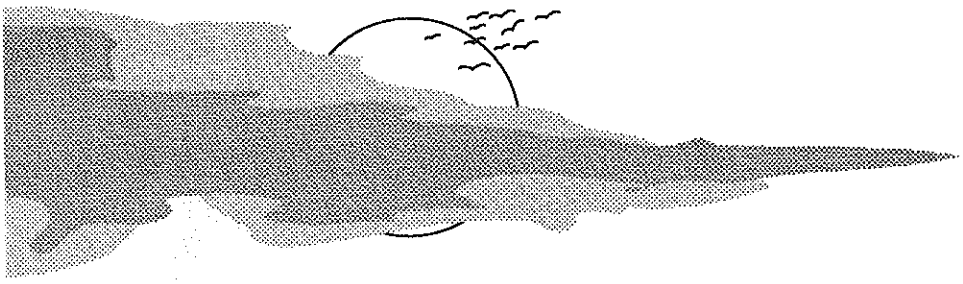
JOINT MEETING

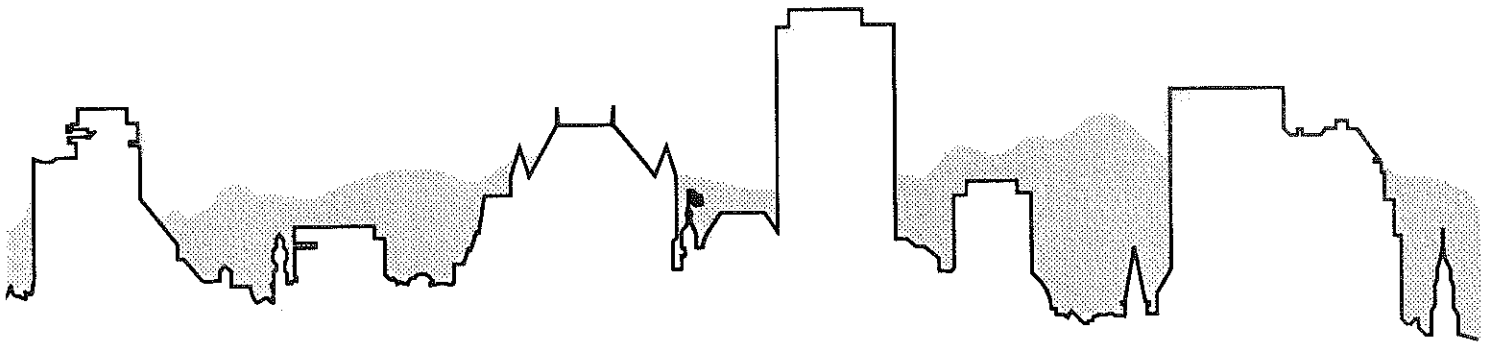
INTERNATIONAL SOCIETY OF CHEMICAL ECOLOGY

and PHYTOCHEMICAL SOCIETY OF NORTH AMERICA

LAVAL UNIVERSITY
QUEBEC CITY, P.Q.
CANADA



11 - 15 August, 1990



OFFICIAL PROGRAMME

UNIVERSITÉ LAVAL

Meeting Co-Chairpersons

Jeremy N. McNeil ISCE
Murray B. Isman PSNA

ISCE Symposium Organisers

Clive G. Jones
Jeremy N. McNeil

PSNA Symposium Organisers

Nikolaus H. Fischer
Murray B. Isman

Local Arrangements

Jeremy N. McNeil
Marthe Lemieux
Lucie Royer

PROGRAMME OUTLINE

Saturday, 11 August	13:30-17:30	Registration
	18:00-19:30	Opening Reception
Sunday, 12 August	09:00-12:30	Symposium
	12:30-14:00	Lunch
	14:00-17:00	Symposium
	17:30-19:45	PSNA Student Paper Competition
Monday, 13 August	09:00-12:30	Symposium Poster Presentations
	12:30-14:00	Lunch
	14:00-17:30	Symposium Poster Presentations
	17:30-19:30	Annual business meetings of ISCE and PSNA
Tuesday, 14 August	08:30-12:15	Oral Presentations
	09:00-12:00	Poster Presentations
	12:15-14:00	Lunch
	14:00-17:00	Oral Presentations Poster Presentations
	18:00	Buses leave for the Château Frontenac
	18:30-19:30	Pay Bar
	19:30-22:00	Banquet
Wednesday, 15 August	08:15-12:15	Oral Presentations

SATURDAY, 11 AUGUST

13:30-19:30 **Registration** - Foyer, Pavillon Lacerte

18:00-19:30 **Opening Reception** - Grand Salon, Pavillon Lacerte

SUNDAY, 12 AUGUST

08:45-09:00 **Official Opening** - Room 1C, Pavillon De Koninck

I.S.C.E SYMPOSIUM PART I

Room 1C, Pavillon De Koninck

Moderator: J.N. McNeil

09:00-09:45 **D.L. Marshall.** The potential for chemical mediation of non-random mating in plants.

09:45-10-30 **I.T. Baldwin.** Ecological constraints on plant chemical defenses: The case of nitrogen and nicotine in *Nicotiana.*

10:30-11:00 *Coffee*

11:10-11:45 **M. Rowell-Rahier and J.M. Pasteels.** Chemical specialization on toxic plants as a defensive tool: Ecological reality or fantasy?

11:45-12:30 **J.H. Himmelman, M. Starr and J.C. Therriault.** Coupling of marine invertebrate spawning with larval food abundance using chemical cues.

12:30-14:00 *Lunch*

SUNDAY, 12 AUGUST

P.S.N.A SYMPOSIUM PART I
Room 1C, Pavillon De Koninck

Moderator: N.H. Fischer

14:00-14:45 **K. Hostettmann and A. Marston.** New developments in the separation of natural products.

14:45-15-30 **J.P. Foley.** Supercritical fluid chromatography and capillary zone electrophoresis for the analysis of natural products.

15:30-16:15 **C. Pidgeon, W. Chae, C. Luo, C.R. Lombardo and P. Low.** Immobilized artificial membranes.

16:15-17:00 **O. Spring.** Trichome microsampling of sesquiterpene lactones for the use of systematic studies.

17:00-17:30 *Coffee*

P.S.N.A STUDENT PAPER COMPETITION
Room 1C, Pavillon de Koninck

Moderator: M.B. Isman

17:30-17:45 **R.R. Aucoin, B.J.R. Philogène and J.T. Arnason.** Antioxidants and antioxidant enzymes as a defense against photo-activated plant secondary compounds in herbivorous insects.

17:45-18:00 **E. De Carolis and V. De Luca.** Partial purification and characterisation of a 2-oxoglutarate dependent dioxygenase from *Catharanthus roseus.*

18:00-18:15 **J.D. Bacheller and J.T. Romeo.** Salinity, soil aeration and herbivory effects on amino acid and betaine chemistry of *Spartina alterniflora.*

SUNDAY, 12 AUGUST

18:15-18:30 **J.I.M. Rajaonarivony, J. Gershenzon and R. Croteau.** Partial purification and characterization of limonene cyclase from *Mentha piperita* L. CV. black mitcham (Peppermint).

18:30-18:45 **D.C. Gagnon and J.T. Arnason.** Neem-based antifeedants and their effect on European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae).

18:45-19:00 **T.C. Morton and J.T. Romeo.** Two new trihydroxypipecolic acids from *Inga paterno* (Mimosoideae).

19:00-19:15 **M.A. Menelaou, N.H. Fisher, M. Hjortso, M. Foroozesh and T. Thibodeaux.** Biosynthetic studies of thiophenes from hairy root cultures of *Tagetes patula* using ^{13}C-labeled sodium acetate.

19:15-19:30 **Ma.E.J. Inciong, E. Yamamoto, L. Davin and N.G. Lewis.** CIS-monolignols and their glucosides in beech bark tissue: Enzymology and their involvement in lignification.

19:30-19:45 **T.J. Savage and R. Croteau.** Characterization of monoterpene cyclase activity in conifers.

MONDAY, 13 AUGUST

P.S.N.A SYMPOSIUM PART II
Room 1C, Pavillon De Koninck

Moderator: N.H. Fischer

09:00-09:45 **J. St. Pyrek.** The role of mass spectrometry in structure elucidation of natural products.

09:45-10:30 **D.L. Smith.** Methods of mass spectrometry for the structure elucidation of natural products.

10:30-11:00 *Coffee*

11:00-11:45 **N.H. Fischer.** New NMR methods in phytochemical studies.

11:45-12:30 **J. Gershenzon.** Biosynthetic methods for plant natural products: New procedures for the study of glandular trichome constituents.

12:30-14:00 *Lunch*

I.S.C.E SYMPOSIUM PART II
Room 1C, Pavillon De Koninck

Moderator: C.G. Jones

14:00-14:45 **N.C. Negus.** Plant chemical cues, phenotypic plasticity and population dynamics.

14:45-15:30 **L.M. Gosling.** The role of scent marking in the assessment of opponents by male mammals.

15:30-16:00 *Coffee*

16:00-16:45 **V.A. Krischik.** Generalized plant defense: Effects on multiple species.

16:45-17:30 **R.K. Colwell.** Rapid discrimination between host plants and non-hosts by hummingbird flower mites on the basis of nectar chemistry.

POSTER PRESENTATIONS

09:00-17:-00 2rd Floor Hall, Pavillon De Koninck
See pages 35-57 for titles and abstracts.

MONDAY, 13 AUGUST

ANNUAL GENERAL BUSINESS MEETINGS

17:30-19:30 **I.S.C.E.** - Room 1B, Pavillon De Koninck

P.S.N.A. - Room 1D, Pavillon De Koninck

TUESDAY, 14 AUGUST

SUBMITTED PAPER SESSION I

Room 1B, Pavillon de Koninck

Moderator: J.T. Arnason

8:30-08:45 **A.M. Zobel and S.A. Brown.** Histological localization of psoralens in seeds and fruits.

8:45-09:00 **A.M. Zobel, S. Louis and K. Mwiraria.** Mechanisms of action of psoralen and xanthotoxin on mitotically active cells.

09:00-09:15 **R.J. Marles, J.T. Arnason, R. L. Compadre and C.M. Compadre.** Applying quantitative structure-activity relationship techniques to natural products: Phototoxic thiophenes.

09:15-09:30 **A. Walther.** Effect of salinity stress on seedling chlorophyll of *Liatris punctata* Hook.

09:30-09:45 **H.E. Nordby and R.E. McDonald.** Relationship of epicuticular wax fatty aldehydes to chilling injury in stored grapefruit.

09:45-10:00 **O.T. Chortyk and R.F. Severson.** The use of GC and GC/MS to characterize the sucrose esters of *Nicotiana* species.

10:00-10:30 *Coffee*

10:30-10:45 **C.A. McIntosh, L. Latchinian and R.L. Mansell.** Flavonone-specific 7-glucosyl-transferase activity in *Citrus paradisi* seedlings: Purification and characterization.

10:45-11:00 **H.E. Khouri.** Protein engineering of papain: Designing new properties into an old enzyme!

TUESDAY, 14 AUGUST

11:00-11:15 **J.A. McCallum, G.H.N. Towers, S. Tachibana, I.E.P. Taylor and T. Arnason.** Photochemistry of hydroxycinnamate esters in grass cell walls.

11:15-11:30 **T. Vogt, D. Strack and B. Ellis.** Sinapine metabolism within the Brassicaceae.

11:30-11:45 **L. Davin, T. Umezawa and N.G. Lewis.** Lignan biosynthesis in *Forsythia* species.

11:45-12:00 **G.H.N. Towers, F. Balza, R. Hartley, M. Rodgers, S. Tachibana, J. McCallum, E. Neeland, V. Rathore and T. Arnason.** Phenylpropanoid photochemistry of grass cell walls.

12:00-12:15 **Y. Hua, M.D. Bentley and B.J.W. Coole.** Triterpenes from two *Betula* species.

12:15-14:00 *Lunch*

TUESDAY, 14 AUGUST

SUBMITTED PAPERS, SESSION II

Room 1D, Pavillon De Koninck

Moderator: J. R. Aldrich

08:45-09:00 **S. Schulz.** Volatile compounds from male butterflies.

09:00-09:15 **S.A. Teale, A. Zhang, G.N. Lanier and F.X. Webster.** Detection and identification of a cryptic pheromone from *Ips pini* (Coleoptera: Scolytidae).

09:15-09:30 **T. Simon and A. Hefetz.** Trail-following responses of *Tapinoma simrothi* (Formicidae: Dolichoderinae) to pygidial gland extracts.

09:30-09:45 **C. Everaerts, A. Perrin and O. Bonnard.** Intraspecific recognition in *Kalotermes flavicollis* (Isoptera, Kalotermitidae).

09:45-10:00 **J.R. Aldrich.** Identification and attractiveness of a major pheromone component for nearctic *Euschistus* Spp. stink bugs (Hemiptera: Heteroptera: Pentatomidae).

10:00-10:30 *Coffee*

10:30-10:45 **M.K. Stowe.** Various araneiod spiders attract fly and/or moth prey.

10:45-11:00 **J. N. McNeil.** Comparison of calling behaviour in two geographically isolated populations of the true armyworm.

11:00-11:15 **D. Rittschof, A. Oliver, R.B. Forward Jr. and B.W. Erickson.** Structure-function studies of crab pheromone mimics.

11:15-11:30 **R.T. Mason and T.H. Jones.** The sex attractiveness pheromone of the guam brown tree snake, *Boiga irregularis*, identified?

11:30-11:45 **D. Müller-Schwarze and P.W. Houlihan.** Pheromonal activity of phenols and ketones in beaver castoreum.

11:45-12:00 **R. Cocke.** Alarm chemosignals and immune processes.

12:00-14:00 *Lunch*

TUESDAY, 14 AUGUST

SUBMITTED PAPER SESSION III

Room 1B, Pavillon De Koninck

Moderator: J.V. Lovett

14:00-14:15 **F.A. Einhellig, I.F. Souza and M.J. Haar.** Inhibition of growth and photosynthesis by sorgoleone.

14:15-14:30 **U. Blum, K. Klein, L.D. Holappa, S-W. Lyu, T.R. Wentworth and L.D. King.** Phenolic acid content of soils from soybean cropping systems.

14:30-14:45 **J.V. Lovett and D. Liu.** Chemical communication and defence in barley. CANCELLED

14:45-15:00 **J.Jiménez-Osornio and J. Kumamoto.** Allelopathic activity of *Chenopodium ambrosioides* essential oil.

15:00-15:15 **L.D. Holappa and U. Blum.** Effects of ferulic acid, a potential allelopathic agent, on the endogenous levels of abscisic acid in tomato.

15:15-15:30 **G.R. Leather.** *Crotalaria juncea* seeds contain a potent phytotoxin.

15:30-16:00 *Coffee*

16:00-16:15 **K.W. Yun, B-S. Kil, and J.-S. Park.** Identification of growth inhibitor from *Artemisia princepts* var. *orientalis*.

16:15-16:30 **Inderjit and K.M.M. Dakshini.** Investigations on some aspects of chemical ecology of cogongrass (*Imperata cylindrica* (L.) Beauv.)

16:30-16:45 **M. Essenberg, G.D. Davis and G. Davila-Huerta.** Biosynthesis of sesquiterpenoid phytoalexins in cotton foliar tissue.

16:45-17:00 **A.M. Brinker and D.S. Seigler.** Identification of piceatannol as a phytoalexin of sugarcane.

TUESDAY, 14 AUGUST

SUBMITTED PAPER SESSION IV

Room 1D, Pavillon De Koninck

Moderator: J.D. Olechno

14:00-14:15 **R. Jullien and F. Perez, J.C. Biemont, A. Ouedrago and J. Pouzat.** Modification of cuticular hydrocarbons in diapausing insects.

14:15-14:30 **L. Varin, N. Brisson, V. De Luca and R.K. Ibrahim.** Molecular cloning of flavonol 3-sulfotransferase (ST) from *Flaveria*.

14:30-14:45 **A. Nahrstedt, S. Franzl and E. Müller.** Enzymology of cyanogenesis in larvae of *Zygaena trifolii* (Zygaenidae - Lepidoptera).

14:45-15:00 **W.S. Bowers and F. Ortego.** Grasshopper spit: What is the nature of its "tobacco juice"?

15:00-15:15 **C. Everaerts**, J.L. Le Quere, Y. Roisin and J.M. Pasteels. Sesquiterpenoids in *Nasutitermes* soldier defensive secretion.

15:15-15:30 **J.D. Olechno, J.R. Thayer, C.A. Pohl, R.W. Slingsby and J.A. Statler.** High performance ion exchange chromatography in the analysis of plant materials.

15:30-16:00 *Coffee*

16:00-16:15 **D. Barron.** Use of HPLC in prefractionation and preparation isolation of natural volatile compounds.

16:15-16:30 **J.D. Olechno, J.M.Y. Tso, A. Wainright and J.R. Thayer.** Capillary electrophoresis: A new weapon in the phytochemists' arsenal.

16:30-16:45 **A.L. Anaya J.M. Ramos and J.A. Arevalo.** Use of an IRGA (infra-red gas analyser) to evaluate the effects of volatile allelochemicals on the gas exchange of leaves.

16:45-17:00 **J.D. Olechno, D.A. Weitz and D. Felix.** Supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) for the analysis of plant materials.

TUESDAY, 14 AUGUST

POSTER PRESENTATIONS

09:00-17:00 2rd Floor Hall, Pavillon De Koninck
See pages 43-57 for titles and abstracts.

18:00 **Buses leave Pavillon Lacerte for the Chateau Frontenac**

18:30 *Pay Bar*

19:30-22:00 **Banquet**

WEDNESDAY, 15 AUGUST

SUBMITTED PAPER SESSION V

Room 1C, Pavillon De Koninck

Moderator: L.P. Brower

08:15-08:30 **D.M. Norris.** Chemical defense in *Glycine max* against polyphagous versus oligophagous herbivores. CANCELLED

08:30-08:45 **C.A. McDaniel.** Major antitermitic compounds from the heartwood of northern white cedar, *Thuja occidentalis* L.

08:45-09:00 **D.P. Richardson and A.C. Messer.** Identification and preparation of anti-insectan dienols from *Dipterocarpus kerrii* tree resins.

09:00-09:15 **S.H. Sennett, A.M. Wright, S.A. Pomponi, J.E. Armstrong and R. Willoughby.** Cellular localization and ecological role of a secondary metabolite from the sponge *Hymeniacidon heliophila*.

09:15-09:30 **E. Rodriguez and R. Wrangham.** Zoopharmacognosy: Non-nutritional use of secondary chemicals by vertebrates. CANCELLED

09:30-09:45 **J. Wyman, Y. Maufette, J. Martel and J.P. Renaud.** Changes in leaf chemistry related to forest decline.

09:45-10:00 **M.B. Isman and M.J. Smirle.** Differential fate of plant allelochemicals in two polyphagous insects.

10:00 *Coffee*

10:30-10:45 **K.S. Johnson and J.M. Scriber.** Phytochemical basis for differential host use by wild silk moths (*Callosamia*: Saturniidae) larvae.

10:45-11:00 **S.Z. Merritt.** Combined effects of amino acids and glucosinolates on preference and performance of the green peach aphid (*Myzus persicae* Sulz.).

11:00-11:15 **N.M. Targett, A.E. Boettcher and T.E. Targett.** Marine plant polyphenols: Their effect on assimilation efficiency in the temperate herbivorous marine fish, *Xiphister mucosus*.

WEDNESDAY, 15 AUGUST

11:15-11:30 **L. Legal, M. Katz, B. Chappe and J.M. Jallon.** Toxicity of *Morinda citrifolia* on *D. melanogaster* and related species.

11:30-11:45 **L.P. Brower.** Costs, benefits and exaptations of cardenolide utilization in the chemical defense of the monarch butterfly, *Danaus plexippus*.

11:45-12:00 **D.E. Champagne and E.A. Bernays.** The role of nutrients in host-plant selection by insects.

Sunday 09:00 **Symposium Paper 1**

THE POTENTIAL FOR CHEMICAL MEDIATION OF NON-RANDOM MATING IN PLANTS

Diane L. Marshall, Dept of Biol., Univ. of New Mexico. Albuquerque, NM, USA.

Plant mating is non-random whenever the actual paternity of seeds is different from that which would be achieved by a random assortment of the pollen available. Non-random mating can alter the fitness of pollen donors, seed parents, and progeny. While it may occur either before or after pollen arrives on stigmas, I will concentrate on post-pollination mechanisms of non-random mating. These include differential pollen germination, pollen tube growth and fertilization and differential seed and fruit filling. While the possibility is little studied, these mechanisms may be mediated by chemical signals from pollen grains, pollen tubes, maternal plants, and embryos, just as animal mating behavior can be affected by pheromonal signals. The chemical signals may mediate competition among pollen donors and/or mate choice by the maternal plants. Recent data from wild radish suggest that all of the mechanisms of post-pollination non-random mating operate. Further, studies of pollen germination suggest direct interference among pollen from different donors. These data will be discussed in terms of data from the literature that suggest chemical mediation of these interactions.

Sunday 09:45 **Symposium Paper 2**

ECOLOGICAL CONSTRAINTS ON PLANT CHEMICAL DEFENSES: THE CASE OF NITROGEN AND NICOTINE IN *NICOTIANA*

Ian T. Baldwin, University at Buffalo, SUNY Buffalo, Buffalo, NY 14260, USA.

Ecologists interested in the patterns of secondary metabolites in plants have proposed a set of hypotheses that range from the largely ecological (Plant Apparancy Theory) to the largely physiological (Carbon-Nutrient Theory) which thoroughly treat the **Inter**specific patterns. However, the **Intra**specific patterns of chemical defense have received less attention. The ecological/physiological constraints on the intraspecific patterns of chemical defense will be explored in the genus*Nicotiana*. Particular emphasis will be placed on the patterns of alkaloid concentration both before and after herbivory and how these patterns fit ecological and physiological predictions. The alkaloidal responses to leaf damage in plants grown over a range of nitrate supply rates suggest that these responses to damage are important in the transport of nitrogen from root to shoot after herbivory. A nitrogen-transport function for a damage-induced alkaloid response highlights the integration of defensive and physiological responses to herbivory in plants.

Sunday 11:00 **Symposium Paper 3**

CHEMICAL SPECIALIZATION ON TOXIC PLANTS AS A DEFENSIVE TOOL: ECOLOGICAL REALITY OR FANTASY?

Martine Rowell-Rahier, Zoological Institute, Rheinsprung 9, Basel 4051, Switzerland and J.M. Pasteels, Faculté des Sciences, ULB, av. F. D. Roosevelt 50, Brussel 1050, Belgium.

The leaf beetles of the genus *Oreina* feed on herbaceous plants of the families Apiaceae and Asteraceae both of which are known to be rich in toxins, e.g. coumarins in the former and alkaloids (pyrrolizidin N-oxides) in the latter. However, only 2 of the 9 species studied so far are able to sequester plant toxins for their own defense. The others biosynthesize costly autogenous cardiac glycosides. We study the food plant preferences and genetic relationships of several species, as well as their mode of chemical defense and reproductive biology, to try to determine what favors the utilization of plant toxins for defense. The results show that sequestration of alkaloids in *O. cacalia* and *O. speciosissima* is associated with a host plant shift and is a derived condition. The process of sequestration is chemically specific. In *O. cacaliae* it has completely replaced autogenous defense, whereas in *O. speciosissima* does not feed on plants rich in PA N-oxides even when they are available. Thus this species is physiologically able to sequester toxins but does not utilize this possibility in the field.

Sunday 11:45 Symposium Paper 4

COUPLING OF MARINE INVERTEBRATE SPAWNING WITH LARVAL FOOD ABUNDANCE USING CHEMICAL CUES

John H. Himmelman and Michel Starr, Dép. biologie, Université Laval, Québec, G1K 7P4 and Jean-Claude Therriault, Ministère des Pêches et des Océans, Mont-Joli, Québec G5H 3Z4, Canada.

We examined the mechanisms coupling spawning (release of gametes or larvae) with the spring phytoplankton bloom in three phylogenetically separated invertebrates, urchins (*Strongylocentrotus droebachiensis*), mussels (*Mytilus edulis*) and barnacles (*Balanus balanoides*). Gamete release in urchins and mussels is triggered by a heat-stable metabolite released by various species of phytoplankton. Mussels require a higher phytoplankton density for a maximum response than urchins, probably because mussels are exposed to higher concentrations of phytoplankton as a result of their filtering activity. The spawning substance is readily extracted from phytoplankton cells using 1 N NaOH and is likely phenolic in nature. Its spectral properties resemble the "yellow substances" previously described for natural sea water. Phytoplankton similarly stimulates the release of nauplii larvae in barnacles but, in contrast to spawning in urchins and mussels, contact with the plankton cells is required. In addition to being a source of food, phytoplankton as a spawning cue probably integrates numerous physical and biotic factors indicating favourable conditions for larval growth and survival. Similar direct coupling of the larval phase with phytoplankton blooms may be common among marine invertebrates.

Sunday 14:00 Symposium Paper 5

NEW DEVELOPMENTS IN THE SEPARATION OF NATURAL PRODUCTS

Kurt Hostettmann and A. Marston, Institute of Pharmacognosy and Phytochemistry, University of Lausanne, rue Vuillermet 2, CH-1005 Lausanne, Switzerland.

Obtention of pure constituents from a crude extract of biological origin remains a crucial step in any research on natural products. The past few years have seen an increasing interest in chromatography systems involving liquid-liquid partition. Droplet counter-current chromatography (DCCC) has found numerous applications but is limited by time considerations and by the choice of solvent systems. The newly emerging technique of centrifugal partition chromatography (CPC) has done much to overcome these limitations and provides a very useful addition to the methods available for the separation of different classes of compounds. CPC has been used for the separation of simple mixtures and complex plant extracts, containing both polar and non-polar constituents (saponins, flavonoids, coumarins, anthranoids and alkaloids).

Sunday 14:45 Symposium Paper 6

SUPERCRITICAL FLUID CHROMATOGRAPHY AND CAPILLARY ZONE ELECTROPHORESIS FOR THE ANALYSIS OF NATURAL PRODUCTS

Joe P. Foley, Dept. of Chemistry, 232 Choppin Hall, Louisiana State University, Baton Rouge, Louisiana, USA.

Two emerging separation technologies, supercritical fluid chromatography (SFC) and capillary zone electrophoresis (CZE), are reviewed and assessed for their applicability to the analysis of natural products. SFC utilizes the unique properties of a supercritical fluid (a gas above its critical pressure compressed beyond its critical pressure) to achieve separations of certain compounds that are impractical or impossible to perform by modern gas or liquid chromatography. Such compounds include those that are thermally unstable and/or nonvolatile (high molecular weight) that do not have a UV chromophore. First reported in 1962, SFC did not come of age until the early 1980's due to advances in the supporting technology. CZE is an even newer separation methodology (late 1980's) that utilizes very high voltages (typically 15-30 kV) applied across long, very small diameter fused silica capillaries (e.g., 1 m long and 100 mm i.d.) to achieve separation of sample constituents via their differential electrophoretic flow velocities. CZE can provide information that is complementary to that provided by chromatographic separations. The differences between CZE and slab gel electrophoresis are, in many ways, analogous to the differences between HPLC and classical column chromatography.

Sunday 15:30 **Symposium Paper 7**

IMMOBILIZED ARTIFICIAL MEMBRANES

C. Pidgeon[1], W. Chae[1], C. Luo[1], C.R. Lombardo[2], P. Low[2], [1]Department of Medicinal Chemistry and Pharmacognosy and [2]Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA.

Immobilized Artificial Membranes (IAM) are solid-phase-membrane-Mimetics. Synthesis of IAM entails bonding cell membrane lipid molecules to solid surfaces at high molecular surface densities. IAM surfaces are intended to mimic the lipid environment of cell membranes and consequently the initial applications of IAM relate to endogenous solute-membrane interactions. Non-chromatographic applications of IAM are reconstitution of phospholipase D (unpublished observation), and the correlation of drug-binding to IAM with drug-transport through human skin. Chromatographic applications include the purification of cytochrome p450, and the purification of other membrane proteins. Although both non-chromatographic and chromatographic applications are evolving, the most useful application(s) of IAM will be for the purification of membrane proteins. Our current understanding of IAM chromatography for the purification of proteins will be presented.

Sunday 16:15 **Symposium Paper 8**

TRICHOME MICROSAMPLING OF SESQUITERPENE LACTONES FOR THE USE OF SYSTEMATIC STUDIES

Otmar Spring, University of Tuebingen, Institute of Biology, Auf der Morgenstelle 1, D-7400 Tuebingen, F.R.G.

Chemical investigation of secondary plant metabolites, in the past, has been conducted by means of intensive extraction of large amounts of plant material and subsequent time consuming spectroscopic measurements. Meanwhile, modern techniques for separaton and analysis of plant extracts have been developed and should be applied in order to reduce costs, to save time and to provide complete data on the biochemical constitution of plants for other scientific disciplines. Advantageously, sesquiterpene lactones - the characteristic compounds of Asteraceae - are often located in special compartments like glandular trichomes from which they can be sampled in almost pure form. HPLC analysis allows establishment of compound profiles of plants in only a few minutes with detection of even minor constituents of less than 1 ppm in tissues. Peak assignments, via reference compounds, provide information on the chemical constitution of a specimen. This enables previously unaffordable broad screening of plant material for investigation of intraspecific variation and chemotaxonomic relationship within complete systematic groups. The potential of this technique and its impact on taxonomy will be discussed with special focus on *Helianthus* and related genera.

Monday 09:00 **Symposium Paper 9**

THE ROLE OF MASS SPECTROMETRY IN STRUCTURE ELUCIDATION OF NATURAL PRODUCTS

Jan St. Pyrek, Division of Medicinal Chemistry and Life Science Mass Spectrometry Facility, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536, USA.

Mass Spectrometry (MS), since its first use for organic compounds, is intensely applied to structural and analytical problems facing natural product chemistry. These applications have made significant contribution to the mutual development of both fields. The simplicity of information provided, great sensitivity, precision, and selectivity of detection are the major virtues of MS methods. In addition, new ways of ion generation and the direct combination with chromatography, permit the extension of MS analysis to vary polar and high molecular weight compounds. In this context a systematic review of major MS methods, the information they provide, and questions they could answer will be attempted.

Monday 09:45 Symposium Paper 10

METHODS OF MASS SPECTROMETRY FOR THE STRUCTURE ELUCIDATION OF NATURAL PRODUCTS

David L. Smith, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, W. Lafayette, IN 47907, USA.

The traditional uses of mass spectrometry for structure elucidation of natural products include determination of molecular weight and elemental composition. Fragmentation patterns have also been used to provide important structural information. During the past decade, mass spectrometric methods have advanced in many areas, especially with respect to the types of compounds that can be analysed. Despite the excitement over these new capabilities (MW determination to 100,000!), traditional methods of mass spectrometry continue to play an important role in the structure elucidation of natural products. This presentation will illustrate the use of traditional as well as new methods of mass spectrometry for determining the structures of acetogenins.

Monday 11:00 Symposium Paper 11

NEW NMR METHODS IN PHYTOCHEMICAL STUDIES

Nikolaus H. Fischer, Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana, 70803, USA.

Recent developments in high-field NMR spectroscopy have provided a powerfull tool for structural and biosynthetic studies of natural products. Computer-controlled pulse sequences for one- and two-dimentional NMR experiments have dramatically increased the sensitivity and efficiency of NMR spectral data. In spite of the many confusing NMR spectroscopist's jargons that have been introduced for the various pulse sequences, emphasis will be on selected examples to illustrate the power of NMR spectroscopy in solving structural and biosynthetic problems of various types of natural products.

Monday 11:45 Symposium Paper 12

BIOSYNTHETIC METHODS FOR PLANT NATURAL PRODUCTS: NEW PROCEDURES FOR THE STUDY OF GLANDULAR TRICHOME CONSTITUENTS

Jonathan Gershenzon, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340 USA.

Many plant terpenoids, phenolics and other natural products are synthesized and accumulated in specialized epidermal structures known as glandular trichomes. Biosynthetic studies of these substances have been hindered by the low incorporation of precursors in *in vivo* experiments and by the difficulties in obtaining active *in vitro* preparations from typical plant extracts due to the instability and low concentrations of the relevant enzymes. I will outline several useful procedures for investigating the metabolism of glandular trichome constituents and describe their application to the study of monoterpene biosynthesis in mints. Techniques for isolating high yields of active enzymes from glandular trichomes will be presented, as well as a protocol for obtaining trichome cell clusters suitable for *in vivo* studies.

Monday 14:00 **Symposium Paper 13**

PLANT CHEMICAL CUES, PHENOTYPIC PLASTICITY AND POPULATION DYNAMICS

Norman C. Negus, Dept. of Biology, University of Utah, Salt Lake City, Utah 84112, USA.

Some herbivorous mammals employ plant chemicals (6-methoxy-benzoxazolinone) in the diet as a cue for timing of reproduction. Since 6-MBOA occurs primarily in monocotyledons, this cuing mechanism is adaptive only for species that specialize on grasses and sedges. The use of such a temporally variable cue selects for phenotypically plastic growth and maturation responses, which in turn results in unstable age distributions. Species that employ plant chemical cues exhibit more profound population fluctuations than species employing other cues (i.e., photoperiod). The limitations and implications will be discussed.

Monday 14:45 **Symposium Paper 14**

THE ROLE OF SCENT MARKING IN THE ASSESSMENT OF OPPONENTS BY MALE MAMMALS

L. Morris Gosling, Mammal Ecology Group, ADAS Central Science Laboratory, MAFF, Jupiter Road, Norwich NR6 6SP, UK.

The scent matching hypothesis of competitor assessment suggests that male mammals may assess opponents by comparing their odour with that of nearby scent marks. Where these scents are the same the opponent is probably a resource holder (a male monopolizing a territory or mates). The receiver should then be reluctant to continue the encounter, since resource holders will generally escalate to defend their resources. Scent marks may thus be conditional threats which resource holders use to reduce defence costs. Predictions of this hypothesis were tested using house mice in experiments simulating interactions between territory owners and intruders. We compared how readily males fought with opponents that either matched or did not match the smell of a scent marked substrate. Scent marked substrates were also manipulated to separate the effect of an opponent's behaviour (as an owner or intruder) from whether or not its odour matched the substrate.

Monday 16:00 **Symposium Paper 15**

GENERALIZED PLANT DEFENSE: EFFECTS ON MULTIPLE SPECIES

Vera A. Krischik, Dept. Entomol., University of Maryland, College Park, MD, USA, 20782.

Since Fraenkel's 1959 paper on the purpose of secondary compounds in plants, a vast literature has accrued discussing the effects of plant chemicals on insects. He also speculated that other biota such as plant pathogenic bacteria and fungi are affected by allelochemicals. The general nature of plant compounds and effects on multiple species such as generalist and specialist herbivores and plant pathogens as well as insect pathogens are studied. Plant chemicals having high general toxicity, such as the alkaloids discussed here, offer plants broad spectrum defense. It seems parsimonious to argue that broad spectrum compounds are employed by plants. In some instances it may appear that specific plant compounds affect either herbivores or plant pathogens, when it actually is the sensitivity of the species to the chemical. In addition, both herbivores and plant pathogens have the ability to induce leaves and alter leaf quality. If a plant chemical has a generalized effect on multiple species, then pathogen or herbivore attack may affect the ability of either group to subsequently colonize a plant. The general nature of plant chemicals may be more ubiquitous than realized. The occurrence of alkaloids in numerous plant families may be a case in point.

Monday 16:45 **Symposium Paper 16**

RAPID DISCRIMINATION BETWEEN HOST PLANTS AND NON-HOSTS BY HUMMINGBIRD FLOWER MITES ON THE BASIS OF NECTAR CHEMISTRY

Robert K. Colwell, Dept. of Ecology & Evolutionary Biology, University of Connecticut, Storrs, CT 06269-3042; Amy J. Heyneman, 2019 Newton Street, Seattle, WA 98112; and Irene Baker (deceased).

Hummingbird flower mites feed on nectar and pollen and reproduce in the flowers of hummingbird-pollinated plants and ride the birds between plants. The mites are highly host-plant specific--many are monophagous. Although local mite species often share the same (polyphagous) hummingbird carrier, fewer than 1 in 200 mites makes a host error when disembarking, with only 1-3 seconds to decide. Every species in some plant families (e.g. Lobeliaceae) have mites, yet flowers of other plant families on the same bird route (e.g. Labiatae) are never occupied. In tests, mites choose (virgin) nectar of their own host species over sugar water, nectar of another mite species' host, or nectar of a "miteless" species--some of which appear to repel mites. In a survey of 40 chemical constituents of host nectar (39 spp.) and miteless nectar (24 spp.), differences in phenolics and amino acids were the statistical discriminators between these two groups of plants.

Sunday 17:30 **Student Presentation 1**

ANTIOXIDANTS AND ANTIOXIDANT ENZYMES AS A DEFENSE AGAINST PHOTO-ACTIVATED PLANT SECONDARY COMPOUNDS IN HERBIVOROUS INSECTS

Richard R. Aucoin, Bernard J.R. Philogène and John T. Arnason. Ottawa-Carleton Institute of Biology, University of Ottawa, Ottawa, Ont., Canada, K1N 6N5.

We have been examining antioxidant defenses in phototoxin-tolerant and phototoxin-sensitive lepidopteran larvae. *Anaitis plagiata* , a specialist feeder on the phototoxic plant *Hypericum perforatum* which contains the extended quinone hypericin, has relatively high levels of the antioxidant enzymes superoxide dismutase, catalase, and glutathione reductase. These enzymes may be inducible defenses against the activated oxygen species which are generated by these phototoxins and are responsible for their toxicity. *Manduca sexta* is particularly sensitive to phototoxins such as hypericin and the thiophene alpha-terthienyl and has relatively low constitutive levels of antioxidant enzyme activity. This sensitivity can be dramatically altered however, with the use of dietary antioxidants such as beta-carotene and vitamin E. The implications of antioxidant defenses for insect herbivory will be discussed.

Sunday 17:45 **Student Presentation 2**

PARTIAL PURIFICATION AND CHARACTERIZATION OF A 2-OXOGLUTARATE DEPENDENT DIOXYGENASE FROM *CATHARANTHUS ROSEUS*

Emidio De Carolis and Vincenzo De Luca, Institut Botanique, Dept. de Biologie, Université de Montréal, Qué. Canada H1X 2B2.

Young leaves of *Catharanthus roseus* contain the enzymes which convert the monoterpenoid indole alkaloid tabersonine by 3 hydroxylations, 2 methylations and 1 acetylation step to vindoline. A novel hydroxylase which catalyses the second to last step in vindoline biosynthesis will be described. Using a recently established enzymatic assay for the hydroxylase, the enzyme was partially purified by ammonium sulfate precipitation, successive chromatography on Sephadex G-100, Hydroxylapatite, Alkyl Superose and Mono Q. The enzyme demonstrated an absolute requirement for 2-oxoglutarate and enzymatic activity was enhanced by ascorbate, establishing it as a 2-oxoglutarate dependent dioxygenase. The hydroxylase was characterised by its strict specificity for position 4 of various alkaloid substrates, K_mS, and M_r .

Sunday 18:00 **Student Presentation 3**

SALINITY, SOIL AERATION AND HERBIVORY EFFECTS ON AMINO ACID AND BETAINE CHEMISTRY OF *SPARTINA ALTERNIFLORA*

John D. Bachelier and John T. Romeo, Department of Biology, University of South Florida, Tampa, FL 33620, USA.

The salt marsh cordgrass,*Spartina alterniflora*, modulates proline and glycinebetaine levels in response to saline stress. The amplitude of this osmoregulation is correlated with plant ecophene, tall growth forms having higher levels than short. The factors responsible are sediment oxidation level, interstitial salinity, and available nitrogen. Our experiment, conducted under conditions of high nitrogen availability, examined single and interactive effects of sediment aeration, interstitial salinity, and herbivory by monophagous *Prokelisia* planthoppers on nitrogen chemistry. Plants were subjected to combinations of: 10 or 20 ppt salinity; daily or weekly flushes; and 0, 50, or 200 insect loads. Sampling was done at 0, 14, 28, 42, and 56 days, and chemicals quantified by HPLC. Data were statistically analyzed by means of multifactorial analyses utilizing Duncan's New Multiple Range test and Student-Newman-Keuls test. Significant findings include: a density-dependent increase in proline and 3 other amino acids in response to planthopper predation.

Sunday 18:15 Student Presentation 4

PARTIAL PURIFICATION AND CHARACTERIZATION OF LIMONENE CYCLASE FROM *MENTHA PIPERITA* L. CV. BLACK MITCHAM (PEPPERMINT)

Jean I. M. Rajaonarivony, Jonathan Gershenzon and Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340 USA.

The cyclization of geranyl pyrophosphate (GPP) is a key step in the biosynthesis of most monoterpenes. The enzymes that catalyze this conversion are known as cyclases. In peppermint, GPP is converted to the monocyclic olefin limonene by the action of limonene cyclase. We partially purified this activity 45-fold from a preparation of epidermal glands. Limonene cyclase has an apparent molecular weight of 55,000, an isoelectric point of 4,35, shows a pH optimum near 6.7 and requires a divalent metal ion, either Mn^{++} or Mg^{++}, for catalysis, with Mn^{++} being preferred. The apparent Michaelis constant for GPP was determined to be 1.7 mM. Both histidine and cysteine residues appear to be essential for catalysis.

Sunday 18:30 Student Presentation 5

NEEM-BASED ANTIFEEDANTS AND THEIR EFFECT ON EUROPEAN CORN BORER *OSTRINIA NUBILALIS* (LEPIDOPTERA: PYRALIDAE)

Diane C. Gagnon and J.T. Arnason, Dept. of Biology, University of Ottawa, 30 George Glinski, Ottawa, Ont., Canada, K1N 6N5.

Neem oil extracted from the neem tree (*Azadirachta indica* A. Juss. (Meliaceae)) and azadirachtin, a triterpenoid purified from neem seeds, were evaluated in field trials for their antifeedant and insecticidal properties to the European corn borer in sweet corn. Corn plants artificially infested with egg masses of corn borer showed that cobs were significantly less damaged when treated with neem oil or azadirachtin, compared to an infested control. Antifeedant activities of azadirachtin and the formulation used in the field trials were tested in a leaf-disk bioassay; growth and development parameters of larvae placed on diet treated with various neem oil concentrations were monitored. The data suggest that neem may offer a safer alternative to the use of synthetic pesticides in controlling the corn borer in sweet corn.

Sunday 18:45 Student Presentation 6

TWO NEW TRIHYDROXYPIPECOLIC ACIDS FROM *INGA PATERNO* (MIMOSOIDEAE)

Timothy C. Morton and John T. Romeo, Department of Biology, University of South Florida, Tampa, FL 33620,USA.

The Mimosoid subfamily of the Leguminosae is known for its array of imino acids based on pipecolic acid. All 4- and 5- monohydroxy and 4,5- dihydroxy sterioxisomers previously have been isolated from various genera. A single trihydroxy pipecolic acid is also known, but from a single species in the Papilionoideae. A phytochemical study of the Mimosoid genus *Inga* has revealed two additional naturally occurring trihydroxypipecolic acids. The new compounds were isolated from *Inga paterno*, native to Mexico and Central America. Alumina column chromatography, followed by high voltage paper electrophoresis and reverse-phase C-18 HPLC yielded small amounts of both compounds. They were characterized by proton and C13 NMR spectroscopy and structures assigned. A distributional survey revealed that both compounds are restricted to a small number of species.

Sunday 19:00 Student Presentation 7

BIOSYNTHETIC STUDIES OF THIOPHENES FROM HAIRY ROOT CULTURES OF *TAGETES PATULA* USING ^{13}C-LABELED SODIUM ACETATE

Marios A. Menelaou, Nikolaus H. Fischer, Martin Hjortso*, Maryam Foroozesh and Tina Thibodeaux. Department of Chemistry and Chemical Engineering*, Louisiana State University, Baton Rouge, Louisiana, USA.

Biosynthetic studies of 5 (but-3-en-1-inyl) 2,2' bithiophene and 5 (4-acetoxy-1-butinyl) 2,2' bithiophene were carried out using *Agrobacterium* rhizogenes-transformed hairy root cultures of *Tagetes patula* . Experiments with [1-^{13}C] and [2-^{13}C] sodium acetate as precursors verified that the two thiophenes follow the acetate pathway as previously proposed based on studies of structurally related compounds using ^{14}C labeled precursors. It was shown that the C-12 thiophenes are derived from C-14 precursors followed by two decarboxylations as previously proposed for the biogenesis of this class of compounds. The interpretation of the ^{13}C NMR spectra of the above thiophenes and the advantages of a combined use of hairy root cultures and ^{13}C labeled precursors for biosynthetic studies will be discussed.

Sunday 19:15 Student Presentation 8

CIS-MONOLIGNOLS AND THEIR GLUCOSIDES IN BEECH BARK TISSUE: ENZYMOLOGY AND THEIR INVOLVEMENT IN LIGNIFICATION

Ma. Estela J. Inciong, Etsuo Yamamoto, Laurence Davin and Norman G. Lewis, Departments of Wood Science and Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0323, USA

American beech bark (*Fagus grandifolia* Ehrh) exclusively accumulates Z-monolignols and their corresponding glucosides, which are biosynthetically derived from E-hydroxycinnamyl alcohols, such as coniferyl alcohol. Although E→ Z isomerization can occur photochemically *in vitro* , the evidence suggests that a novel isomerase is involved *in vivo*. Beech bark glucosyltransferase exhibits a strong substrate preference for Z-, and not E-, monolignols. Recent progress in the purification and characterization of this unusual enzyme will be discussed. Finally, the nature of the inter-monomeric bonding environments in beech bark lignins, together with a proposed role for Z-monolignos, is presented.

Sunday 19:30 Student Presentation 9

CHARACTERIZATION OF MONOTERPENE CYCLASE ACTIVITY IN CONIFERS

Thomas J. Savage and Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340 USA

Oleoresins from conifers contain a complex mixture of monoterpene olefins. While the composition of conifer resins has been widely studied in relation to tree defense and taxonomy, little is known about the monoterpene cyclase activities that produce these natural products. Methods for the isolation of terpene cyclases from conifers will be described, and the partial purification and characterization of these enzymes will be discussed.

Tuesday 08:30 Oral Presentation 1

HISTOLOGICAL LOCALIZATION OF PSORALENS IN SEEDS AND FRUITS

Alicja M. Zobel and Stewart A. Brown, Department of Chemistry, Trent University, Peterborough, Ont., Canada, K9J 7B8.

Three psoralens of umbelliferous plants were determined on the fruit surface, and in rutaceous and leguminous plants on the seed surface. Concentrations varied among and within the species, from traces to 40 micrograms/g fresh weight -- 0.28 to 43% of the total. On *Ruta graveolens* seeds the concentration was very low compared to that on the leaf surface. Dry fruits of the Umbelliferae contained higher concentrations within the tissue than on the surface, and still higher within the seed. Autofluorescent microscopy and isolation of fruit and seed parts revealed a fluorescent layer on the surface of umbelliferous fruits and seeds, and on the surface of the embryo. Crystals were visible in some longitudinal glands of dry fruits of *Heracleum*, *Angelica* and *Seseli*, and also on the seed covers of the Umbelliferae. *Psoralea* contained crystals on the surface of the embryo.

Tuesday 08:45 Oral Presentation 2

MECHANISMS OF ACTION OF PSORALEN AND XANTHOTOXIN ON MITOTICALLY ACTIVE CELLS

Alicja M. Zobel, Dept. of Chemistry, Sharon Louis and Koome Mwiraria, Dept. of Biology, Trent University, Peterborough, Ont., Canada, K9J 7B8.

In darkness and under UV, two plant furanocoumarins affected mitoses and morphology of mitotically active cells. Embryonic meristem of *Allium cepa* root tips and animal cell cultures showed fewer mitoses, but more chromosomal aberrations and blockage of some phases of mitosis. Even after 1h in the presence of UV, psoralen and xanthotoxin caused abnormal chromosome separation leading to chromosomal aberrations in sister cells, and complete cessation of mitosis after 3h. In darkness mitotic spindle aberrations were visible after 3h, but the primary mechanism after 1h was on the endomembrane system. The first responses were in the cell membrane, nuclear envelope, and endoplasmic reticulum, changing their structure and causing fragmentation. We suggest that although the effect of UV is on DNA, as is well-known, the primary reaction, which occurs in darkness, is on the endomembrane system.

Tuesday 09:00 Oral Presentation 3

APPLYING QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP TECHNIQUES TO NATURAL PRODUCTS: PHOTOTOXIC THIOPHENES

Robin J. Marles and J. Thor Arnason, Biology Dept., University of Ottawa, Ottawa, Ont., Canada, K1N 6N5, and R. Lilia Compadre and César M. Compadre, Dept. of Biopharmaceutical Sci., College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA.

Reports on the relationship between chemical structure and biological activity of plant secondary metabolites generally describe structural features essential for activity and, in qualitative manner, how various modifications to the molecule enhance or detract from the level of activity. Such information, while useful, does not allow quantitative prediction of the level of activity or specificity of new natural or synthetic analogues of the lead compound. By preparing a large number of synthetic analogues of naturally occurring phototoxic thiophenes, determining their differences in selected biologically relevant physical parameters, and their phototoxicity against a variety of organisms, statistical models have been developed to reliably predict the phototoxicity and specificity of novel thiophene analogues.

Tuesday 09:15 Oral Presentation 4

EFFECT OF SALINITY STRESS ON SEEDLING CHLOROPHYLL OF *LIATRIS PUNCTATA* HOOK

Alina Walther, Dept. of Biology, University of Regina, Regina SK, S4S 0A2, Canada.

L. punctata is a nonhalophytic herbaceous prairie perennial. It depends on seed for establishment on new sites, now increasingly subject to secondary soil salinization. Although seed germinated readily in high NaCl or Na_2SO_4 solutions, radicle development was inhibited. Viable seedlings from lower concentrations were raised in vermiculite irrigated with salt-enriched 1/2-strength nutrient solution under a 14 h 25°/15° regime. Chl was extracted with 80% acetone. Salinity depressed the Chl content of mature cotyledons without affecting the a/b ratios. The first foliage leaves were not significantly affected but older leaves showed a similar effect of mild-to-moderate salinity stress on Chl content. The near-constancy of the Chl a/b ratios was also observed in another prairie species, *Glycyrrhiza lepidota* (Walther, 1989, Plant Physiol. vol. 89, No. 4, S1062) suggesting an equivalent effect on Chl formation under NaCl- or Na_2S0_4-induced salinity stress.

Tuesday 09:30 Oral Presentation 5

RELATIONSHIP OF EPICUTICULAR WAX FATTY ALDEHYDES TO CHILLING INJURY IN STORED GRAPEFRUIT

Harold E. Nordby and Roy E. McDonald, U.S. Department of Agriculture, ARS. 2121 Camden Road, Orlando, FL, USA 32803.

Grapefruit is quite susceptible to chilling injury (CI), e.g., rind pitting or brown staining, when stored 3 or more weeks at 1-5C. Squalene and other terpenoids have been shown to be natural protectants against CI, being synthesized when grapefruit are held 7 days at 15°C prior to storage. Under storage conditions conducive to CI, the level of epicuticular wax C_{28}-C_{34} linear aldehydes decreased dramatically. This weakening of the "outer coat" is postulated to make the membranes more susceptible to CI. Conditioning grapefruit 7 or more days at 15°C stimulated synthesis of C_{24}-C_{26} aldehydes. This replacement of longer chain with shorter chain aldehydes is believed to be a complement to the protective action of squalene against CI.

Tuesday 09:45 Oral Presentation 6

THE USE OF GC AND GC/MS TO CHARACTERIZE THE SUCROSE ESTERS OF *NICOTIANA* SPECIES

O.T. Chortyk and R.F. Severson, Tobacco Quality & Safety Research Unit, USDA, ARS, P.O. Box 5677, Athens, Georgia, 30613, USA.

The glandular trichomes on the surface of green leaves of most *Nicotiana* plants produce a gummy exudate. We have identified the major constituents of this cuticular material to be terpenes and sucrose esters. Sucrose esters (SE) of some *Nicotiana tabacum* varieties have demonstrated both antibiotic and plant growth regulating activities. However, their most important function is the liberation, on smoking, of the potent Turkish tobacco flavor and aroma compounds, methylvaleric and methylbutyric acids. Accordingly, we are investigating the SE of the *Nicotiana* species, with the objective of finding promising species to cross with and enhance the aromas of commercial tobacco varieties. GC and GC/MS methodology used in the characterization of these SE will be described. Details will be given for the analyses and differentiation of five different SE structural types.

Tuesday 10:30 Oral Presentation 7

FLAVANONE-SPECIFIC 7-GLUCOSYLTRANSFERASE ACTIVITY IN *CITRUS PARADISI* SEEDLINGS: PURIFICATION AND CHARACTERIZATION

Cecilia A. McIntosh*, Lilian Latchinian**, and Richard L. Mansell*, *Dept. Biology, University of South Florida, Tampa, FL, USA 33620, ** Dept. Biology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

The isolation and characterization of a flavanone-specific 7-0-glucosyl-transferase (GT) and its resolution from other GT's in grapefruit seedlings will be described. This new enzyme in the subclass 2.4.1 catalyzes the glucosylation of the 7-OH group of naringenin to form prunin and has been purified (943 fold) by fractional precipitation with ammonium sulfate and successive chromatography on Sephadex G-100, hydroxyapatite, UDP-glucuronic acid agarose, Mono Q, and Mono P columns. It has a pH optimum of 7.5-8.0, an apparent pI of 4.3, and apparent Mr of 54.9 kd. This GT has an expressed specificity for the 7-position of the flavanones naringenin (Km_{app} 62 µM; Km_{app} UDPG 51 µM) and hesperetin (Km_{app} 124 µM; Km_{app} UDPG 243 µM) and did not accept other flavone, flavonol, or chalcone aglycones.

Tuesday 10:45 Oral Presentation 8

PROTEIN ENGINEERING OF PAPAIN: DESIGNING NEW PROPERTIES INTO AN OLD ENZYME!

Henry E. Khouri, Protein Engineering Section, Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montréal, Québec, Canada H4P 2R2

Papain from the latex of the papaya tree (*Carica papaya* L.) has been the most intensively studied of all cysteine proteases. Its amino acid sequence is known and its structure has been determined to 1.65 Å resolution by X-ray crystallography. This makes the enzyme an ideal candidate for protein engineering. A synthetic gene coding for papain was expressed in a Baculovirus-insect cell system. Through site directed mutagenesis several papain mutants designed to investigate the mechanism of papain were produced in this system. Based on amino acid alignment with other known cysteine proteases, selected modifications into papains S_2 subsite were designed with the aim of altering papains substrate specificity. Characterization of the mutants thus produced and their implication with respect to industrial application will be discussed.

Tuesday 11:00 Oral Presentation 9

PHOTOCHEMISTRY OF HYDROXYCINNAMATE ESTERS IN GRASS CELL WALLS

J.A. McCallum[1], G.H.N. Towers[1], S. Tachibana[1], I.E.P. Taylor[1] and T. Arnason[2], [1]Botany Dept, University of British Columbia, Vancouver, B.C. V6T 2B1. [2]Dept. Biology, University of Ottawa, Ottawa, Ont., K1N 6N5, Canada.

Although earlier work has shown that UV-irradiation can bring about isomerization and dimerization of hydroxycinnamate esters in grass cell walls in vivo the influence of such reactions on the structure, development and degradation of the walls is uncertain. Our present studies have examined the effects of UV-irradiation on cell walls of Barley (*Hordeum vulgare*) and hydroxycinnamate esters in relation to degradation of walls and esters by crude and purified enzymes from *Aspergillus niger*. Our findings indicate that hydrolysis of cell walls and esters by xylanases and aromatic esterases respectively is significantly reduced by UV-irradiation. These results support earlier suggestions that in vivo E-Z isomerization of hydroxycinnamate esters may have significant effects on the conformation of hemicellulosic polymers in the monocot cell wall and thus their susceptibility to enzymatic modification

Tuesday 11:15 **Oral Presentation 10**

SINAPINE METABOLISM WITHIN THE BRASSICACEAE

Thomas Vogt[1], Dieter Strack[2] and Brian Ellis[1], [1] University of British Columbia, Dept. of Plant Science, Vancouver V6T 2A2, Canada, [2] Institut für Pharm. Biologie der TH Braunschweig, 3300 Braunschweig, FRG.

Sinapoylcholine (sinapine) is the main phenolic compound in the seeds of many members within the Brassicaceae. Its metabolic pathway during maturation and germination of seeds has been demonstrated in *Raphanus sativus* (red radish) to involve high energy UDP-glucose esters instead of CoA-esters as intermediates. Members of the Brassicaceae like *Brassica napus* (rape-seed, canola) are important crops, the seed of which, after extraction of the oil, is used as protein rich animal food. This meal still contains high amounts of phenolics, especially sinapine, which are undesirable and therefore restrict its use. A new approach to reduce the sinapine content in the seeds may be achieved by genetically engineering a reduction in the amount or activity of the key biosynthetic enzyme 1-sinapoylglucose: choline sinapoyl transferase (SCT), which is responsible for the high accumulation of sinapine during seed maturation. The metabolic pathway of sinapine in *B. napus* is thought to likely resemble that demonstrated earlier in *R. sativus*. In this report the strategy for the purification of SCT from *B. napus* is outlined as a first step towards cloning the corresponding structural gene and development of an antisense RNA construct.

Tuesday 11:30 **Oral Presentation 11**

LIGNAN BIOSYNTHESIS IN FORSYTHIA SPECIES

Laurence Davin, Toshiaki Umezawa and Norman G. Lewis. Departments of Wood Science and Forest Products and Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0323, USA.

Lignan biosynthesis has long been proposed to occur via oxidative coupling of two phenylpropanoid monomers in a manner analogous to that of lignins (i.e. via an H_2O_2-requiring peroxidase). However, lignans are normally enantiomerically pure, whereas lignins have no measurable optical activity. No explanation has been profferred to account for the stereochemical control leading to optical activity of lignans, other than that the reaction is somehow enzymatically mediated. The biosynthetic routes to the forsythia lignans, (+) pinoresinol, (-) secoisolariciresinol and (-) matairesinol have been investigated. We have established that (+) pinoresinol formation does not occur via mediation of a typical H_2O_2-requiring peroxidase. In addition, evidence for the role of (-) secoisolariciresinol (or an oxidised equivalent) as a central pivotal intermediate in the biogenesis of butyrolactone lignans, such as (-) matairesinol, is discussed.

Tuesday 11:45 **Oral Presentation 12**

PHENYLPROPANOID PHOTOCHEMISTRY OF GRASS CELL WALLS

G.H.N. Towers, Felipe Balza, Roy Hartley*, Michael Rodgers, Sanro Tachibana, John McCallum, Ed Neeland, Vic Rathore and Thor Arnason**. Botany Department, University of British Columbia, Vancouver, B.C. 6VT 2B1. *USDA, Athens, GA, USA. ** Biology, University of Ottawa, Ottawa, Ont., K1N 6N5, Canada.

In addition to pohotoisomerization, the hydroxycinnamic acids, esterified to cell wall hemicelluloses in barley, corn and other grasses, undergo photodimerization to produce phenolic truxillic and truxinic esters on transfer of plants from dark to light. The significance of these photochemical events in relation to wall structure and to utilization by herbivores will be discussed.

Tuesday 12:00 **Oral Presentation 13**

TRITERPENES FROM TWO *BETULA* SPECIES

Yun Hua, Michael D. Bentley, and Barbara J.W. Cole, Department of Chemistry, University of Maine, Orono, Maine, 04469, USA.

Triterpenes from the outer bark of yellow birch, *Betula alleghaniensis* , and black birch, *Betula lenta* , have been isolated and identified. From *B. alleghaniensis*, we have isolated 17 lupane triterpenes, including 29-nor-20-oxolupan-3-one-28-ol, lupan-3-one-20,28-diol, 29-nor-20-oxolupan-3-one, and lup-20 (29)-ene-28-ol-30-al-3-one as new natural products. From *B. lenta*, we have isolated 10 lupane triterpenes, including lup-20 (29)-ene-30-al-3,28-diol as a new natural product.

Tuesday 8:45 **Oral Presentation 14**

VOLATILE COMPOUNDS FROM MALE BUTTERFLIES

Stefan Schulz, Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, FRG

(1S,3R,6R,9S,10S)-9,10-Epoxytetrahydroedulan is the main compound in the volatile secretions of male scent organs of several species of *Euploea* (Danainae) butterflies. (1R*,3S*,6R*)-8-Oxodihydroedulan was identified in *Danaus plexippus* . The new monoterpenes, 8-hydroxy-6,7-dihydro-isogeraniol and -isonerol, could be identified as the minor components of the hairpencil bouquet of *Danaus chrysippus.* In males of the related Helioconidae acyclic esters, lactones and terpenes dominate the scent bouquets. In *Heliconius erato*, pentyl- and hexyl esters of 2,3-dihydrofarnesenic acid were identified as the major components of the extracts of scent organs. In *Heliconius hewittsonii* scent organs contain at least 32 different lactones. The major component of the abdominal secretion of males of the satyrid *Elymnias thryallis* proved to be the new monoterpene, 2-(2-hydroxy-2-methyl-3-butenyl)-4-methylfuran. Straight chain cis- and trans-2-5-dialkyltetrahydrofurans containing 27 -35 carbon atoms could be identified from several butterfly families. They occurred in species specific mixtures in different concentrations in the scent organs of males of heliconids, danaids, ithomiids and nymphalids. A new class of surfactants could be identified from the body extracts of *Creatonotos transiens* and from the scent organs of *Mechanitis lysimnia*:: esters of long chain fatty acids with N,N-dimethylaminoethanol. Identification procedures and syntheses of the compounds will be presented, biological functions of some compounds will be discussed.

Tuesday 09:00 **Oral Presentation 15**

DETECTION AND IDENTIFICATION OF A CRYPTIC PHEROMONE FROM *IPS PINI* (COLEOPTERA: SCOLYTIDAE).

Stephen A. Teale, Aijun Zhang, Gerald N. Lanier and Francis X. Webster, State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210, USA.

A major new pheromone was isolated from *Ips pini* through a sequential fractionation and bioassay procedure. The interaction of several peculiarities of the system made detection of the new pheromone elusive; it co-eluted with a major capillary GC peak in Porapak Q collections of volatiles, it is present and active in minute relative quantities, and its activity varies seasonally. The structure of the isolated active compound was elucidated through MS, IR, and NMR spectrometry. It is active as a synergist with ipsdienol.

Tuesday 09:15 Oral Presentation 16

TRAIL-FOLLOWING RESPONSES OF *TAPINOMA SIMROTHI* (FORMICIDAE: DOLICHODERINAE) TO PYGIDIAL GLAND EXTRACTS

Tovit Simon and Abraham Hefetz, Department of Zoology, Tel-Aviv University, Ramat-Aviv, 69978 Tel-Aviv, Israel

The pygidial (anal) gland was found to be the source of trail-pheromone in the ant *Tapinoma simrothi*. Bioassays conducted with fractionated pygidial gland secretion indicated that the fraction containing iridodials and iridomyrmecin is responsible for the trail-pheromone activity. Thus workers of *T. simrothi* may utilize the same glandular exudate for alarm and trail following. At high emission rates from a point source, the ants responded in alarm, e.g., rushed to the source with open mandibles and raised abdomen. At low concentrations and when drawn as a line, the ants followed the secretion calmly. Trails of *T. simrothi* are long-lived, having a biological half life between 10 and 19 days. Quantitative studies of the evaporation rates of the iridodials by gas chromatography resulted in a half life of 11 days, agreeing with the biological data. The implications of the use of the same glandular secretion for alarm and food recruitment will be discussed.

Tuesday 09:30 Oral Presentation 17

INTRASPECIFIC RECOGNITION IN *KALOTERMES FLAVICOLLIS* (ISOPTERA, KALOTERMITIDAE)

C. Everaerts, A. Perrin and O. Bonnard, Laboratoire de Zoologie, URA CNRS 674, Université de Bourgogne, 21000 Dijon, France.

As a rule, criminality does not exist inside a termite society. Being an exception to this rule, the primitive termite *Kalotermes flavicollis* is characterized by a special form of intra-nest aggressivity. In this species, the society is strictly monogynic. When the pair of reproductives disappears, many of the remaining individuals transform into replacement sexuals. While all non-transformed individuals are non-aggressive, neotenic individuals are very aggressive to all other neotenics but not to other castes. Fights occur between neotenics until only one pair survives. This behavior involves the existence of special recognition cues between neotenics and other castes, and such differences may be correlated to the involved recognition cues.

Tuesday 09:45 Oral Presentation 18

IDENTIFICATION AND ATTRACTIVENESS OF A MAJOR PHEROMONE COMPONENT FOR NEARCTIC *EUSCHISTUS* SPP. STINK BUGS (HEMIPTERA: HETEROPTERA: PENTATOMIDAE)

Jeffrey R. Aldrich, USDA-ARS, Insect Hormone Laboratory, Bldg. 467, Beltsville, Maryland 20705, USA.

Methyl (*2E, 4Z*)-decadienoate is the major male-specific volatile of *Euschistus conspersus, E. tristigmus, E. servus, E. politus*, and *E. ictericus* . In a sixth species, *E. obscurus*, this ester is a relatively minor male-specific component, with the major component being tentatively identified as methyl 2,6-dimethyltetradecanoate. Females, males, and nymphs of *E. conspersus, E. tristigmus, E. servus* and *E. politus* were significantly attracted to methyl (*2E,4Z*)-decadienoate in field tests conducted in California and Maryland. Tests in Maryland also demonstrated that parasitic tachinid flies use the *Euschistus* unsaturated methyl-ester as a host-finding kairomone. It is not yet clear what role minor volatile components of *Euschistus* play in reproductive isolation.

Tuesday 10:30 **Oral Presentation 19**

VARIOUS ARANEIOD SPIDERS ATTRACT FLY AND/OR MOTH PREY

Mark K. Stowe, c/o J. Reiskind, Dept. of Zoology, Univ. of Florida, Gainesville, FL 32611, USA.

A variety of recent work supports the hypothesis that "bolas spiders" (species in the araneid subfamily Mastophorinae) mimic female moth sex pheromone blends and attract their prey (male moths). I discuss new work which suggests that aggressive mimicry is employed by a variety of genera in the superfamily Araneoidea, that some of the spiders attract Dipteran as well as Lepidopteran prey, and that these spiders evolved aggressive mimicry independently of each other.

Tuesday 10:45 **Oral Presentation 20**

COMPARISON OF CALLING BEHAVIOUR IN TWO GEOGRAPHICALLY ISOLATED POPULATIONS OF THE TRUE ARMYWORM

Jeremy N. McNeil, Département de biologie, Université Laval, Ste-Foy, P.Q., Canada, G1K 7P4.

In both Europe and North America the true armyworm, *Pseudaletia unipuncta* extends its summer range northward, into areas far beyond their overwintering sites. It has been hypothesised (McNeil, 1987. Insect Sci. and its Appl. 8:591) that the delay in female calling behaviour, as well as male receptivity to the female sex pheromone, observed under short day, cool temperature conditions permits both northward (spring) and southward (fall) migratory flights. A existence of a permanent population in the Azores provides an opportunity to test the hypothesis that, in non-migrant populations, the prereproductive period will be shorter than that of migrant populations. Data in support of this hypothesis will be presented and the ecological implications discussed.

Tuesday 11:00 **Oral Presentation 21**

STRUCTURE-FUNCTION STUDIES OF CRAB PHEROMONE MIMICS

D. Rittschof, A. Oliver, R.B. Forward Jr., and B.W. Erickson*. Duke Univ. Marine Laboratory, Beaufort NC 28516. *UNC, Chapel Hill, NC 27514, USA.

Synchrony of larval release in brachyuran crabs is orchestrated by pheromones released from hatching eggs. Here we examine the potency of pheromone mimics - arginine carboxyl terminus tripeptides and of individual L-amino acids. Thresholds for responses ranged from attomolar for the most potent tripeptides and nanomolar for the most potent individual amino acids. Tests altering the position of glycine and either isoleucine or leucine showed the position and nature of the hydrophobic side chain were important. Thresholds increased 6 orders of magnitude through the test series GIR, IGR, GLR and LGR. Amino acids with charged R groups were ineffective. Those with polar R groups had millimolar to micromolar thresholds. Those with large hydrophobic side chains were most potent. The untested tripeptide GMR should be the most potent peptide mimic. The dynamic range of the chemically mediated response will be discussed with respect to the hypothetical structure of the receptor.

Tuesday 11:15 Oral Presentation 22

THE SEX ATTRACTIVENESS PHEROMONE OF THE GUAM BROWN TREE SNAKE, *BOIGA IRREGULARIS* , IDENTIFIED?

Robert T. Mason and Tappey H. Jones, Laboratory of Biophysical Chemistry, National Hearth, Lung, and Blood Institute, NIH, Bethesda, MD 20892 USA.

The brown tree snake of Guam is an introduced pest species responsible for catastrophic ecological, environmental, and public health problems on the island. This colubrid snake is known to have extirpated at least three species of birds and the remaining species are considered threatened. We have previously identified a series of saturated and monounsaturated methyl ketones as the sex attractiveness pheromones of a related colubrid, the red-sided garter snake. Utilizing this paradigm and methodology, a similar mixture of methyl ketones was identified in *Boiga irregularis* . The predominant components of this mixture were identified as six new long chain ketodienes, Z,Z-6-26-pentatriacontadien-2-one, Z-Z-8,26-pentatriacontadien-2-one, Z,Z-6-27-hexatriacontadien-2-one, Z,Z-8,27-hexatriacontadien-2-one, Z,Z-6-28-heptatriacontadien-2-one, and Z,Z-8,28-heptatriacontadien-2-one. Their structures were determined from chemical and spectral means.

Tuesday 11:30 Oral Presentation 23

PHEROMONAL ACTIVITY OF PHENOLS AND KETONES IN BEAVER CASTOREUM

D. Müller-Schwarze and Peter W. Houlihan, College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, USA.

Twenty-four single castoreum components and their mixtures were bioassayed with free-living beaver of three different populations in New York during four seasons. An artificial scent mark was placed in a family territory and the residents responded to this "mark of the intruder" with approach, sniffing, pawing, and scent marking in turn. Five compounds were active. These were 4-ethylphenol, 1,2-dihydroxybenzene, 4-methoxyphenol, acetophenone, and 3-hydroxyacetophenone. Mixtures of 6 or 24 compounds were as active as whole castoreum. The active compounds appear to be diet-derived and interact in additive as well as redundant fashion.

Tuesday 11:45 Oral Presentation 24

ALARM CHEMOSIGNALS AND IMMUNE PROCESSES

Robert Cocke, Department of Psychiatry, University of Rochester, Rochester, N.Y. 14642, USA.

Exposure to alarm chemosignals from stressed conspecifics results in physiological reactions that alter the immune response in laboratory mice. Lymphocyte proliferation following mitogen stimulation is altered in tissue samples from mice exposed to stress odors. Exposure for 24 hours results in suppression of T cell proliferation and suppression of B cell proliferation is seen following 48 hours exposure. Evidence suggesting that these immune system reactions to alarm chemosignals may play a role in disease resistance will be discussed.

Tuesday 14:00 **Oral Presentation 25**

INHIBITION OF GROWTH AND PHOTOSYNTHESIS BY SORGOLEONE

Frank A. Einhellig, Itamar F. Souza, and Milton J. Haar, Dept. of Biology, University of South Dakota, Vermillion, South Dakota 57069, USA.

Root exudates of *Sorghum bicolor* contain an abundance of hydrophobic p-benzoquinones named sorgoleone. The objective of this study was to determine possible toxicity and physiological effects of sorgoleones on plants in order to evaluate their action in allelopathy. Root exudates containing sorgoleone were collected from *S. bicolor* seedlings five days after germination. Sorgoleone concentrations as low as 10 μM inhibited plant growth in bioassays using *Lemna minor*, *Eragrostis tef*, *Abutilon theophrasti*, and *Setaria viridis*. Photosynthesis was significantly reduced in *Glycine max* leaf disks vacuum infiltrated with 10 μM sorgoleone. These data indicate sorgoleones contribute to the allelopathic effects of *S. bicolor*.

Tuesday 14:15 **Oral Presentation 26**

PHENOLIC ACID CONTENT OF SOILS FROM SOYBEAN CROPPING SYSTEMS

Udo Blum, Kristofer Klein, Lynn D. Holappa, Seung-Won Lyu, Thomas R. Wentworth, Dept. of Botany, Doug A. Worsham, Dept. of Crop Science, and Larry D. King, Dept. of Soil Science, North Carolina State University, Raleigh, NC 27695, USA.

Soil samples were obtained from wheat/no-till, wheat/conventional tillage, and fallow/conventional tillage at 30 day intervals during July and October of 1989. Soil extracts were obtained by autoclaving flasks containing 50 g of soil and 100 ml water for 45 min. Centrifuged and filtered supernatants were adjusted to pH 5 or adjusted to pH 2, centrifuged, and then adjusted to pH 7. HPLC analysis was used to isolate and quantify 7 common phenolic acids. Folin & Ciocalteu's reagent was used to determine total phenolic acid content. Individual phenolic acids and total phenolic acid content of soils were highly correlated. Phenolic acid content of 2.5 cm core samples from the wheat/no-till system were significantly higher than the samples from other cropping systems. Phenolic acid content and water potential of extracts was related to the observed inhibition of crimson clover germination.

Tuesday 14:30 **Oral Presentation 27**

CHEMICAL COMMUNICATION AND DEFENCE IN BARLEY

John V. Lovett and Deli Liu, Dept. of Agronomy and Soil Science, University of New England, Armidale, N.S.W., Australia, 2351.

Allelopathic potential has been demonstrated in the major temperate cereal crops, barley, oats, rye and wheat. Allelochemicals may be liberated during life or from the residues of these, and other, crops after death. These compounds play a wider role in chemical communication with a range of organisms than their association with allelopathy, only, would imply. Techniques developed for the study of allelopathy and other chemical communication by barley will be described. Data will be presented on examples of chemical communication in barley which have defensive connotations. These data are part of a programme which aims to define new selection criteria for this important crop.

Tuesday 14:45 Oral Presentation 28

ALLELOPATHIC ACTIVITY OF *CHENOPODIUM AMBROSIOIDES* ESSENTIAL OIL

Juan Jiménez-Osornio, Junji Kumamoto, Dept. of Botany and Plant Sciences, University of California, Riverside. Riverside CA 92521 and Christian Wasser Phytochemistry Laboratory, Dept. of Ecology and Evolutionary Biology, University of California, Irvine. Irvine CA 92717, USA.

The activity of extracts of *Chenopodium ambrosioides* was examined and it was found that the active compound was volatile. Two ml per Petri dish (9 cm diameter) of *C. ambrosioides* essential oil inhibited totally germination and hypocotyl growth of *Amaranthus hypocondriacus* . At lower concentrations germination is only retarded though hypocotyl growth is inhibited 60% in comparison to the control. Steps to separate and identify the allelopathic compound(s) are described along with the possible effects of these allelochemicals in nature.

Tuesday 15:00 Oral Presentation 29

EFFECTS OF FERULIC ACID, A POTENTIAL ALLELOPATHIC AGENT, ON THE ENDOGENOUS LEVELS OF ABSCISIC ACID IN TOMATO

Lynn D. Holappa and Udo Blum, Dept. of Botany, North Carolina State University, Raleigh, NC. 27695-7612, USA.

Wild type and *flacca* (mutants deficient in abscisic acid) tomato plants of the isogenic parent line, *Lycopersicon esculentum* Mille. cv. Ailsa Craig were treated with multiple treatments (at 2-day intervals) of ferulic acid concentrations (0.0, 0.2, 0.4, and 0.8 mM) in nutrient solution. Regression analysis indicated that ferulic acid was inhibitory to leaf elongation rates and plant water utilization of both taxa. As ferulic acid concentrations were increased concurrent decreases in leaf elongation and water utilization were observed. In addition, both wild type and *flacca* plants exhibited increases in endogenous levels of abscisic acid as measured by indirect immunoassay.

Tuesday 15:15 Oral Presentation 30

CROTALARIA JUNCEA SEEDS CONTAIN A POTENT PHYTOTOXIN

Gerald R. Leather and Leonard E. Forrence, USDA-ARS, Forth Detrick, Bldg. 1301, Frederick, MD USA 21701

Crotalaria juncea (sunn hemp) is a legume with allelopathic potential. In our search for natural phytotoxins, we found that a water-soluble compound(s) extracted from the seeds of sunn hemp was toxic to *Lemna sp.* in bioassay. Fifty parts per million of a semi-purified extract inhibited *L. obscura* frond production 50%. Leafy spurge (*Euphorbia esula*), an invading noxious weed of rangelands in the USA and Canada is the target of alternative control techniques. Leafy spurge in sand culture produced only 25% of normal growth when two seeds of sunn hemp were planted in the same pot. Eight sunn hemp seeds inhibited the leafy spurge growth 88%. We are exploring the use of sunn hemp as an allelopathic crop plant for reducing leafy spurge infestations. With further development, the phytotoxin may be suitable as a natural herbicide.

Tuesday 16:00 **Oral Presentation 31**

IDENTIFICATION OF GROWTH INHIBITOR FROM *ARTEMISIA PRINCEPTS* VAR. *ORIENTALIS*

Kyeong Won Yun, Bong-Seop Kil, Dept. of Biology, Wonkwang University, Iri, Chonbuk, 570-749 and Jong-Sei Park, Doping Control Center, KAIST, Seoul, 135-090, Republic of Korea.

To find out growth inhibitor of the wormwood, *Artemisia princeps* var. *orientalis* , aqueous extract and volatile substances from the donor were treated to several selected receptor species included some bacteria and fungi. The results suggested that their inhibitory values of their growth were proportional to the concentration of the extract and the essential oil. It was, therefore, confirmed that the chemical substances of wormwood plant showed toxic activity biologically. The GC/MS method was employed to characterize the possible phytotoxic substances from the donor. Twenty chemical compounds from the water soluble fraction and the volatile mass were identified. The bioassays have done with chemicals identical to the gas-chromatically detected chemical compounds from the donor. The results obtained will be discussed.

Tuesday 16:15 **Oral Presentation 32**

INVESTIGATIONS ON SOME ASPECTS OF CHEMICAL ECOLOGY OF COGONGRASS (*IMPERATA CYLINDRICA* (L.) BEAUV.)

Inderjit and K.M.M. Dakshini, Department of Botany, University of Delhi, Delhi -11007, India.

To understand the mode of competitive mechanism of the weed, cogongrass [*Imperata cylindrica* (L.) Beauv.], its effect on nutrient availability and the components of mycoflora of its rhizosphere soils, as well as nodulation characteristics of *Melilotus parviflora* Desf. were investigated. Additionally, the effect of the leachate of the leaves and the roots of the cogongrass on percent germination and seedling characteristics of radish, mustard, tomato and fenugreek were examined. Further to assess the qualitative and quantitative differences in the phytochemical components, the leachates were analysed through High Performance Liquid Chromatography (HPLC). It was observed that out of Cu^{++}, Ca^{++}, Mg^{++}, Na^{+}, $K^{+}Zn^{++}$,Cl and $PO^{3-}{}_4P$ analysed, the weed appeared to control the availability of $PO^{3-}{}_4P$, Cl and Cu^{++}. Furthermore, of the 21 fungi recorded in the soils from the rhizosphere of the weed, decrease in the number of colonies (per gm of soil) of *Aspergillus niger* , *A. fumigatus* , *A. terreus* and *Penicillium chrysogenum* and increase in *A. flavus* and *Trichoderma viridis* was examined. The inhibition in nodule number and weight, acetylene reduction activity, root length and root/shoot ratio of *M. parviflora* were recorded. Percent seed germination, root and shoot length, fresh and dry weight of seedlings of different seeds were affected by the leachate of root and leaves. It was found that root leachate was more inhibitory than leaf leachate. However, the inhibition was higher in soil+leaves than soil+root leachate. HPLC analysis established that four compounds were contributed by the weed to the soil system even though their concentration varied in various leachate. It is surmised that these compounds cause allelopathic inhibition of growth characteristics of the seeds tested. Significance of the data vis-à-vis the competitive potential of the grass will be discussed.

Tuesday 16:30 **Oral Presentation 33**

BIOSYNTHESIS OF SESQUITERPENOID PHYTOALEXINS IN COTTON FOLIAR TISSUE

Margaret Essenberg, Gordon D. Davis, and Guadelupe Davila-Huerta, Dept. of Biochemistry, Oklahoma State University, Stillwater, OK 74075-0454, USA.

During the hypersensitive resistant response of leaves and cotyledons of cotton (*Gossypium hirsutum*) to incompatible races of the bacterial pathogen *Xanthomonas campestris* pv. *malvacearum* , phytoalexins accumulate in the fluorescent, necrotic host cells closest to intercellular colonies of the pathogen. The most potent of these structurally related, photoactivatable phytoalexins is 2,7-dihydroxycadalene (DHC). Putative biosynthetic precursors to DHC include *trans* -7-hydroxycalamenene, reported last year, and related compounds of molecular weights 216 and 222, which have been purified by normal phase HPLC on silica. A bacterial blight-resistant cotton line lacking lysigenous blands, WbM*gl* , possesses very low constitutive levels of sesquiterpenoids, but produced high levels of the phytoalexins after infection. Cell-free preparations from inoculated and $CaCO_3$-infiltrated control cotyledons of WbM*gl* , supplemented with cofactors, converted 1-^{3}H-farnesyl pyrophosphate to several hexane/chloroform-extractable, radioactive products. The predominant labeled product from the control extract cochromatographed with farnesol, whereas other products predominated from the extract of inoculated cotyledons. The FPP-converting enzyme from the latter extract has been partially purified. (Supported in part by the Oklahoma Agricultural Experiment Station and NSF Grant DMB 86-16650).

Tuesday 16:45 **Oral Presentation 34**

IDENTIFICATION OF PICEATANNOL AS A PHYTOALEXIN OF SUGARCANE

Anita M. Brinker and David S. Seigler, Dept. of Plant Biology, University of Illinois, Urbana, Illinois 61801, USA.

Sugarcane (*Saccharum* spp.) responds to infection by the fungus *Colletotrichum falcatum* , the causal agent of the disease red rot, by producing a red substance. Previous workers isolated the anthocyanidin luteolinidin from this red substance and suggested that the compound might play a role in disease resistance, but evidence that this was the case was not conclusive. In our studies, the most active component of extracts of infected sugarcane was identified as piceatannol (3,3', 4,5' -tetrahydroxy-stibene). This compound is produced by sugarcane in response to infection but apparently not in response to wounding alone.

Tuesday 14:00 **Oral Presentation 35**

MODIFICATION OF CUTICULAR HYDROCARBONS IN DIAPAUSING INSECTS

Jullien, R. and F. Perez, Laboratoire de Chimie Structurale Organique, Université de Paris-Sud, Bâtiment 410-415, 91405 Orsay, and J. C. Biemont, A. Ouedrago and J. Pouzat, Institut de Biocénotique Expérimentale des Agrosystèmes, Université François Rabelais, 37200 Tours, France.

It is now generally accepted that insect cuticular hydrocarbons are able to furnish valuable taxonomic information. Effectively the results of the comparison of cuticular hydrocarbons from *Acanthoscelides obtectus*, *A. obvelatus* and *Callosobruchus maculatus*, agree with the classification of these three species which are in the same subfamily of the Bruchidae. However, some differences between *A. obvelatus* (using exclusively diapausing insects) and its twin species *A. obtectus* stimulated us to study whether diapause has an effect on cuticular hydrocarbon repartition. The first results presented here concern the species *Callosobruchus maculatus* for which it is relatively easy to simultaneously obtain flying (sexually diapausing) or flightless (sexually active) insects.

Tuesday 14:15 **Oral Presentation 36**

MOLECULAR CLONING OF FLAVONOL 3-SULFOTRANSFERASE (ST) FROM *FLAVERIA*.

Luc Varin[1], Normand Brisson[2], Vincenzo De Luca[2] and Ragai K. Ibrahim[1], [1]Plant Biochemistry Lab, Department of Biology, Concordia University, Montréal H3G 1M8, Canada and [2]Department of Biochemistry, University of Montréal, Montréal H3C 3J7, Canada.

In order to greatly reduce glucosinolate biosynthesis in *Brassica napus* (Canola), we propose to divert the pool of the sulfate donor, 3'-phosphoadenosine 5' phosphosulfate (PAPS) towards the synthesis of novel sulfated metabolites, the flavonol sulfates. A flavonol 3-ST was purified from *F. chloraefolia* , partially sequenced and a monospecific polyclonal anti 3-ST antibody was raised. A cDNA library was constructed, screened with the anti 3-ST antibody and a positive clone was isolated and sequenced. The identity of the 3-ST clone was confirmed by expression in *E. coli* of flavonol 3-ST activity.

Tuesday 14:30 **Oral Presentation 37**

ENZYMOLOGY OF CYANOGENESIS IN LARVAE OF *ZYGAENA TRIFOLII* (ZYGAENIDAE - LEPIDOPTERA)

Adolf Nahrstedt, Sylvia Franzl and Elisabeth Müller, Institut für Pharmazeutische Biologie, University of Münster, FRG.

All developmental stages of the moth *Zygaena trifolii* liberate hydrocyanic acid (HCN) when violated. The glucosides linamarin and lotaustralin are the source of HCN by a two step degradation. The enzymes involved were investigated using the larvae of *Z. trifolii* . A specific ß-glucosidase consisting of two subunits catalyses the hydrolysis of the substrates to form the corresponding cyanohydrins; a flavin containing hydroxynitrile lyase then catalyses the decomposition to HCN and acetone and methylethylketone resp. The activity of both enzymes is evenly distributed in different tissues with more than 80% in the hemolymph. Thus the larvae dispose of an effective enzyme system to producing HCN from their endogenous substrates.

Tuesday 14:45 **Oral Presentation 38**

GRASSHOPPER SPIT: WHAT IS THE NATURE OF ITS "TOBACCO JUICE"?

William S. Bowers and Felix Ortego, Dept. of Entomology, University of Arizona, Tucson, Arizona 85721, USA.

Grasshoppers posses numerous defensive artifices including coloration, autotomy, saltatorial mobility, flight and offensive chemical secretions. When disturbed the grasshopper *Schistocerca americana* regurgitates a vomitus highly repellant to insects especially ants. Chemical and biological relationships of the secretion will be discussed.

Tuesday 15:00 **Oral Presentation 39**

SESQUITERPENOIDS IN *NASUTITERMES* SOLDIER DEFENSIVE SECRETION

C. Everaerts[1], J.L. Le Quere[2], Y, Roisin,[3] & J.M. Pasteels[3] ([1]: Laboratoire de Zoologie, URA CNRS 674, Université de Bourgogne, 21000 Dijon, [2]: Laboratoire de Recherche sur les Arômes, INRA, 21000 Dijon, France, [3] Laboratoire de Zoologie, Université Libre de Bruxelles, 1050 Bruxelles, Belgique.

In *Nasutitermes*, the defensive secretion secreted by the soldier frontal gland is composed of a terpenic mixture which acts as an irritant and entangling weapon, and sometimes as alarm pheromone. Now there is plenty of information aobut the mono - and diterpenes contained in these resins, but only a few studies have mentionned the existence of sesquiterpenes in these secretions. In seven Neo Guinean *Nasutitermes*, we have found no less than twelve sesquiterpene hydrocarbons, and in one species, *N. novarumhebridarum*, the sesquiterpenic fraction is bigger than the monoterpenic one. The possible roles and taxonomic implications of the sesquiterpenic fraction in *Nasutitermes* defensive secretion will be discussed.

Tuesday 15:15 **Oral Presentation 40**

HIGH PERFORMANCE ION EXCHANGE CHROMATOGRAPHY IN THE ANALYSIS OF PLANT MATERIALS

Joseph D. Olechno, James R. Thayer, Christopher A. Pohl, Rosanne W. Slingsby and John A. Statler, Dionex Corp., 501 Mercury Dr., Sunnyvale, CA 94086, USA.

Many compounds of plant origin are extremely polar (or ionic) and tend to be non-volatile. These attributes tend to make these compounds difficult to analyze by either reversed phase HPLC or gas chromatography. Traditional ion exchange techniques (e.g., CM- or DEAE-cellulose) tend to yield poor resolution and low efficiency. New polymer microbead based columns coupled with improved detection techniques allow the analysis of many plant derived materials with speed, efficiency and resolution. Ion exchange separations of numerous plant derived analytes will be shown including: HPAE-PAD analyses of plant derived mono- and oligosaccharides; acid labile non-protein amino acids analyzed at elevated pH; cation exchange separations of alkaloids and anthocyanins; plant protein analyses. Pulsed amperometry, suppressed conductivity, absorbance, fluorescence and post-column derivatization will all be considered in detection schemes.

Tuesday 16:00 **Oral Presentation 41**

USE OF HPLC IN PREFRACTIONATION AND PREPARATIVE ISOLATION OF NATURAL VOLATILE COMPOUNDS

Denis Barron, Pharmacognosy Laboratory, Faculty of Pharmacy, Joseph Fourier University-Grenoble I, 38706 La Tronche Cédex, France.

Capillary GC represents the method of choice for the analysis of aroma constituents, whereas HPLC is superior to GC in the preparative isolation of volatile constituents. In addition, HPLC is an efficient method for the prefractionation of complex extracts prior to GC analysis. The elution profiles of strawberry jam aroma constituents have been compared using two types of HPLC supports, silica gel, and diol-bonded silica. HPLC on diol allowed the recovery of all aroma constituents and resulted in the separation of two main fractions. The first fraction contained hydrocarbons and carbonyl compounds while alcohols and lactones were present in the second fraction. Further fractionation of the diol head fraction was achieved on silica gel. Combined HPLC on diol and silica gel columns has been applied to the semi-preparative isolation of some strawberry jam aroma constituents.

Tuesday 16:15 **Oral Presentation 42**

CAPILLARY ELECTROPHORESIS: A NEW WEAPON IN THE PHYTOCHEMISTS' ARSENAL.

Joseph D. Olechno, Jacqueline M.Y. Tso, Ann Wainright and James R. Thayer, Dionex Corp., 501 Mercury Dr., Sunnyvale, CA 94086, USA.

Most analytical scientists have mistakenly categorized capillary electrophoresis (CE) as a technique solely for the separation of proteins and nucleic acids. CE can also be used for a broad range of analytes regardless of size or charge. All liquid chromatographic techniques have corresponding CE equivalents, e.g., micellar electrokinetic capillary chromatography (MECC) separates small neutral molecules on the basis of their partition coefficients, capillary zone electrophoresis (CZE) separates on the basis of charge/mass ratios and ion exchange electrokinetic capillary chromatography (IEECC) separates on the basis of ion exchange. Only recently has CE been applied to plant samples. Examples of flavanoid, coumarin (with fluorescence detection) and alkaloid separations will be shown. A brief overview of the technique will be incorporated.

Tuesday 16:30 **Oral Presentation 43**

USE OF AN IRGA (INFRA-RED GAS ANALYSER) TO EVALUATE THE EFFECTS OF VOLATILE ALLELOCHEMICALS ON THE GAS EXCHANGE OF LEAVES

A.L. Anaya, Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600, 04510 México, J.M. Ramos and J. Arevalo, UAM-Xochimilco, Calz. del Hueso 1100, 04960 México.

Volatile compounds from organic extracts of different fresh leaves were collected by flushing air through them, and then injected to an IRGA open system. The changes on CO2 assimilation and H2O transpiration exchange on attached leaves were recorded. The possible mode of action and their biological significance are discussed. The potential for the use of IRGA systems to study the effect of volatile substances on gas exchange of leaves in situ is analyzed.

Tuesday 16:45 **Oral Presentation 44**

SUPERCRITICAL FLUID EXTRACTION (SFE) AND SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) FOR THE ANALYSIS OF PLANT MATERIALS

Joseph D. Olechno, Douglas A. Weitz and Dale Felix, Dionex Corp., 501 Mercury Dr., Sunnyvale, CA, 94086.

While both SFE and SFC have become accepted techniques in analytical science, their applications for plant analyses have been few and far between. Non-volatile or heat labile compounds, unacceptable for analysis by gas chromatography, can often be chromatographed with SFC. Extraction with supercritical carbon dioxide eliminates the need for halogenated solvents and eliminates the need for solvent evaporation. Recent work illustrating the efficacy of the technique for a number of analytes in a wide variety of plant matrices will be shown. Included will be the analysis of capsicum species for capsaicin, a comparison of expressed seed oils, analyses of Tagetes-derived acetylenics and comparisons of supercritical extractions versus those performed with traditional solvents. A brief review of the techniques and the analyses most benefited by their use will be included.

Wednesday 08:15 Oral Presentation 45

CHEMICAL DEFENSE IN *GLYCINE MAX* AGAINST POLYPHAGOUS VERSUS OLIGOPHAGOUS HERBIVORES

Dale M. Norris, 642 Russell Laboratories, University of Wisconsin, Madison, WI 53706, USA.

It was hypothesized that readily distinguishable chemistries in *Glycine max* provide defense against polyphagous versus oligophagous insects. Resistance in a "man-made" cultivar Davis to the oligophagous *Epilachna varivestis* is attributable to a mixture of flavonoids; however, resistance in a wild introduction PI 227687 to the very polyphagous *Trichoplusia ni* requires additional volatile essential oils. In Davis, man unknowingly made an attractive "death trap" for *E. varivestis* . Some implications of our findings regarding evolution of plant-insect interactions will be discussed.

Wednesday 08:30 Oral Presentation 46

MAJOR ANTITERMITIC COMPOUNDS FROM THE HEARTWOOD OF NORTHERN WHITE CEDAR, *THUJA OCCIDENTALIS* L.

Clarence A. McDaniel, USDA, US Forest Service, Southern Forest Experiment Station, P.O. Box 2008, GMF, Gulfport, MS 39505, USA.

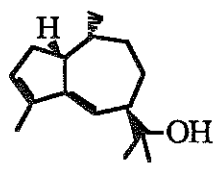
The heartwood of Northern White Cedar, *Thuja occidentalis* L., exhibits a natural resistance to attack by the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). Solvent extracts of the heartwood were separated into acidic, phenol-tropolone, and neutral fractions; and the antitermitic activity was found to be in the neutral fraction. The major compounds in this fraction were identified by GC/MS as the sesquiterpene alcohols occidentalol, -eudesmol, occidiol (occidenol), and occidol. These compounds were isolated by semi-preparative scale reverse-phase HPLC. Laboratory tests for antitermitic activity included cellulose pad tests, and vacuum-impregnating the compounds at approximately 1 percent and 0.5 percent by mass into sapwood of sweetgum, *Liquidambar styraciflura* L. All of the compounds above exhibited some antitermitic activity.

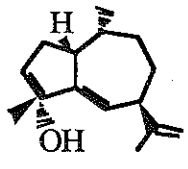
Wednesday 08:45 Oral Presentation 47

IDENTIFICATION AND PREPARATION OF ANTI-INSECTAN DIENOLS FROM *DIPTEROCARPUS KERRII* TREE RESINS

David P. Richardson, Department of Chemistry, Williams College, Williamstown, MA. 01267, USA and Adam C. Messer, School of Food and Nutritional Science, University of Shizuoka, 395 Yada, Shizuoka, 422 Japan.

Two previously uncharacterized sesquiterpene dienols (**1** and **2**), originally isolated from *Dipterocarpus kerrii* resin, were prepared from a-gurjunene in order to extend earlier structural studies and to confirm termiticidal activity. Preparation of **2** involved a novel one step oxidation of a-gurjunene with m-chloroperoxybenzoic acid. Spectroscopic studies established that **2** is a nonconjugated diene and that the C-4 tertiary alcohol center has α-configuration. Biossays with Indonesian termites (*Neotermes* ? *dalberglae*) demonstrated that **2** was more toxic than **1**, resulting in a 50% mortality in 7 days. The sesquiterpene dienols appear to arise from biotransformation of α-gurjunene.





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Wednesday 09:00 Oral Presentation 48

CELLULAR LOCALIZATION AND ECOLOGICAL ROLE OF A SECONDARY METABOLITE FROM THE SPONGE *HYMENIACIDON HELIOPHILA*

Susan H. Sennett, Amy E. Wright, Shirley A. Pomponi, J.E. Armstrong and Robin Willoughby, Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Ft. Pierce, FL, 34946, USA.

The sponge *Hymeniacidon heliophila* was selected to investigate cellular localization of secondary metabolites, production of metabolites by associated microbes, and feeding deterrence by secondary metabolites. Mature and larval forms of *H. heliophila* contain one major secondary metabolite which has been isolated and its structure elucidated. Density gradient fractionation yielded enriched cell fractions which were examined by light and electron microscopy to determine cell type. Analysis of fraction extracts suggests that the compound is localized in the archaeocytes. Production of the compound by microbial associates was also addressed. Extracellular bacterial associates separated from the sponge cells during the density fractionation did not contain the compound, nor was the compound detected in seven intracellular bacterial strains isolated from *H. heliophila* . Pinfish (*Lagodon rhomboides*) rejected pieces of whole sponge and sponge homogenate in preliminary feeding assays. Further assays are in progress to determine if the secondary metabolite has a role in this feeding deterrence.

Wednesday 09:15 Oral Presentation 49

ZOOPHARMACOGNOSY: NON-NUTRITIONAL USE OF SECONDARY CHEMICALS BY VERTEBRATES

Eloy Rodriguez, Phytochemistry and Toxicology Lab, University of California, Irvine, and Richard Wrangham, Dept. of Anthropology, Harvard University, Cambridge, MA 02138, USA.

Phytochemical and chemical ecology studies have clearly established that animals and insects have evolved a variety of adaptations for avoiding plants that contain toxins or repellents. In some cases, insects are known to sequester the substances and use them for defensive purposes. In this presentation, we present our recent chemical and ecological findings on tropical animals that use plant chemicals for therapeutic or stimulatory purposes. Wild chimpanzees from Africa have been shown to consume plants that contain potent anhelminthics and antibiotics; a primitive herbivorous bird, (hoatzin), from the Amazonas consumes a mixture of very toxic and primitive plants from the Amazon and wild monkeys from Brazil swallow large quantities of tree resins for possible stimulatory and therapeutic purposes.

Wednesday 09:30 Oral Presentation 50

CHANGES IN LEAF CHEMISTRY RELATED TO FOREST DECLINE

J. Wyman, Y. Maufette, J. Martel, G.R.E.F., UQAM, Montréal H3C 3P8, and J.P. Renaud, C.R.A., MPAQ, Québec, G1P 3W8, P.Q., Canada.

North American forests have been subjected to forest decline and one of the main species affected by this perturbation is *Accer saccharum*. Such stress will lead to changes in the leaf chemistry, which may have an impact on the phytophagous insects associated with these trees. This study was carried out in the south-west of Québec (300 km east of Montréal) in an area hardest hit by forest decline. 72 trees found in 18 different stands were sampled throughout the growing seasons of 1987 and 1988. Leaf samples were analyzed for total nitrogen, sugars (glucose, sucrose, frutose, ribulose), polyphenols, and tannins. Statistical analyses indicated no significant difference in total nitrogen concentrations between leaves of healthy and declining trees. However, significantly higher amounts of both sugars and polyphenols were noted in declining trees. This can have a potential impact on the nutritional ecology of a single key species or the structure of the whole insect community.

Wednesday 09:45 Oral Presentation 51

DIFFERENTIAL FATE OF PLANT ALLELOCHEMICALS IN TWO POLYPHAGOUS INSECTS

Murray B. Isman and Michael J. Smirle, Department of Plant Science, University of British Columbia, Vancouver, B.C. Canada V6T 2A2.

Using radiolabelled allelochemicals, we examined the metabolism and fate of several putatively toxic plant natural products in two polyphagous insects, the migratory grasshopper *Melanoplus sanguinipes* (Orthoptera, Acrididae), and the variegated cutworm, *Peridroma saucia* (Lepidoptera, Noctuidae). We sought to test the hypothesis that the more evolutionarily advanced lepidopteran would depend primarily on metabolism to avoid toxicity, whereas the orthopteran would depend primarily on physical barriers to absorption from the gut (Berenbaum and Isman, Experientia **45**, 229 [1989]). Both insects were fed the thiophene alpha-terthienyl, the furanocoumarin xanthotoxin, and the cardenolide digitoxin. Both insects metabolized xanthotoxin and digitoxin to a great extent, but the thiophene was metabolized to only a minor degree in the cutworm and excreted unmetabolized by the grasshopper. The fate of individual allelochemicals in polyphagous insects is best described as idiosyncratic.

Wednesday 10:30 Oral Presentation 52

PHYTOCHEMICAL BASIS FOR DIFFERENTIAL HOST USE BY WILD SILK MOTHS (*CALLOSAMIA*: SATURNIIDAE) LARVAE

K.S. Johnson and J. Mark Scriber, Department of Entomology, Michigan State University, East Lansing, MI 48824, USA.

The genus *Callosamia* is composed of three species with varying host breadths, with the most polyphagous (*C. promethea*) feeding on plants from numerous plant families, an oligophagous species (*C. angulifera*) which uses both Lauraceous and Magnoliaceous hosts, and a monophagous species (*C. securifera*) which is restricted to a single Magnoliaceous host, sweetbay (*Magnolia virginiana*). Hostplant specialization may be advantageous in terms of predator free space, higher rates of ingestion, enhanced assimilation efficiencies, or improved detoxification of plant tissues. Although sweetbay occurs within the ranges of the two *Callosamia* species with broader feeding habits, they have not been reported to use it as a host. First instar survival and relative growth rate of *C. promethea* and *C. angulifera* on sweetbay is low, with 100% mortality occurring by the end of the third instar. Fresh foliage was extracted and bioassayed for activity against these two silkmoth species. Preliminary results suggest a phytochemical component for differential host use within the genus. Identification of active compounds is underway.

Wednesday 10:45 Oral Presentation 53

COMBINED EFFECTS OF AMINO ACIDS AND GLUCOSINOLATES ON PREFERENCE AND PERFORMANCE OF THE GREEN PEACH APHID (*MYZUS PERSICAE* SULZ.)

Stewart Z. Merritt, Dept. of Biology, University of Michigan, Ann Arbor, MI, 48109, USA.

Although nutrients and allelochemicals co-occur in plants, their combined effects on insect herbivores have rarely been examined. I used artificial diets to test for effects of amino acids and glucosinolates, separately and in combination, on the preference and performance of the green peach aphid (*Myzus persicae* Sulz.). In choice tests, first instar nymphs failed to discriminate among diets, even though nymph performance was highest on diets high in amino acids and low in glucosinolates. However, because adults preferred diets that gave high nymph performance, nymphs were born where high quality diets were located. Thus, adult choice may compensate for the inability of nymphs to choose high quality diets.

Wednesday 11:00 Oral Presentation 54

MARINE PLANT POLYPHENOLS: THEIR EFFECT ON ASSIMILATION EFFICIENCY IN THE TEMPERATE HERBIVOROUS MARINE FISH, *XIPHISTER MUCOSUS*

N.M. Targett, A.E. Boettcher and T.E. Targett, Graduate College of Marine Studies, University of Delaware, Lewes, Delaware, USA.

We have previously shown that polyphenolic compounds, found commonly in temperate phaeophytes, negatively affect assimilation efficiency of the temperate marine herbivorous fish, *Xiphister mucosus* . This study examines the effects of distinct phenolic size classes using the commercially available phenolics, phloroglucinol and tannic acid, and phenolic size fractions derived from the phaeophytes *Fucus distichus* and *Fucus vesiculosus* (<10,000, 10,000-100,000, and >100,000) on *X. mucosus* . Both commercially available and naturally occurring phenolics were assayed at concentrations of 1% plant wet weight, an average total phenolic level which occurs naturally for many temperate brown algal species. At this concentration, phloroglucinol exhibited no significant effect on assimilation efficiency. Tannic acid enhanced assimilation, perhaps due to a pH effect. Phenolic containing *Fucus* extracts showed a negative dose-dependent response. However, upon fractionation, the response at a given concentration was dependent upon phenolic size, thus demonstrating that phenolic chemical nature (size) as well as phenolic concentration is important in determining the effect of these compounds on fish digestive processes.

Wednesday 11:15 Oral Presentation 55

TOXICITY OF *MORINDA CITRIFOLIA* ON *D. MELANOGASTER* AND RELATED SPECIES

L. Legal*, M. Katz**, B. Chappe*** and J.M. Jallon*. *BGE, CNRS, 91198, Gif sur Yvette. ** Biologie des populations, Univ. Paris VI, VII, Place Jussieu, 75005, Paris, France. *** ICSN, CNRS, 91198, Gif sur Yvette, France.

Morinda citrifolia is the host plant of *D. sechellia* while it is highly toxic for the other species of the subgroup (*D. melanogaster, D. simulans, D. mauritiana*). Only the ripe fruit is toxic for the three last species while the rotten fruit may serve as an host plant for all species. We have found significant differences in reactivity to the toxic fruit between species. Moreover, for *D. melanogaster* there is an intraspecific variability between strains. First investigations showed that a genetic factor controlling this reactivity is sex linked. The toxine of the ripe fruit is poorly volatile and of a moderate polarity. The whole toxicity can be extracted by acetone.

Wednesday 11:30 Oral Presentation 56

COSTS, BENEFITS, AND EXAPTATIONS OF CARDENOLIDE UTILIZATION IN THE CHEMICAL DEFENSE OF THE MONARCH BUTTERFLY, *DANAUS PLEXIPPUS*

Lincoln P. Brower, Department of Zoology, University of Florida, Gainesville, Florida, U.S.A. 32611.

The selective bases for the evolutionary origin and individual utilization of *de novo* and plant derived chemical defenses in insects continues to be debated. My paper will review the nature of the evidence that bears on the hypothesis of metabolic costs and will provide new experimental data that cardenolides do in fact exact a cost when ingested by first instar larvae of the monarch butterfly feeding on milkweed (*Asclepias humistrata*).

Wednesday 11:45 Oral Presentation 57

THE ROLE OF NUTRIENTS IN HOST-PLANT SELECTION BY INSECTS

Donald E. Champagne and Elizabeth A. Bernays, Dept. of Entomology, University of Arizona, Tucson Az. 85721, USA.

Current research emphasizes deleterious or deterrent roles of plant secondary metabolites, and tends to assume plants are nutritionally adequate for most insects. However, nutritional deficiencies can also affect host-plant selection. The acceptability of spinach to the grasshopper *Schistocerca americana* rapidly declines with successive contacts in a classic aversion learning pattern. Grasshoppers cannot metabolize the D7 or D22 phytosterols of spinach; when usable cholesterol or sitosterol is added aversion learning does not occur. After 24 h on spinach, grasshoppers prefer glass fibre discs with added cholesterol or sitosterol to those with the D22 sterol stigmasterol or sucrose controls. Detection of sterol quality occurs via gut feedback, not chemoreception. In this situation secondary metabolites likely provide a distinctive taste which is associated with the aversive stimulus ("bad" sterols) to facilitate rapid learning.

Poster 1

CHANGES IN BIRCH LEAF CHEMISTRY ASSOCIATED WITH WATER STRESS AND GYPSY MOTH FEEDING

A. Tomczyk, Department of Entomology, Warsaw Agricultural University, Warsaw, Poland and D.G. Nielsen, Department of Entomology, The Ohio State University-OARDC, Wooster, Ohio 44691, USA.

Two micropropagated clones of paper birch (*Betula papyrifera* Marsh.) were evaluated for response to water stress and partial defoliation by gypsy moth [Lymantria dispar (L.)] larvae, under greenhouse conditions. Two separate cohorts of 4th instars were placed on plants ca. 10 days apart to investigate influence of previous feeding on subsequent larval performance. Leaf samples were taken at specified intervals to measure the impact of water deficit and insect feeding on leaf sugars, amino acids, proteins and phenols. The clones responded differently, both in leaf chemistry and insect performance.

Poster 2

INTERSEXUAL PHEROMONAL COMMUNICATION OF THE EUROPEAN CORN BORER MODIFIED BY HUMIDITY CONDITIONS

Lucie Royer and Jeremy N. McNeil. Dept. de biologie, Université Laval, Ste-Foy, P.Q., Canada, G1K 7P4

We examined the effect of relative humidity conditions on *O. nubilalis* mating success over time, the calling behaviour of virgin females from emergence through their sixth night of calling and the flight response of 3-day-old males to three pheromone concentrations in a flight tunnel. The incidence of mating was significantly less the first two days following emergence at low than at high humidity conditions. The mean age at which females first initiated calling was independent of relative humidity conditions. However on subsequent nights of calling, fewer virgin females called under low compared with high humidity conditions, and those that did began later in scotophase. Furthermore, they spent less time calling. The maximum male response to the sex pheromone occurred at lower and lower relative humidity conditions as the pheromone concentration increased. The evolutionary and ecological significance of these results will be discussed.

Poster 3

ANTIMICROBIAL AND NEMATOCIDAL COMPOUNDS FROM SOME NORTHERN ROSACEAE PLANTS

Yuko Yoshizawa, Satoru Kawaii and Junya Mizutani, Mizutani Plant Ecochemicals Project, Research Development Corporation of Japan, Eniwa RBP Center Building, Megumino Kita 3-1-1, Eniwa-shi, Hokkaido, 061-13, Japan

Ethyl acetate extracts from more than 250 wild plants in Hokkaido, a northern island in Japan, were surveyed for their antimicrobial and nematocidal activities, for the purpose of understanding defence mechanisms of wild plants. Among the extracts, more than 90 samples exhibited one and/or both activities. Some typical northern Rosaceae plants such as *Sorbaria sorbifolia* var. *stellipila* , *Spiraea salicifolia* and *Malus baccata* var. *mandshurica* showed strong activities in both examinations. The active components are isolated. Their structure and characteristics will be described.

Poster 4

TWO DIMENSIONAL ANALYSIS OF INTRACELLULAR IONIZED CALCIUM IN THE NEMATODE, *CARNORHABDITIS ELEGANS.*

Satoru Kawaii, Yuko Yoshizawa and Junya Mizutani, Mizutani Plant Ecochemicals Project, Research Development Corporation of Japan, Eniwa RBP Center Building, Megumino Kita 3-1-1, Eniwa-shi, Hokkaido, 061-13, Japan.

In order to monitor changes in the intracellular concentration of ionized calcium in the nematode *Caenorhabditis elegans* , an esterified fluorescent indicator (Fura 2-AM) which penetrates into the cells and binds specifically to $Ca^{2}+$, was used. The fluorescent image of the Fura 2 stained nematode obtained by illuminating with light at 340 nm or 380 nm is displayed on a video screen using an SIT (silicon intensifier tube) video camera. The video signal from the camera is then processed with an image analyzing system. The effects of some northern Japanese plants extracts on the changes in $Ca^{2}+$ concentration are described.

Poster 5

THRYSIFLORIN, A NOVEL PHENALENONE PIGMENT FROM *WACHENDORFIA THRYSIFLORA* BER. (HAEMODORACEAE)

Gamal Dora and Michael Edwards, School of Pharmacy, University of Connecticut, Storrs, CT, USA, and William Campbell, Department of Chemistry, University of Cape Town, South Africa

From the yellow flowers of this plant we have isolated a phenalenone pigment, mp 218-222°; *v*max 1624 cm^{-1} (H-bonded CO);MW 304.0735. Cal. for $C_{19}H_{12}O_4$, 304.0732; H-NMR 8.21 (1H; 8.1Hz), 7.95(1H; 8.0Hz), 7.53 (1H; s), 7.41 (1H; 8.1Hz), 7.35 (1H; d 8.0Hz, 7.22 (2H; d 8Hz), 6.88 (2H; d 8Hz), to which the structure 2,6-dihydroxy-9-(4'-hydroxyphenyl) phenalenone was initially assigned. Unequivocal synthesis of this from 6-methoxyphenalenone has proved that this is not the correct structure for the natural product, and we now propose 2,4-dihydroxy-9-(4'-hydroxyphenyl) phenalenone, as the structure for thrysiflorin. This structure better fits the spectral data and agrees with the current biosynthetic hypothesis.

Poster 6

CHEMICAL BASIS OF CANNIBALISM IN AN HERBIVOROUS INSECT

Franz Bogner & Thomas Eisner, Neurobiology & Behavior, Cornell University, Ithaca, NY, USA.

Larvae of the moth *Utetheisa ornatrix* sequester pyrrolizidine alkaloids from their foodplants (Leguminosae; genus *Crotalaria*). They retain the chemicals through metamorphosis, and as adults transmit them in part to the eggs. All developmental stages are protected against predators as a result. Both males and females contribute to the alkaloidal endowment of the eggs. The male's endowment is transferred to the eggs by the female via the sperm package. By use of a pheromone derived from the alkaloid, males announce to females during precopulatory interaction how much alkaloid they can donate by seminal infusion. *Utetheisa* larvae in nature compete for the seeds of the foodplant, where the alkaloids are primarily stored, and they are unequally successful in acquiring alkaloids. In laboratory experiments we have shown that larvae can make up for systemic shortfalls in alkaloid by resorting to cannibalism. They consume pupae and eggs, feeding preferentially on those that contain alkaloid. Alkaloid-free pupae and eggs can be rendered vulnerable to cannibalism if artificially supplemented with alkaloid. Moreover, the predisposition to cannibalism in larvae is specifically triggered by systemic alkaloid deficiency.

Poster 7

DISTRIBUTION OF DIPEPTIDES CONTAINING D-ALANINE IN GRAMINEAE CROPS

Hisashi Manabe, Aizu Junior College of Fukushima Prefecture, Aizuwakamatsu, Fukushima 965, Japan.

In Japonica type rice plants, D-alanine (D-Ala) exists in the form of dipeptides, D-alanylglycine (D-Ala-Gly) and D-alanyl-D-alanine (D-Ala-D-Ala): D-Ala-Gly content was high (few µmol/g fr. leaves) and D-Ala-D-Ala content was low (one tenth of D-Ala-Gly content or less). These results made me examine all *Oryza* species (rice) and other well-known Gramineae crops (11 species) for dipeptides containing D-Ala.

Oryza species were classified into 3 groups; type I, II and III. Type I plants contain high levels of D-Ala-Gly and a low level of D-Ala-D-Ala. Type II plants contain only D-Ala-D-Ala and they can be further classified into IIa, IIb and IIc according to the amount of D-Ala-D-Ala they contained (IIa > IIb > IIc). Type III plants do not have any dipeptides containing D-Ala. Gramineae crops except rice were suggested to be classified into IIc or III because D-Ala-Gly was not detected in any of these crops and a low leverl of D-Ala-D-Ala was detected in only two species.

Poster 8

RELATIONSHIP OF FOLIAR GLUCOSINOLATE PROFILES IN CANOLA AND MUSTARDS TO GROWTH AND FEEDING OF THE BERTHA ARMYWORM, *MAMESTRA CONFIGURATA*

Cathy McCloskey and Murray B. Isman, Department of Plant Science, University of British Columbia, Vancouver, B.C., Canada V6T 2A2

The recent interest in developing new, chemically altered varieties of canola has prompted questions regarding the susceptibility of these new plants to insect attack. Five canola (*Brassica napus* and *B. campestris*) and three mustard (*B. juncea* and *Sinapis alba*) cultivars were evaluated for insect resistance against the bertha arm;yworm, *Mamestra configurata* Wlk. Foliar glucosinolate profiles were found to be related to larval growth and feeding on excised foliage of field-grown plants. The relative growth rate of this insect was negatively correlated with levels of isothiocyanate (ITC)-producing glucosinolates in the foliage. There was no correlation between consumption rate and levels of these substances. However, there appears to be a positive relationship between consumption rate and foliar levels of indolyl glucosinolates. Trends suggested by these results have been investigated further using pure compounds added to meridic diet.

Poster 9

BIOCHEMICAL INTERACTIONS BETWEEN CITRUS ROOTS AND THE SUGARCANE ROOTSTALK BORER, *DIAPREPES ABBREVIATUS*

Jeffrey P. Shapiro, U.S. Department of Agriculture, Agricultural Research Service, Horticultural Research Laboratory, Orlando, Florida 32803, and Kelsey R. Downum, Dept. of Biology, Florida International University, Miami, Florida 33199, USA.

Larvae of the root weevil *Diaprepes abbreviatus* feed on both small and large roots of citrus trees. Young larvae eat entire small roots, while older larvae strip the bark from large roots, girdling and killing them. Putative allelochemicals such as the pyranocoumarins exist in substantial concentration in roots but have no discernable effect on larvae. To determine the roles and significance of hemolymph in phytochemical detoxification or delivery, we are studying the involvement of larval hemolymph proteins in pyranocoumarin uptake, transport, and distribution.

Poster 10

NEW ISOFLAVONOIDS IN THE ROOT BARK OF *PISCIDIA ERYTHRINA* (JAMAICAN DOGWOOD: LEGUMINOSAE)

S. Tahara*, M. Moriyama and J. Mizutani, Dept. of Agr. Chem., Hokkaido Univ., Sapporo 060, Japan, and J.L. Ingham, Dept. of Food Sci., Univ. of Reading, Reading RG6 2AP, England. *Present address: Biol. Dept., Concordia Univ., Montréal, P.Q., Canada, H3G 1M8.

Further investigation of constitutive isoflavonoids in the root bark of *P. erythrina* (Leguminosae; Papipionoideae-Lonchocarpeae) has revealed the presence of some complex compounds. One of these is a coumaranochromanone derivative (3,5,7,4'-tetrahydroxy-5'-methoxy-3'-prenylcoumaranochroman-4-one) which belongs to a new class of naturally occurring isoflavonoids. Another novel compound is an amino-substituted isoflavone (4'-amino-5,7,3'-trihydroxy-5'-methoxy-2',6'-diprenyl-isoflavone). The characterization of these new compounds, as well as the evidence for the occurrence of 5,7,3',4',5'-pentaoxygenated isoflavones in this arboreal legume will be presented.

Poster 11

THE PHYTOTOXICITY OF PHENOLIC COMPOUNDS IN SOIL SYSTEMS: USING DENSITY-DEPENDENT GROWTH AS A BIOASSAY

Jodi R. Shann and Missy Watkins, Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-006, USA.

Allelopathic interactions are often difficult to separate from situations of resource competition. However, Wiedenhamer *et al*. (1989. *J. Applied Ecol.* 26:613) introduced the use of deviations from the density-dependent growth laws to partition out the inhibition associated with the presence of an allelochemical. In this study, we used that deviation from density-dependent growth as a bioassay to determine the phytotoxicity of soils treated with phenolic acids. One hundred grams of greenhouse soil (Taylor mix: peat, sand, river clay) were placed in 6X6 cm pots, planted with wheat (*Triticum aestivum* var. Caldwell) and given 18 hour days. After germination, plants were thinned to densities of 1, 5, 10, 15, 20 and 30 plants per pot and then treated with concentrations of ferulic, caffeic, *p*-hydroxybenzoic, or vanillic acid. Plants and soils were harvested at 3 and 6 weeks. Dry and wet shoot weights were used as the growth parameters. Soils were subjected to water and alkali extractions that were then analyzed for phenolic acids by HPLC. Result will be presented.

Poster 12

ISOLATION OF A NEW VINDOLINE DIMER FROM *CATHARANTHUS ROSEUS* VAR. LITTLE BRIGHT EYE. ARE VINDOLINE DIMERS ARTEFACTS OF ISOLATION?

John Balesevich and Greg Bishop, Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, S7N 0W9, Canada.

A new dimeric indole alkaloid, called supaine (**1**), was isolated from the aerial parts of *Catharanthus roseus* var. little bright eye. The structure of the new alkaloid was elucidated by comparison of its spectral data with that of the established compounds, vindolicine and secologanin. Corroboration for the assigned structure was obtained by analyses of products obtained from acidic hydrolyses of **1** as well as from its acetylation. Reaction of vindoline with various aldehydes under appropriate conditions afforded the corresponding vindoline dimers which were analogous to vindolicine and **1** thus raising the question of whether these latter vindoline dimers were artefacts produced during the isolation sequence.

Poster 13

THE EFFECT OF DENSITY AND METHOD OF KILL IN WEED SUPPRESSION PROVIDED BY A RYE COVER CROP

Leslie A. Weston, Dept. of Horticulture, University of Kentucky, Lexington; John B. Masiunas, Dept. of Horticulture, University of Illinois, Urbana; and Stephen C.Weller, Dept. of Horticulture, Purdue University, West Lafayette, USA.

Allelopathic rye cover crops may have potential to provide excellent weed control in tomato production systems. The effect of rye seeding density and method of kill on rye biomass persistence, weed control and tomato yield were determined in Kentucky, Illinois and Indiana locations in 1989 and 1990. Mowing alone did not adequately control the rye cover crop in any location and adversely affected tomato yields whereas greatest yields were obtained in plots treated with glyphosate. Rye density, method of kill and location interacted to determine weed pressure with greatest weed biomass occurring in mowed, low density rye plots in Kentucky. Although rye biomass did not substantially decrease during the growing season, weed control was only satisfactory for 4 to 8 weeks after rye kill; suggesting that other factors such as allelochemical concentration contributed to weed control.

Poster 14

AN EXAMINATION OF PHYTOALEXIN PRODUCTION IN THE HALOPHYTES *SPARTINA ALTERNIFLORA* AND *PHRAGMITES AUSTRALIS*

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This study examined phytoalexin production in the response of the halophytes *Spartina alterniflora* and *Phragmites australis* to abiotic and biotic stress. Field studies indicated that natural populations of *Phragmites* and *Spartina* contained compounds which were active *in vitro* against two marine fungi. Laboratory studies were undertaken to determine if these naturally occurring antifungal metabolites could be induced in sterile plant and callus cultures using abiotic (mechanical wounding) and biotic (fungal inoculation) stresses. Abiotic stress was ineffective in inducing a phytoalexin response in any of the laboratory cultures. *Phragmites* plants and callus treated biotically contained an antifungal metabolite not present in controls; however,*Spartina* sterile plants did not respond to the biotic treatments. Results of these studies suggest that a biotic stress such as microbial invasion can induce the formation of antifungal metabolites in halophytic plants. Characteristics of the antifungal metabolite produced in the treated *Phragmites* cultures will be described.

Poster 15

OVIPOSITION STIMULANTS IN THE COCCOID CUTICULAR WAXES OF *APNYTIS YANONENSIS*

Shozo Takahashi, Pesticide Research Institute, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan.

Aphytis yanonensis, a parasitic wasp of the arrowhead scale, *Unaspis yanonensis* was introduced to Japan to control *U. yanonensis*. *A. yanonensis* recognizes a host insect by antennal contact and deposits eggs on the insect host underneath the scale. Ovipositional behavior is induced by perceiving the cuticular wax of the host insect, *U. yanonensis*, and other coccoids. Chemical composition of the cuticular wax was analyzed and oviposition stimulants were isolated following a bioassay using *A. yanonensis*. All cuticular wax that actively stimulated the ovipositional behavior of *A. yanonensis* were found to be a mixture of wax esters with a composition similar to the cuticular wax of *U. yanonensis*.. The latania scale, *Hemiberlesia lataniae* was found to be a good substitute host for successful rearing of *A. yanonensis* in the laboratory. The major component (52.5%) of the scale wax was a mixture of triglycerols of molecular weight from 666 to 890.

Poster 16

PUDDLING BEHAVIOR: MASSIVE FLUID THROUGHPUT LEADS TO SODIUM SEQUESTRATION IN A MOTH

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Male butterflies and moths are commonly attracted to standing water sources to drink, a behavior known as "puddling". The notodontid moth *Gluphisia septentrionis* exhibits the behavior in extreme form. In the course of several hours of puddling, male *Gluphisia* forcibly pulse through their bodies a volume of water equivalent to over 600 times their mass (up to 38.4 mL). As indicated by cationic analyses of imbibed and ejected fluids, and of the body itself, the net result of puddling is a systemic gain in sodium. Male *Gluphisia* concentrate sodium in their reproductive system, presumably for eventual nuptial transfer to females (as documented for a butterfly by other researchers). Rapid fluid throughput and ionic absorption in male *Gluphisia* are enabled by oral and enteric structural specializations.

Poster 17

ROLE OF 2,4-DIHYDROXY-7-METHOXY-1,4-BENZOXAZIN-3-ONE (DIMBOA) IN THE RESISTANCE OF A MAIZE TO WESTERN CORN ROOTWORM, *DIABROTICA VIRGIFERA VIRGIFERA* (LECONTE) (COLEOPTERA; CHRYSOMELIDAE)

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2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), the major hydroxamic acid present in corn, was studied for its effects on western corn rootworm, *Diabrotica virgifera virgifera* (LeConte). Exogenously applied DIMBOA showed a strong effect on western corn rootworm larvae feeding on corn seedlings. The LC50 (lethal concentration for 50% mortality) value (fiducial limits) was 153.2 ppm (107.7-208.9). The deleterious effects of DIMBOA on western corn rootworm larvae are possibly due to feeding deterrence and toxicity. In replicated pot trial tests during two growing seasons, two corn lines from CIMMYT collections, ITR 3872 with high DIMBOA content in roots, and NTR-2 Ger. 4042 with low DIMBOA content in roots, were evaluated for resistance to western corn rootworm larvae. The results indicated that the high DIMBOA line stressed western corn rootworm larvae to produce inferior size and number of adults, and high DIMBOA line showed significantly less damage than low DIMBOA line NTR-2 Ger. 4042 in most plant growth parameters measured.

Poster 18

(Z)-10-HEPTADECEN-2-ONE BIOSYNTHESIS IN *DROSOPHILA BUZZATII*

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(Z)-10-heptadecen-2-one, the major aggregation pheromone component of *Drosophila buzzatii*, is produced and stored in the ejaculatory bulb of males. The ketone first appears in the bulb at 2-3 days of age, increases most rapidly from 4-6 days, and plateaus at 7-8 days. We have proposed two alternative pathways for (Z)-10-heptadecen-2-one biosynthesis starting from various fatty acid precursors. Excised ejaculatory bulbs incubated for 18 hours in pH 6.5 buffer fortified with coenzymes produce (Z)-2-heptadecen-2-one comparable to live males. Fatty acids are being identified as possible pheromone precursors from changes in the fatty acid profiles of incubated ejaculatory bulbs. Preliminary experiments on incorporation of radiolabled fatty acid precursors will be presented.

Poster 19

REEXAMINATION OF THE FEMALE-PRODUCED SEX PHEROMONE OF THE PEACH TWIG BORER, *ANARSIA LINEATELLA* ZELLER (LEPIDOPTERA: GELECHIIDAE)

Jocelyn G. Millar, Thomas C. Baker, Dept. of Entomology, University of California, Riverside, CA 92521 and Richard E. Rice, Dept. of Entomology, University of California, Davis, CA 95616, USA.

The peach twig borer is a major pest of stone fruits and almonds in California. Developing resistance to pesticides and/or the possible loss of pesticide registrations has created a pressing need for alternative management strategies. Recent mating disruption trials using blends of E5-10:Ac and E5-10:OH, the components identified in pheromone gland extracts by Roelofs et al. (1975), were not completely sucessful. We have reexamined the sex pheromone of the peach twig borer and wish to report: a) the identification and field testing of minor components from the pheromone gland; b) determination of the pheromone blend produced by calling females; c) field testing of analogs and homologs of the pheromone components as synergists and behavioral antagonists.

Poster 20

SCREENING OF HOKKAIDO PLANTS FOR ANTIFEEDANT ACTIVITY, USING AN IMPROVED LEAF-DISK BIOASSAY

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Chemical elucidation of plant allelochemicals and study of their physiological mode of action are two fundamental steps for understanding the complex relationships between plants and other organisms. In our search for new bioactive compounds, we have undertaken a survey of Hokkaido plants where we have used an improved leaf-disk antifeedant bioassay against *Spodoptera litura* (Tobacco cutworm). The principal improvement consists of a more accurate measurement of consumption rates, using numerisation of leaf-disk surfaces through a video-computer interface. Among the 250 samples assayed, 33 were found to present a significant feeding-deterrent activity. The method, as well as the results of the screening, are presented.

Poster 21

DEFENSIVE SUBSTANCES FROM TROPICAL PLANTS I

Labunmi Lajide, Pierre Escoubas and Junya Mizutani, Mizutani Plant Ecochemicals Project, Research Development Corporation of Japan, Eniwa RBP Center Building, Megumino Kita 3-1-1, Eniwa-shi, Hokkaido 061-13, Japan.

Crude methanolic extracts of *Pavetta crassipes*, *Parkia clappertoniana* and *Aristolochia albida* were examined for insect feeding deterrent activities against the armyworm larvae, *Spodoptera litura* using a choice leaf-disk bioassay technique. TLC bioautography was also used for activity against the fungus *Cladosporium herbarum* . Only *A. albida* extracts exhibited strong activity against *S. litura* and *C. herbarum.* The results of the bio-assay using an improved technique in quantification and the compounds responsible for the activities in *A. albida* will be discussed.

Poster 22

NITROGEN REGULATION OF ANTHOCYANIN COMPOSITION IN GRAPE (*VITIS VINIFERA* L.) CELL SUSPENSION

François Cormier and Chi Bao Do, Food Research and Development Centre, Agriculture Canada, 3600 Casavant Blvd. West, St-Hyacinthe (Québec) Canada J2S 8E3.

Recent restriction of the use of synthetic red dyes in food has activated research on plant pigments. We investigated the effects of nitrogen source on the formation of anthocyanins to develop methods for the production of red pigments by *Vitis vinifera* cell suspension culture. When cells were grown in B5 basal medium anthocyanin accumulation was low. Decreasing nitrate concentration and increasing the ratio of the carbon source to nitrate ions in the culture medium enhanced significantly the level of anthocyanins, mainly peonidin 3-glucoside. The composition of anthocyanins was also influenced by the concentration of ammonium ions. The formation of acylated anthocyanin, namely peonidin 3-p-coumaroyl glucoside was promoted by the high concentration of ammonium ions. Nitrogen source can be one of the important culture factors and may be useful in controlling of anthocyanin composition and production.

Poster 23

^{1}H and ^{13}C NMR ASSIGNMENTS OF THE MAJOR ANTHOCYANINS FROM A *VITIS VINIFERA* CELL SUSPENSION CULTURE

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A cell suspension culture of *Vitis vinifera* cv Gamay Fréaux var teinturier was studied for its anthocyanins. The structure of the three major anthocyanins, which together account for approximately 85-90% of the total, was determined on small quantities of material using 1D and 2D NMR techniques in both normal and inverse modes of detection. ^{1}H and ^{13}C NMR spectra were completely assigned for cyanidin 3-b-D-glycopyranoside, peonidin 3-b-D-glycopyranoside and peonidin 3-(6-p-coumaryl)-b-D-glycopyranoside, as well as for the commercial product malvidin 3,5-di-b-D-glycopyranoside, which was used for comparison purposes.

Poster 24

FORMATION OF INSECT ALKALOIDS FROM PLANT DEHYDRO-PYRROLIZIDINES BY ARCTIID MOTHS (LEPIDOPTERA)

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Callimorphine plus two new pyrrolizidine alkaloids ("creatonotine", "isocreatonotine") were isolated from adults of *Creatonotos transiens* and shown to be synthesized in both sexes by esterification of retronecine fed to larvae. Feeding ester alkaloids to *Creatonotos* larvae lead to degradation of plant PAs and reesterification of the resulting necine, i.e. also to the formation of creatonotines and callimorphine. However, there were quantitative differences and, in part, sex-bias in the rates of transformation due to different PAs. In any case, this is the first demonstration of hydrolysis of ester alkaloids and subsequent reesterification of the necine base with necic acids of insect origin by PA-adapted insects.

Poster 25

PURIFICATION AND PROPERTIES OF PHENYLALANINE AMMONIA-LYASE FROM ELICITOR-TREATED JACK PINE CELL CULTURE

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Phenylalanine ammonia-lyase (PAL, E.C.4.3.1.5) appears to be involved in the accumulation of phenolic compounds in pine suspension cultures in response to ectomycorrhizal fungal elicitation. PAL has been purified to homogeneity from *Pinus banksiana* suspension cultures using anion-exchange and chromatofocussing FPLC. Physical and kinetic characterization of the enzyme has revealed that pine PAL is similar to PAL from other plant sources. Chromatographic analyses have been used to identify possible isoenzymic changes in pine PAL in response to elicitation by an ectomycorrhizal fungus.

Poster 26

FEEDING DETERRENCY OF *AUSTROEUPATORIUM INULAEFOLIUM* (COMPOSITAE) AGAINST *SITOPHILUS ORYZAE* (COLEOPTERA, CURCULIONIDAE) IN RELATION TO INSECT ADAPTATION

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Methanol, dichloromethane-acetone, and hezane leaf extracts of the title plant were found to be strong feeding deterrents against tropical strains of the rice weevil *S. oryzae* (L.). This was determined by using short term (24 h) two choice bioassays on whole wheat kernels with concentrations as low as 0.5% w/w of extract. However, longer exposure of the test insects in the two choice testing arena (7 days) resulted in undifferentiated acceptance of treated/control kernels with all extracts. The possible implication of these observations in terms of inadequacy of established feeding deterrency bioassays of storage insects will be discussed.

Poster 27

TANNIN CHEMISTRY IN RELATION TO HERBIVORY

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The impact of tannins on herbivory has been difficult to assess in part because the tannins are such a diverse group of compounds. We have evaluated the chemical and biochemical properties of tannin *in vitro*, and have used that information to understand the nutritional effects of tannins. For example, nitrogen utilization in deer is adversely affected by the gallotannin found in fireweed flowers, but is unaffected by commercial gallotannin (tannic acid). The fireweed tannin is higher molecular weight than the commercial tannin, and is more likely to form insoluble tannin-protein complexes. The low molecular weight galloyl esters in tannic acid are presumably hydrolyzed in the rumen of the deer and thus do not affect nitrogen economy. In monogastric mammals such as rats even tannic acid diminishes apparent protein digestibility.

Poster 28

BIOACTIVE PHENOLIC CONSTITUENTS FROM THE LEAVES OF *MAGNOLIA VIRGINIANA*

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We have identified plant compounds that prevent larvae of unadapted swallowtail butterfly species (Lepidoptera: Papilionidae) from feeding on *Magnolia virginiana* (Magnoliaceae). This tree is native to the southeastern United States and, although is a host for certain *Papilio* species, contains compounds that strongly deter feeding by *Papilio palamedes* caterpillars. Our bioassay-directed fractionation of *M. virginiana* leaf extracts identified high activity in a fraction comprised of two neoligan compounds. One of these compounds, magnolol, has been reported to occur in other members of the Magnoliaceae. The second compound, a biphenyl ether of magnolol, has to our knowledge not been previously identified in *Magnolia* species. In addition to having bioactivity against a lepidopteran species, preliminary studies indicate that both compounds are toxic to *Aedes aegypti* larvae and greatly inhibit bacterial growth.

Poster 29

LOCALIZATION OF PHENOLIC COMPOUNDS IN EPIDERMIS AS THE FIRST CELLULAR BARRIER BETWEEN THE PLANT BODY AND ITS ENVIRONMENT

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Epidermal vacuoles of many of the 50 species examined had phenolic compounds, as did trichomes, if present. Epidermis originates via protoderm from tunica initials, at the very top of the shoot apex. *Crategus* and *Vitis* contained phenolics in the tunica; others only started to contain them in the vesicles and vacuoles of the protoderm. These vacuoles arise from the ER by: (1) separation of small vesicles from ER cisternae followed by their fusion, leading to vacuoles of increasing size, or (2) dilation of large areas of ER sheet, with the rapidly increased space of the cisternae leading directly to formation of large vacuoles. In the present case a connection of the still increasing ER with the large central vacuole was recognizable.

Poster 30

IDENTIFICATION OF HAIRPENCIL SECRETION FROM *MAMESTRA BRASSICAE* (L.) MALE (LEPIDOPTERA, NOCTUIDAE) AND ELECTROANTENNOGRAM STUDIES

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The male *Mamestra brassicae* hairpencils are everted during courtship in the vicinity of the female and this seems to increase female acceptance. Hairpencil extracts of 3-day-old males were analysed by capillary gas chromatography (GC) and by GC-mass spectrometry. The extracts were found to consist of 6 components. Benzaldehyde, 2-methyl propanoic acid, 2- methyl butanoic acid and phenol were present in the extracts as well as the previously identified benzyl alcohol and phenyl ethanol. Electroantennograms were recorded on male and female antennae in response to stimulation by hairpencil compounds. Male and female antennae responded to each chemical but the female responses were significantly higher than those of the males.

Poster 31

C-GLYCOSYLFLAVONES FROM *CENTAURIA PALLESCENS* L.

Mamdouh M. Abou-Zaid, Department of Biology, University of Ottawa, Ottawa, Ont., Canada K1N 6N5.

The flavonoids of the Compositae have been of ongoing interest as this family contains a number of medicinal plants. The present study deals with the separation and identification of the C-glycosylflavones of *Centauria pallescens* L. (Compositae). Fresh plant material was collected in August 1988, from St. Catharines, Sinai, Egypt. The leaves and stems were extracted with 70% EtOH, followed by isolation on a series of polyamide, polyvinyl-polypyrolidone (PVPP) and Sephadex LH-20 columns. The C-glycoside mixture was isolated by HPLC using a prep. C-18 column and a MeOH : H20 : HCOOH solvent system. Pure compounds were subjected to physical analysis (UV, ^{1}H-NMR, ^{13}C-NMR and FAB-MS). Two (of a total of six) of the C-glycosylapigenins have been identified as isovitexin (6-C-glucosylapigenin) and vitexin (8-C-glucosyl-apigenin). The remaining four are under further investigation.

Poster 32

FLAVONOIDS FROM THE LEAVES OF *ACER RUBRUM* L. AND *A.SACCHARUM* L.

Mamdouh M. Abou-Zaid and Constance Nozzolillo, Department of Biology, University of Ottawa, Ottawa, Ont. Canada K1N 6N5.

Leaves (1 Kg. Fr. wt.) of *Acer rubrum* L. (red maple) and *Acer saccharum* L. (sugar maple) [family *Aceraceae*] were collected in September 1989, from mature trees in the city of Ottawa and extracted with 70% MeOH. The methanolic extracts were fractionated on a polyvinylpolypyrrolidone (PVPP) column using water followed by increasing concentrations of methanol. PVPP fractions were further separated by paper chromatography. Final purification was achieved on Sephadex LH-20 column. Pure compounds were tested for purity by HPLC using a C-18 column and a MeOH: $H_2$0 : HCOOH solvent system. The glycosidic flavonoids were subjected to chemical and physical investigations (UV, ^{1}H-NMR, ^{13}C-NMR and FAB-MS). Kaempferol 3-0-glucoside, quercetin 3-0-glucoside, gallic acid and acylated glycosides of kaempferol and quercetin were isolated and identified.

Poster 33

THE CHEMICAL RELATIONSHIP BETWEEN COTTON AND BOLLWORM (*PECTINOPHORA GOSSIPIELLA*).

Cao Ri Qiang and Yu Hui Min, Dept. of Biology, Nanjing University, Nanjing, Jiangsu, China.

We used a variety of chemical analyses combined with bollworm bioassays to determine the effects of different cotton plant extracts upon the feeding, growth and development of cotton bollworms. Alcohol extracts of cotton bolls stimulated feeding and increased growth rate, decreased mortality and increased pupation and emergence rates. These chemicals were crucial for emergence and thus, for further reproduction. The chemicals involved were isolated by a variety of techniques and were found to be sesquiterpenes and long chain fatty acids and esters. Both water extract of cotton bolls and alcohol extracts of cotton leaves were toxic to cotton bollworm larvae. These inhibited feeding, decreased growth rates and increased larval mortality. Various analytical techniques showed that there are at least 10 toxins in the leaves and 8 in the boll. These include carboxylic acids, alkaloids, aromatic carboxlic acid esters, flavonoids, and quinones Future research will further investigate the nature and effects of these naturally occurring toxins and stimulants.

Poster 34

BIOACTIVITY OF SOME CANARIAN LAURACEAE EXTRACTS AGAINST THE SUGARCANE BORER, *HIEROXESTIS SUBCERVINELLA* Wilk. (LEPIDOPTERA: LYONETIIDAE) AND THE CANARIAN PINE CATERPILLAR, *MACARONESIA FORTUNATA* B. & AL. (LEPIDOPTERA: LYMANTRIIDAE).

Azucena González-Coloma, Instituto de Productos Naturales Orgánicos, CSIC, Avda. Astrofísico F. Sánchez 2, 38206 La Laguna, Tenerife, Canary Islands, Spain and Raimundo Cabrera, Dpto. de Fitopatología, UDI La Laguna, Tenerife, Canary Islands, Spain.

Interest in the possible application of natural products to insect pest management is increasing with increasing environmental problems related to chemical pest control. Plants of the family Lauraceae are dominant in the wet forest ("Laurel forest") of the Canary Islands, a remant flora of the Tertiary era. We have previously shown that the toxicity of a methanolic extract of the tree *Persea indica* (Lauraceae) to laboratory mice was due to the presence of the diterpenes ryanodiol and cinnceylanol. To investigate the potential insecticidal properties of this plant species, its hexane, chloroform and methanol extracts were incorporated into artificial diets at various doses to the larvae under study in no choice tests. Results on larval mortality, growth and potential use as a pest control agent will be discussed.

Poster 35

TRANSFORMATION OF *ESCHSCHOLTZIA CALIFORNICA* : ROOTS, EMBRYOS AND ALKALOIDS

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Seedlings of *E. californica* were found to be susceptible to infection with a variety of *Agrobacterium rhizogenes* strains. Some strains induced adventitious root formation on greater than 60% of the test seedlings. In addition to roots many of the infected seedlings also showed the development of embryos at the wound site. Control seedlings showed a very low frequency of root formation but never showed embryo production. Root and embryo cultures, established from the infected seedlings were examined for growth and alkaloid accumulation. Benzophenanthridine alkaloids accumulated to relatively high levels in the roots (> 0.3% dry weight), but embryos showed little accumulation until further tissue development. The effect of nitrogen and carbohydrate source on embryo development will also be discussed.

Poster 36

INSECTICIDAL MODE OF ACTION OF AN EXTRACT OF THE BARK OF THE PAW PAW, ASIMINA TRILOBA, CONTAINING THE LINEAR ACETOGENIN, ASIMICIN.

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A purified extract (F020) containing the linear acetogenin, asimicin, from the bark of *Asimina triloba* , was investigated as a biologically active substance to the European cornborer, *Ostrinia nubilalis* and the tobacco hornworm, *Manduca sexta* . Significant aberrations in mortality, growth and fecundity were evident. Toxicity of the compound was apparent through a comparison of the nutritional indices determined for *O. nubilalis* between the consumption and topical application of F020. Consumption index, relative growth rate, efficiency of conversion of ingested food and efficiency of conversion of digested food decreased in all cases, suggesting a true toxin rather than an antifeedant. The approximate digestibility was not significantly different as the dose of F020 increased, indicating that the digestive process is not being affected. The effects of this novel plant product observed in the present study represents one of the first investigations of acetogenins as a class of natural insecticides and may lead to the development of a useful pest control agent.

Poster 37

RESOURCE AVAILABILITY, PHENOLIC PRODUCTION, AND HERBIVORY IN THE HAYSCENTED FERN (*DENNSTEADTIA-PUNCTILOBULA*)

Cary D. Dustin and Gillian A. Cooper-Driver. Biology Dept., Boston University, 5 Cummington St., Boston, MA, USA 02215

The Hayscented Fern can be found growing in a range of habitats including fields, forest gaps, and forests. Eight field sites representing a range of habitat types were studied in the White Mountains of New Hampshire, USA. Results will be presented showing how levels of light, soil moisture, and soil nitrogen in various habitats relate to the production of phenolic compounds. Levels of phenolics in leaves varied significantly among habitats and were most closely correlated with light availability (r=.64), while phenolic levels correlated with soil moisture (r=.52) and soil nitrogen (r=.40) to a lesser degree. A negative exponential relationship was found between phenolic levels and C:N in leaves. An inverse relationship (r=.57) was found between the amount of phenolics present and insect herbivory.

Poster 38

WHY IS THE NOCTURNAL GEOMETRID, *HOMOCHLODES FRITILLARIA* , A FERN SPECIALIST?

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Homochlodes fritillaria is a fern specialist. The major host of the larvae is bracken fern, *Pteridium aquilinum* . In areas where bracken is absent, *H. fritillaria* colonizes the closely taxonomically related weedy fern species, *Dennstaedtia punctilobula* (the hay-scented fern). Although closely related taxonomically bracken and hay-scented fern show major differences in their secondary chemistry. The latter synthesizes coumarins and salicylic acid, compounds not commonly isolated from ferns. The relative amounts of coumarin and salicyclic acid effect both colonization and feeding of the hay-scented fern by the geometrid larvae. Concentrations of these compounds can be changed by manipulation of the environement in which these ferns grow.

Poster 39

USE OF POLYMERIC ADSORBENTS FOR ON LINE EXTRACTION OF BENZOPHENANTHRIDINE ALKALOIDS IN CELL CULTURES

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The production of sanguinarine, a benzophenanthridine alkaloid, by plant cell cultures may represent an interesting alternative to agricultural supply of this product. A key requirement for an efficient bioprocess is that the product of interest be released from cells into the medium so that an on line extraction can be performed. Many techniques have been used to achieve the *in situ* removal: solvent extraction, ion-exchange, adsorption, membrane and physical methods. Among these, the use of polymeric adsorbents like XAD resins seems to be very promising. In this presentation, the theoretical aspects of the adsorption/desorption process for different benzophenanthridine alkaloids onto XAD resins will be examined: the influence of the pH, the adsorption capacity, the importance of contact time and type of contact (batch and fluidized bed) of 2 alkaloids (sanguinarine and chelerythrine) onto 2 resins (XAD4 and XAD7) will be presented. The choice of eluent and concentration effect involved in the desorption step will also be discussed.

Poster 40

A BIOPROCEES FOR ON-LINE REMOVAL OF SANGUINARINE FROM SURFACE-IMMOBILIZED POPPY CELLS.

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Cells lines of Papaveraceae species, including *Eschscholtzia californica, Sanguinaria canadensis* , and several *Papaver* representatives, were examined for their ability to produce benzophenanthridine alkaloids. Maximal alkaloid production was evaluated by elicitation of the cells with either a fungal (*Botrytis*) homogenate or chitin. Alkaloid yields as high as 2.5% of cell dry weight were observed. A number of cell lines from different species were successfully surface-immobilized and grown on a polyester matrix in 2 or 6 L bioreactors. Growth in the bioreactors and in suspension were comparable. On-line removal of alkaloids from elicited immobilized cultures was achieved by recirculating the medium through a column containing neutral adsorbent resin (SAD-7). In many cases more than 50$ of the total alkaloids produced by the elicited cells were located on the resin.

Poster 41

WEEVIL REPELLENT CONSTITUENTS OF OCIMUM SUAVE LEAVES AND EUGENIA CARYOPHYLLATA CLOVES

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The leaves of Ocimum suave and the cloves of Eugenia caryophyllata , used as grain protectants in parts of East Africa, yield essential oils that are repellent to the maize weevil, Sitophilus zeamais, in olfactometric assays. Eugenol, a common constituent of the two, was found to be a potent repellent of the weevil. Eugenol was more repellant than the synthetic commercial compound, DEET (N,N-diethyltoluamide) and the two analogues of eugenol, methyl eugenol and isoeugenol. Results from this study demonstrate a possible scientific rationale for the use of these plants as grain protectants by communities in East Africa.

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