Notes:

28. SEX PHEROMONE GLANDS IN *TOXOTRYPANA CURVICAUDA* (DIPTERA: TEPHRITIDAE)

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Males of *T. curvicauda* were dissected to locate sex pheromone glands like in other Tephritid species. These glands may be modified salivary glands, pleural glands, anal glands and rectal diverticula. Abdominal portion of salivary gland in males of *T. curvicauda* of different ages was studied by SEM to see the cellular column arrangement. Histology of organs involved in pheromone production was studied.

Sexually dimorphic salivary glands are present in males; these glands correspond to type II [Nation, 1974. Ann. Entomol. Soc. Amer. 67(5): 731-34]. In the pleural region of abdominal segments 3, 4 and 5, there are epidermic enlarged cells like pleural glands in other tephritid species. There is no evidence of anal glands and rectal diverticula.

Macerated salivary glands and green papaya fruit attract significantly more gravid females than fruit alone in bioassays. The difference is not significant with pleural glands and green papaya fruit. There is evidence by gas chromatography for sex pheromone in abdominal portion of salivary glands.

Notes:

29. PHYTOALEXINS IN SAPWOOD OF *CRYPTOMERIA JAPONICA*

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Cryptomeria japonica was inoculated by two strains of *Guignardia cryptomeriae*, causal fungus of Guignardia die-back of Japanese cedar. The pathogenicity of strain MA18 was strong and that of MA19 was weak. Rapid progresses of hyphal elongation of both strains, necrosis in inner bark and discoloration of sapwood were stopped at 2 weeks after inoculations. At that time, hyphal development in sapwood of strain MA18 was 2 to 6 times larger than that of strain MA19. The area of necrosis in inner bark and discoloration in sapwood were almost the same as that of hyphal invasion. Norlignans and related chemicals were detected as accumulated compounds in reaction zone. Antifungal activities of methanol extracts obtained from the reaction zone barrier to *G. cryptomeriae* were examined. Several active spots were appeared on *tlc*. Main phytoalexin in the barrier was identified as hinokiresinol from the analyses of its *nmr* and *ms* data. It rapidly accumulated in reaction zone barrier within 2 weeks after inoculation, but increasing rate of its amount was slow down after that time. It was already detected in discolored wood of *C. japonica* attacked by larvae of some boring insects, such as *Semanotus japonicus*.

Notes:

30. THE NATURAL INSECTICIDE AZADIRACHTIN, PRODUCED BY PLANT TISSUE CULTURE

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The natural insecticide azadirachtin, is obtained from the seeds of the neem tree (*Azadirachta indica*), grown widely in tropical areas. Yields of 0.5 to 0.8% of azadirachtin can be found in the seed kernels, but for commercial use, it must be separated from large quantities of oil (30-40% of the weight of kernels), and seed cake (30-45%).

We have been examining the possibility of producing azadirachtin from neem tissues directly (at ENEA) as a source of the insecticide. Initial work proved that azadirachtin could be produced in the tissue cultures, but a first, only in very low concentrations. However, by changing the physical and growth conditions, we have been able to improve the yield 50 times. Other compounds of interest which are known to be naturally occurring in the neem kernels have also been identified in the cultures. Isolated compounds are being assayed for biological activity.

Periodic sampling of media over a two week span has given an identification of the rate of formation of the important triterpenoids. Such data will be used in the study of the biological pathway of formation of the compounds.

Notes:

31. CHEMO-ATTRACTANTS FOR OVIPOSITION IN *TOXORHYNCHITES* MOSQUITOES

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Toxorhynchites mosquitoes do not blood feed and their larvae are predatory on other mosquito larvae. For this reason, it has been suggested that *Toxorhynchites* may be useful as biological control agents of the mosquitoes that are vectors of human pathogens such as malaria, dengue, yellow fever and lymphatic filariasis. This type of biological control has been attempted several times, sometimes successfully. *Toxorhynchites* mosquitoes naturally oviposit into broken nut shells, cocoa husks, holes in bamboo and pools of water in bromeliads. They are also known to oviposit frequently into tyres.

Chemical oviposition cues in *Toxorhynchites* mosquitoes were investigated using electroantennograms (EAGs). The stimuli were breakdown products of rotting plant material (skatole, 4-methylcyclohexanol, phenol, m-cresol, o-cresol, p-cresol and indole) and extracts of water which had contained prey larvae (*Aedes aegypti*). Oviposition bioassays were then carried out to determine the behavioural activity of these compounds. These data will be presented, together with conclusions concerning their possible use in biocontrol schemes.

Notes:

32. PHYSIOLOGICAL AND BEHAVIOURAL RESPONSIVENESS OF THE BITING MIDGE *CULICOIDES NUBECULOSUS* TO SEMIOCHEMICALS IN RELATION TO AGE, PHYSIOLOGICAL CONDITION AND NUTRITIONAL STATUS

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Experiments were carried out with Y-tube olfactometers to investigate how the behavioural sensitivity of the biting midge *Culicoides nubeculosus* varied according to age, physiological condition (teneral or mated and if recently blood fed (females only) and nutritional status (sugar fed or starved). Whether or not any observed changes in behaviour were related to changes in the sensitivity of the peripheral nervous system were investigated by making electroantennographic measurements. Three test odour sources were selected. These were a mammalian host-related odour (1-octen-3-ol), honey solution and volatiles from virgin female midges (*i.e.* sex pheromone producing). Significant differences between the behavioural but not the electrophysiological sensitivities of the different insect classes were recorded.

Notes:

33. SWEET GALE (*MYRICA GALE*): A NATURAL REPELLENT FOR PESTS OF MAN AND LIVESTOCK

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Electroantennogram (EAG) measurements and Y-tube olfactometer bioassays revealed the repellent properties of *Myrica gale* oil against a range of insect pest species. Firstly, gravid female greenbottle (*Lucilia sericata*) and bluebottle (*Calliphora erythrocephala*) flies during the period of their life cycle when they are actively searching for an oviposition site. EAG thresholds were 0.1% for both species and behavioural thresholds were 10^{-3} % and 10^{-2} % *M. gale* oil respectively. Secondly, haematophagous biting midges (*Culicoides nubeculosus* and *C. impunctatus*) and the yellow fever mosquito, *Aedes aegypti*; all test insects were mated, host-seeking females. EAG thresholds ranged between 10^{-4} % and 10^{-3} % and behavioural thresholds were 1% *M. gale* oil for both species. Comparisons with DEET and citronellol indicated a higher efficacy of *M. gale* with the range of insects investigated. The repellent properties of *M. gale* against the Scottish Highland biting midge, *C. impunctatus* were confirmed in field biting-trials with human subjects.

Notes:

34. OCCURRENCE AND BIOGENETIC RELATIONS OF SESQUITERPENES IN SENECEONEAE PLANTS AND THEIR ROLE IN A PROPOSED CHEMICAL DEFENCE AGAINST HERBIVORES

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Sesquiterpenes of the eremophilane type are together with pyrrolizidine alkaloids and benzofuranes characteristic constituents of plants of the tribe Senecioneae (family Asteraceae). Insect antifeeding effect of eremophilanes and of their furano or lactone derivatives has been described. However, nothing was known how these compounds act on herbivores that are feeding from Senecioneae plants. Therefore, a series of selected compounds from four plant species: *Adenostyles alliariae*, *Adenostyles alpina*, *Petasites albus* and *Senecio fuchsii* have been tested on the food choice of the specialized leaf beetles *Oreina cacaliae* and *Oreina speciosissima* and also of the generalist snail *Arianta arbustorum*.

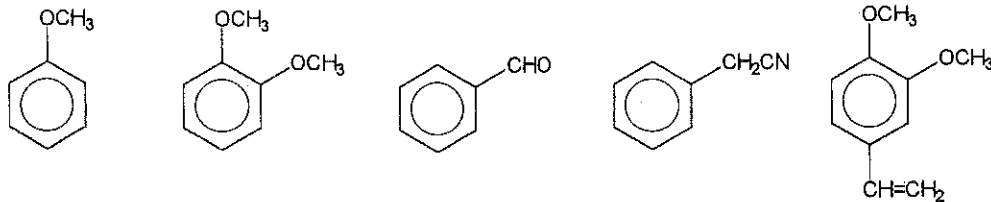
The active compounds in the leaf extracts were analyzed and identified, either by HPLC analysis with reference compounds, or by isolation and structural analysis of the individual compounds. Bakkenolide A, cacalol and a new compound, cacalol-trimer, were identified as active constituents. Biogenetic relations of the active compounds to the main Senecioneae eremophilanes were proposed and a possibility of formation of extraction artifacts will be discussed.

Notes:

35. MATURATION ACCELERATING COMPONENTS OF THE ADULT MALE PRODUCED AGGREGATION-MATURATION PHEROMONE SYSTEM OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA*

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Previously, we reported that accelerated maturation of young adults of both sexes of gregarious *Schistocerca gregaria* was associated with the volatiles of mature gregarious males, the major components of which were anisole, veratrole, benzaldehyde, phenylacetonitrile and 4-vinylveratrole (Chemoecology 1993 159-164). Subtraction bioassays we recently carried out with blend of these compounds show varying activities of the components as maturation accelerants. Of particular interest is the finding that phenylacetonitrile, the most potent aggregant, is also the most potent maturation accelerant. However, not all components show such dual effects. Thus, persimony associated with the adult pheromone blend appears to be due to two overlapping sub-blend.



Notes:

36. LOCALIZATION OF SECONDARY METABOLITES ON THE SURFACE OF *PSEUDOGNAPHALIUM* SPP.

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Electronic microscopy of some Chilean *Pseudognaphalium* spp. shows the characteristic presence of glandular and non-glandular trichomes on the surface of the leaves and stems. The combination of these superficial structures has been associated to the plant defensive mechanism against the attack of some insects.

The resinous exudate from *Pseudognaphalium cheiranthifolium*, *P. heterotrichum*, *P. robustum* and *P. vira vira*, obtained by dipping the fresh plant material in cold CH_2Cl_2 shows the presence of flavonoids and diterpenoids. From *P. cheiranthifolium* the following compounds were isolated: 3-methylgalangin, 3-methylalhusin, 5,8-dihydroxy-3,6,7-trimethoxyflavone, ent-16-kauren-19-oic acid, ent-3 β -hydroxy-16-kauren-16-oic acid and 13-*epi*-sclareol. A mixture of two acyl flavonoids was also isolated, the structural determination of these two compounds is now in progress. From *P. heterotrichum*, in addition to the compounds isolated from *P. cheiranthifolium*, 3,7-dimethylgalangin, and 9(11),16-kauradien-19-oic acid were isolated. From the *P. vira vira* only ent-16-kauren-19-oic acid was isolated.

HPLC and TLC studies have shown that the flavonoids and diterpenoids are stored in the head of the glandular trichomes.

Notes:

37. ANTIMICROBIAL ACTIVITY OF THE RESINOUS EXUDATES OF *HELIOTROPIUM* SPP

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Species of *Heliotropium* of the *Cochranea* section are endemic to the coastal hills of northern and central Chile. As many of the plants of that geographic area, they characteristically produce a resinous exudate that covers leaves and stems as a defense mechanism against extreme environmental conditions. The resinous exudates of *Heliotropium* spp shows the presence of flavonoids and aromatic geranyl derivates. The antimicrobial activity of these exudates in the development of *Escherichia coli* K-12 is shown in table 1 and the activity of the pure compounds isolated from *Heliotropium sinuatum* and *Heliotropium filifolium* is shown in table 2.

TABLE 1

Resinous exudates	time*(h)
control	0,60
standard (phenol)	1,23
solvent (DMSO)	0,89
<i>H. huascoense</i>	3,19
<i>H. megalanthum</i>	1,68
<i>H. filifolium</i>	3,25
<i>H. sinuatum</i>	1,65
<i>H. stenophyllum</i>	2,24
<i>H. chenopodiaceum</i> v. <i>ericoideum</i>	0,95
<i>H. chenopodiaceum</i> v. <i>typica</i>	0,67

* duplication time of *E. coli*

TABLE 2

Compounds	time*(h)
5,7,4'-trihydroxyflavanone	1,26
5,7-dihydroxy-3-methoxyflavone	1,07
5,3',4'-trihydroxy-7-methoxyflavanone	1,29
5,7,4'-trihydroxy-3,3'-dimethoxyflavone	1,00
5,4'-dihydroxy-3,7,5'-trimethoxyflavone	0,80
5,7-dihydroxyflavanone	0,83
3,5,7-trihydroxy-4'-methoxyflavanonol	2,59
filifolinol	1,11
filifolinol senecionate	0,75

* duplication time of *E. coli*

Filifolinol and filifolinol senecionate were the only pure compounds that shows increase of the antimicrobial activity after irradiation for 90 minutes at 366 nm. Table 3:

TABLE 3

Compounds	time*(h)
filifolinol	1,38
filifolinol senecionate	1,71

These results are in agree with the hypothesis that some compounds located in the surface of plants are activated by sun light.

Notes:

38. COLLATERAL SENSITIVITY OF P-GLYCOPROTEIN RICH MULTIDRUG-RESISTANT CELLS TO LIGNANS

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Lignans and their monomers can synergize a number of synthetic and natural insecticides, including carbamates, pyrethrins, azadirachtin and coumarins. As inhibitors of detoxification enzymes (PSMO's) in insects, lignans can increase the level of unmetabolized insecticides. In addition, lignans can increase the uptake of drugs selectively by multidrug-resistant cell with enhanced membrane-bound P-glycoprotein efflux pump. Both modes of action could contribute to alleviate resistance mechanisms in insects and other systems.

Notes:

39. EFFECT OF TWO TERPENES FROM MEXICAN PLANTS ON FALL ARMYWORM *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

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Plant metabolites as allomones are the insecticides of the future. Secondary plant metabolites such as terpenes are very important in plant response to fungi, bacteria and other pathogens. These terpenes may play an important role as new insecticide molecules.

Two terpenes from Mexican plants, piquerol A and 2B hydroxy 15, 16 epoxientlambda-8 (17), 13(16), 14 thriene (from *Brichellia* sp.) were examined in order to know their effect on fall armyworm *Spodoptera frugiperda* (Lep. Noctuidae).

125, 250 and 500 ppm of each one, were tested on artificial diet at laboratory conditions. Terpenes were isolated at the Instituto de Química of the Universidad Nacional Autónoma de México. There were 2 treatments and 4 replicates on random design.

Next table shows mortality of *S. frugiperda* larvae (10 per replicant) (by Abbot) at 24 hours.

Piquerol A		<i>(Brichellia sp)</i>	
Dose	Mortality %	Dose	Mortality %
125 ppm	87	125 ppm	23
250 ppm	87	250 ppm	23
500 ppm	90	500 ppm	17

¹Becario COFAA-IPN

Notes:

40. BIOLOGICAL ACTIVITY OF PLANTS ON *SPODOPTERA FRUGIPERDA* LARVAE

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Plants since long time ago, are sources of botanical insecticides. One approach to the control of pests in future agriculture, is the use of biodegradable natural plant compounds such as the botanical insecticides, azadirachtin, sabadilla and some others in place of synthetic insecticides such as malathion or acephate. Biotic Products Development Center of National Polytechnic Institute (CeProBi-IPN), is interested in those biological compounds from plants, that may be antifeeding, deterrents or insecticides, and take care of environmental and potential health hazards associated with heavy use of conventional pesticides.

We evaluated the biological activity of 5 plants from Mexico (Morelos state) *Leucopremma mexicana* (Caricaceae) known as "cuaguayote", *Okenia hypogaea* (Nyctaginaceae) known as "cacahuete de zorro", *Bromelia hemisphaerica* (Bromeliaceae) known as "timirichi", *Lupinus* sp. and *Lupinus montanus* (Fabaceae) as possible source of biological insecticides. Dried and ground seeds of *L. mexicana*, *B. hemisphaerica*, *Lupinus* sp., *L. montanus* and leaves of *O. hypogaea* were added (15%) to an artificial diet. Each treatment was arranged with 3 replicates and 30 larvae as total. Larval weight and length were recorded at 10 days and survival at 21. Pupation were recorded at the end experiment.

Treatments	10 days		21 days	
	WEIGHT g	LENGTH mm	MORT. %	PUPATION %
<i>L. mexicana</i>	0.1769	21	58	33
<i>O. hypogaea</i>	0.0273	11	60	0
<i>B. hemisphaerica</i>	0.0300	10	26.7	74
<i>L. montanus</i>	0.0410	11	16.7	64
<i>Lupinus</i> sp	0.0269	10	16.6	76
Control	0.1367	18	0	100

Results show a high insecticide activity of leaves of *O. hypogaea* and seeds of *L. mexicana* when they are eaten by larvae. Biochemical analysis are carry on to separate the insecticide compound.

*Becario COFAA-IPN

Notes:

41. BIOLOGICAL ACTIVITY OF EXTRACTS OF *RICINUS COMMUNIS* ON FALL ARMYWORM *SPODOPTERA FRUGIPERDA* (LEP.: NOCTUIDAE)

María Elena Valdés-Estrada¹, Alfredo Jiménez-Pérez¹, Rodolfo Figueroa-Brito and Federico Castrejón-Ayala¹. Centro de Desarrollo de Productos Bióticos-IPN. Apartado Postal 24, Apartado Postal 24, Yauatepec, Morelos, México

Noctuidae family is one of the major pests in the world. Many of their members are important pests in almost all kind of agriculture. In Mexico, the fall armyworm *Spodera frugiperda*, is the most important pest in subsistence com crops. It's control is based on synthetic insecticides and very few farmers can use it. The Centro de Desarrollo de Productos Bióticos (CEPROBI) is interested in all those alternative pesticide from natural origin. The objective of this research was to study the effect of *Ricinus communis* on *S. Frugiperda*. Fresh and dry leaves of *R. communis* were extracted in different conditions: Hexane (H); Acid-ethanol (Ath) at room temperature and at 78°C, Dichloroethane (D) and an aqueous-acid 2N solution (AW), and tested on first instar larvae. Extracts were added to an artificial diet at 15%. Larval weight and mortality were recorded at 7 and 23 days, pupation and emerging rate at the end of its' larvae stage.

Treatment	10 days			23 days			Pupation rate at 24 days	Pupation rate after 24 days
	% Mortality	Weight (g)	Length (mm)	% Mortality	Weight (g)	Length (mm)		
Dry (D)	0	0.014	9.0	16	0.155	16.3	33	33
Fresh (D)	20	nm	5.1	26.6	0.02	8.8	0	0
Dry (Ath)	0	nm	5.6	10	0.03	12.8	0	0
Fresh (Ath)	30	nm	5.7	57	0.02	9.7	0	0
Dry (H)	0	0.005	6.6	0	0.150	19.9	0	0
Dry (AW)	0	nm	5.0	0	0.03	12.9	0	0
Fresh (AW)	0	nm	5.4	0	0.04	13.0	0	0
Control	0	0.064	15.4	0	0.167	18	53	100

nm: non measurable

Notes:

42. ANTIFEEDANT ACTIVITY OF THE LEAVES EXTRACT OF THE NATIVE COLOMBIAN PLANT *BERBERIS MONGUIENSIS* SP.NV. L.A.C. (BERBERIDACEAE)

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Recent advances in Natural Products research suggest the rational investigation for bioactive compounds from native plants in order to decrease and control the present extensive application of wide spectra synthetic pesticides, whose poisonous residues seriously affect health and environment, inducing resistance in several noxious insects. In this field we are looking for potencial insecticidal, antifeedant and related properties of the extracts of the therapeutically used plants belonging to the genus *Berberis* (Berberidaceae) from Chile and Colombia¹⁻³. The genus *Berberis* is widely distributed in America and there are a lot of species with recognized popular applications as antibacterials, antimalaric and insecticidal activities⁴. Extracts of the roots and leaves of *B. glauca*, *B. monguiensis*, *B. montana* and *B. coletiodes* have been evaluated against the 3th and 4th instar larvae of *Spodoptera sumia* (Lepidoptera) and *Drosophila melanogaster* (Diptera) by contact and ingestion bioassays, in choice and no-choice tests. The chromatographic separation of the active extract of the leaves of the species *B. monguiensis* led to isolation and identification of three alkaloidal derivatives of the bisbenzylisoquinolinic type (bbi)⁵ belonging to the series of isothalicberine, previously reported in *B. laurina* and *B. chilensis*⁶. The active fraction and two of the compounds isolated led to notable physical and behavioral changes in the insects. These results will be discussed together with the structural analysis based on data from spectroscopical techniques (IR, UV, ¹H-NMR and 2D-COSY experiments). We are greatly indebted to Colciencias (008-94) and Fondecyt (1941117) for financial support.

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Notes:

43. ASPECTS ON BIOASSAY GUIDED FRACTIONATION OF COMPLEX PLANT EXTRACTS USING ADSORPTION CHROMATOGRAPHY AND ACCELERATING GRADIENT ELUTION

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For quite some time we have been studying the effect of polarity gradients in connection with liquid straight phase adsorption chromatography. Our results have lead us to higher loadings than is common practice in most laboratories. The method enables a rapid group separation of crude plant extracts in amounts sufficient for further investigations. Equipment and methodology will be presented as a poster. Examples including a highly efficient method for isolating azadirachtin from ground Neem seeds by combined extraction and chromatography in the same column will be shown.

The oral presentation of the poster will emphasize the advantages of using gradients with a strong eluant to enable the detection and isolation of constituents that are present in relatively small amounts in the extracts. The pit falls of using isocratic and stepwise gradient elution will be illustrated by computer simulated chromatography.

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4		To Sales		200	0
5		To Purchases	200		0
6		To Salaries	100		0
7		To Rent	50		0
8		To Interest	25		0
9		To Dividend	125		0
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