

# Second Asia-Pacific Conference on Chemical Ecology

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## Scientific Program

Session A - Plenary Lectures & Chemical Communication System and Behaviour

Session B - Natural Products Chemistry

Session C - Biosensor and Chemoreception

Session D - Application of Bioactive Compounds

Session E - Kairomone and Allomone

Session F - Alleopathy in Agroecosystem

Session G - Plant and Animal Interaction

Session H - Chemical Ecology in Agroecosystem

- Session I - Chemical Ecology in Aquatic Ecosystem
- Session J - Chemical Ecology in Forest Ecosystem
- Session K - Chemical Ecology in Urban Entomology
- Session L - Molecular Ecology

**< PLENARY LECTURES >**

Kenji Mori	Organic synthesis as a tool in life sciences
Thomas Hartmann	Pyrrolizidine alkaloids in plants and adapted insects: functional and evolutionary aspects

**SESSION A**

**< Chemical Communication System and Behaviour >**

Aijun Zhang	A-1	Chemicals affecting the behavior of the Asian longhorned beetle, <i>Anoplophora glabripennis</i> (Coleoptera: Cerambycidae)
Tadakazu Nakashima	A-2	Chemical communication in <i>Edoclitia excrescens</i> (Lepodoptera: Hepialidae)
Richard Vickers	A-3	Delayed mating and its significance in mating disruption treatments
Toru Yamasaki	A-4	Tannin toxicity to minute animals
Y.S. Chow	A-5	Mating behavior in a local blister beetle, <i>Epicauta hirticornis</i> (Coleoptera: Meloidae)
Jia-Wei Du	A-6	The diversity of chemical communication systems between the resistant and susceptible strains of cotton bollworm, <i>Helicoverpa armigera</i>
Chul-Sa Kim	A-7	Are the probing stimulants a host recognition substance for rice leafhoppers and planthoppers? – Isolation and identification of the probing stimulant from the rice plant for <i>Nephotettix cincticeps</i> Uhler
Soichi Kugimiya	A-8	Nuptial secretion of the male German cockroach: Pheromonal role of lipid components in courtship
Alvin Kah-Wei Hee	A-9	Comparative attraction of three <i>Bactrocera dorsalis</i> (Diptera: Tephritidae) complex sibling species to methyl eugenol

**SESSION B**

**< Natural Products Chemistry >**

Takeshi Kitahara	B-1	Synthesis of JH-biosynthesis inhibitors and phytosiderophores
William Kitching	B-2	Allenic hydrocarbons from Australian (Melolonthine) scarab beetles

Shigefumi Kuwahara	B-3	Synthesis of some insect pheromones
Patricia Hayes	B-4	Efficient synthesis of the plakortones bicyclic lactones from the sponge <i>Plakortis halichondrioides</i>
Nobuhiro Shimizu	B-5	New function and synthesis of $\beta$ -acaridian and identification of the new monoterpene from <i>Caloglyphus polyphyllae</i>
Mary T. Fletcher	B-6	Investigations into the biosynthesis of spiroacetals in <i>Bactrocera cacuminata</i> and <i>B. cucumis</i> : Mapping [ <sup>10</sup> O]-Oxygen incorporation from dioxygen

### POSTERS

Phan Phuoc Hien	B-7	Researches on the formation, translocation and accumulation of Rotenone from <i>Derris elliptica</i> Benth
M. Z. Mohd. Daud	B-8	Anthocyanin of <i>Carissa carandas</i>
Hisashi Omura	B-9	$\beta$ -Nitrostyrene: natural product as defense secretion of millipede, <i>Eucondylodemus elegans</i>

### SESSION C

#### < Biosensor and Chemoreception >

Jan Van der Pers	C1	Insects linked to electronics make powerful sensors
Thomas C. Baker	C-2	Odor source location using a discriminating insect antennal biosensor array coupled with a wind-direction indicator
Kyung Saeng Boo	C-3	Electroantennographic responses of the oriental tobacco budworm, <i>Helicoverpa assulta</i> , to plant volatile compounds
D.M. Suckling	C-4	Strengths and weaknesses of field electroantennograms as biosensors
Tae-Soo Chon	C-5	Automatic behavioral monitoring through training with artificial neural networks to detect characteristic patterns of response behavior of aquatic insects after sublethal treatments of insecticide in semi-natural conditions

### SESSION D

#### < Natural Products Chemistry >

Gerhard Gries	D-1	Basic research in insect communication ecology enhancing the potential for management of target insects
Hsiao-Yung Ho	D-2	Sex pheromones of the stink bugs <i>Chlorochroa ligata</i> , <i>C.</i>

		<i>sayi</i> and <i>C. uhleri</i> (Hemiptera: Pentatomidae)
Agus Dana Permana	D-3	Quantification and utilisation of insect growth parameter in bioassay of insect growth inhibitor
Philipp Kirsch	D-4	Superior insect controls with plant derived attractant baits
Sadahiro Tatsuki	D-5	Comparison of reproductive characteristics between rice-feeding and water-oats-feeding populations in <i>Chilo suppressalis</i> (Lepidoptera: Crambidae)
Soelaksono Sastrodihardjo	D-6	The progress on the development of neem products as insecticides
Max Suckling	D-7	Can pheromone traps help in biological control of insects and weeds?
Didier Rochat	D-8	Effect of red palm weevil mass trapping with synthetic pheromone in traditional Iranian date palm groves
Park Jong Ho	D-9	Field trapping of <i>Chrysopa cognata</i> (Neuroptera: Chrysopidae) with some synthetic chemicals in Korea
Phan Phouc Hien	D-10	Rotenone bio-activator controlling vegetable insect pests and predatory fish

#### POSTERS

Radzali Muse	D-11	The potential of <i>Clausena excavata</i> and <i>Citrus</i> Spp. Essential oils as an anti-T lymphoblastic leukaemia (CEM-SS cell line)
Mohd Aspollah Sukari	D-12	Chemical constituents and bioactivity of <i>Aegle marmelos</i> (Rutaceae)
Mei-Lan Tan	D-13	Cytotoxic effects of <i>Periskia corrugata</i> extracts against different classes of cell lines
Shaïda F. Sulaiman	D-14	New acylated flavonols from <i>Mimosa pigra</i>
Philipp Kirsch	D-15	Novel new attractant and formulations for control and monitoring of tephritid fruit flies
Bong Chui-Lien	D-16	Field evaluation of <i>Beauveria bassiana</i> for the control of cocoa pod borer in Sabah, Malaysia

#### SESSION E

##### < Kairomone and Allomone >

Eric Jang	E-1	<i>Anastrepha</i> spp. kairomones
Todd E. Shelly	E-2	Attractants and the mating success of male Mediterranean fruit flies

Philipp Kirsch	E-3	Kairomone-based insect control: effective, selective and environmentally secure
Shao Fang Wang	E-4	Chemical basis of host resistance in <i>Trifolium glanduliferum</i> to redlegged earth mite

### POSTERS

Shaida F. Sulaiman	E-5	Antimycotic activity of <i>Alpinia galanga</i> extracts against <i>Microsporium canis</i> : Ultrastructural changes by scanning electron microscope
Shaida F. Sulaiman	E-6	Antibacterial and phototoxic activity of the <i>Triphasia trifolia</i> extracts

### SESSION F

#### < Allelopathy in Agroecosystem >

Franck E. Dayan	F-1	Strategies for improvement of crop allelopathy with transgenes
James E. Pratley	F-2	Allelopathy in agriculture – the Australian experience
Chou Chang-Hung	F-3	Allelopathic potential of <i>Macaranga tanarius</i>
Maria Olofsson	F-4	Genetic control of allelopathy in rice

### SESSION G

#### < Plant and Animal Interaction >

Isao Kubo	G-1	Insect phenol oxidase inhibitors from plants
Keiichi Honda	G-2	Oviposition and host shift in Danaid butterflies mediated by plant chemicals
Keng-Hong Tan	G-3	Improved sex appeal, antipredation and pollination – compelling incentives in the <i>Bactrocera</i> fruit fly and <i>Bulbophyllum</i> orchid relationship
Florian Schiestl	G-4	Mechanisms of pollinator attraction in sexually deceptive orchids
Hiroshi Sasagawa	G-5	Co-evolution of plant and insect: the Oriental orchid ( <i>Cymbidium floribundum</i> Lindl) controls the social behavior of the Asian honey bees
R. Nishida	G-6	Multiple semiochemical roles of <i>neo</i> -Clerodane diterpenes in plant-insect interactions

M.J. Potter	G-7	Glucosinolates in Canola: A path to resistance as well as biofumigation
Fengming Yan	G-8	Behavioral responses of cotton bollworm and the Asian corn borer to volatiles of transgenic Bt cotton

### POSTERS

Hasnah Osman	G-9	Cytotoxicity evaluation of the herbal <i>Phyllanthus</i> extracts using brine shrimp lethality bioassay
Hajime Ono	G-10	Oviposition stimulants and deterrents controlling host preference in two Rutaceae-feeding swallowtail butterflies
Masami Takemura	G-11	Probing stimulants of <i>Megoura crassicauda</i> from the narrowleaf vetch ( <i>Vicia angustifolia</i> )
You-Ju Jin	G-12	Changes of volatiles from drought stressed ash-leaf maple ( <i>Acer negundo</i> Linn.) and their olfactory response to Asian longhorned beetle (ALB, <i>Anoplophora glabripennis</i> Motsch)
Li-Yan Ping	G-13	Phenolic changes in tree leaves exposed to natural and synthesized wound-induced volatiles

### SESSION H

#### < Chemical Ecology in Agroecosystem >

Stephen C. Welter	H-1	Alternative dispensing technologies for pheromone mating disruption of codling moth in pome fruit and nut crops
Philipp Kirsch	H-2	Attract and kill technology: A “knock out” in application of pheromones for pest management.
Toshimi Kobayashi	H-3	Practical use of pheromone mating disruption
Suk-Ling Wee	H-4	Intra- and inter-specific attraction, mating behaviour and hybridization between <i>Bactrocera carambolae</i> and <i>B. papayae</i>
Lai-Keng Chan	H-5	Detection of aromatic compounds from the cell suspension cultures and the <i>in vitro</i> plantlets of <i>Cymbopogon nardus</i>
Eric Jang	H-6	Improved semiochemicals for fruit fly detection and control
Chau Chin Hung	H-7	Assessment of using sex pheromone for control of the carambola fruit borer, <i>Eucosma notanthes</i> Meyrick, at carambola orchards
Syed Abd. Rahman	H-8	Trapping and mating disruption of the diamondback moth, <i>Plutella xylostella</i> (L.) using sex pheromones
Hideshi Naka	H-9	Sex pheromone components of the Japanese persimmon

		moth, <i>Stathmopoda masinissa</i> (Lepidoptera: Stathmopodidae)
Kyeung Sik Han	H-10	Sex pheromone composition of the summer fruit tortrix <i>Adoxophyes orana</i> in Korea

### POSTERS

Lai Keng Chan	H-11	<i>in vitro</i> propagation of <i>Spilanthes acmella</i> , a bio-insecticide herb
Chee Weng Chan	H-12	Detection of juvenile hormone III (JH 3) from <i>in vitro</i> <i>Cyperus aromaticus</i>
Eric Jang	H-13	Improved new male attractant for melon fly
John E. Dunley	H-14	Use of pheromone mating disruption as a tactic for insecticide resistance management in pear and apple
Philipp Kirsch	H-15	Controlling tree fruit pests with last call: innovative attract and kill technology
Philipp Kirsch	H-16	Last call attract and kill technology shows promise in vegetable pest management

### SESSION I

#### < Chemical Ecology in Aquatic Ecosystems >

Bruce Bowden	I-1	The effect of bleaching on metabolite chemistry in soft corals
Tatsuo Higa	I-2	Unstable and masked metabolites of marine organisms: their ecological implication
Pei-Yuan Qian	I-3	Larval settlement and metamorphosis of <i>Hydroides elegans</i> in response to chemical cues: a model system for studying chemical ecology of marine invertebrate larvae
Georg Pohnert	I-4	Wound-activated chemical defense in phytoplankton
Li-Ling Koh	I-5	Antifungal properties of Singapore gorgonians: a preliminary study
Nicole de Voogd	I-6	The abundance of bioactive sponges in the Spermonde Archipelago
Siti Akmar Khadijah A. Rahim	I-7	Induction of larval metamorphosis of the red sea urchin, <i>Pseudocentrotus depressus</i> by the microbial films
Tilman Harder	I-8	Induction of larval attachment and metamorphosis in <i>Hydroides elegans</i> by marine bacterial metabolites

## POSTERS

Georg Pohnert	I-9	Rapid wound-activated transformation of the green algal defensive metabolite caulerpenyne
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### SESSION J < Chemical Ecology in Forest Ecosystem >

Zhang Zhen	J-1	Sex pheromone for monitoring the pine sawfly, <i>Diprion jingyuanensis</i> Xiao et Chang (Hymenoptera: Diprionidae)
Kiyoshi Nakamuta	J-2	Floral attractant and sex pheromone for a cryptomeria borer, <i>Anaglyptus subfasciatus</i> (Coleoptera: Cerambycidae)
Yong-Ping Huang	J-3	The sex pheromone of <i>Celypha pseudolaxicola</i> Liu (Tortricidae: Lepidoptera) and its field application
Xian-Zuo Meng	J-4	Sex pheromone of the carpenterworm, <i>Holcocerus insularis</i> (Lepidoptera: Cossidae): Identification and field trials
Fauziah Abdullah	J-5	Analysis of volatiles from <i>Monochamus alternatus</i> Hope (Coleoptera: Cerambycidae)
A. Sivapragasam	J-6	Biopesticidal effects of sentang, <i>Azadirachta excelsa</i> (Jack), extracts on some major insect pests of vegetables in Malaysia

## POSTERS

Philipp Kirsch	J-7	Attractants for the larger pine shoot beetle, <i>Tomicus piniperda</i>
Philipp Kirsch	J-8	Intercept panel trap a novel trap for monitoring forest coleoptera
Philipp Kirsch	J-9	Attract and kill technology for management of European pine shoot moth, <i>Rhyacionia buoliana</i> , and Western pine shoot borer, <i>Eucosma sonomana</i>

### SESSION K < Chemical Ecology in Urban Entomology >

Nan-Yao Su	K-1	Use of insect growth regulators for population control of subterranean termites
Jeffrey Aldrich	K-2	Chemical attraction of nuisance species of yellowjacket wasps (Hymenoptera: Vespidae)



Masayuki Sakuma	K-3	Homing the German cockroaches to a virtual pheromone source
Junwei Zhu	K-4	Development of attractants from natural products for controlling fruit flies

**POSTER**

Philipp Kirsch	K-5	Attract and kill technology to control ticks
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**SESSION L**  
**< Molecular Ecology >**

David Haymer	L-1	Molecular ecology of ribosomal ITS1 polymorphisms in <i>Helicoverpa armigera</i> populations in Thailand
Sujinda Thanaphum	L-2	Molecular ecology of the <i>Bactrocera tau</i> species complex
Jean-Marc Jallon	L-3	Polymorphism of <i>Drosophilla</i> pheromone: Molecular studies

**POSTER**

Shaida F. Sulaiman	L-4	Phytochemical versus molecular evaluation of <i>Phyllanthus niruri</i> L.
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# PLENARY LECTURES

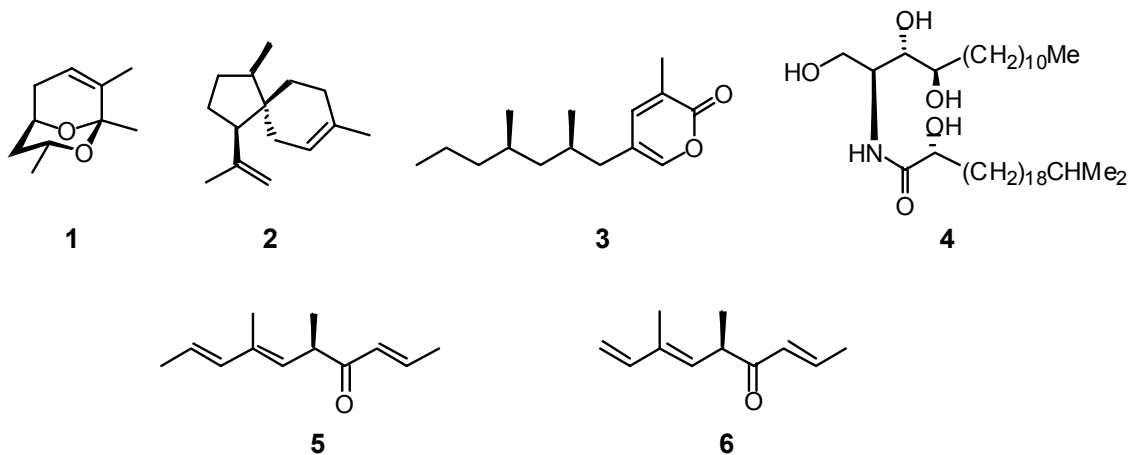
## ORGANIC SYNTHESIS AS A TOOL IN LIFE SCIENCES

Kenji Mori

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Organic synthesis provides the final proof or disproof of the structures proposed for semiochemicals and bioregulators. Through organic synthesis we can provide pure semiochemicals and bioregulators in amounts sufficient for biological studies. The following examples will be discussed.

- (1) Male secretion (**1**) of the swift moth *Endoclita excrescens*.
- (2) Aggregation pheromone (**2**, proposed structure) of the broad-horned flour beetle *Gnatorcerus cornutus*.
- (3) Female sex pheromone (supellapyrone, **3**) of the brownbanded cockroach *Supella longipalpa*.
- (4) Female sex pheromone (**4**) of the hair crab *Erimacrus isenbeckii*.
- (5) Female sex pheromone (**5**) of the Israeli pine bast scale *Matsucoccus josephi* and its mimics (**6**).



## **PYRROLIZIDINE ALKALOIDS IN PLANTS AND ADAPTED INSECTS: FUNCTIONAL AND EVOLUTIONARY ASPECTS**

Thomas Hartmann

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Pyrrolizidine alkaloids (PAs) are typical plant secondary constituents with scattered occurrence in the plant kingdom. They are part of the plant's defense against insect herbivores. During the course of evolution insects from various unrelated taxa acquired the ability to cope with this chemical defence barrier and frequently even learned to sequester PAs from their host plants and use them for their own benefit. The biochemistry of PA sequestration in three different insects is compared, i.e., arctiid moths (Lepidoptera) and two leaf beetles (Coleoptera), the palaeartic genus *Oreina* and the neotropical genus *Platyphora*. The three insects developed completely different strategies to store and maintain plant derived PAs for their own defense. However, in any case they prevent accumulation of the pro-toxic tertiary alkaloid. To understand the evolutionary origin of the PA pathway in plants and the evolution of insect adaptations to plant PAs a molecular approach on the gene level is necessary. First results are discussed: (1) the first pathway-specific enzyme of PA biosynthesis was shown to be derived by gene duplication from a ubiquitous enzyme (gene) of completely different function and to be integrated into PA biosynthesis; (2) the enzyme which in larvae of PA-adapted lepidopterans specifically transforms the ingested toxic tertiary PAs into the non-toxic *N*-oxides was shown to be structurally related to genomic sequences of still unknown functions from *Drosophila*. The molecular data are discussed in the context of recruitment of ubiquitous primary genes for functions in secondary metabolism and insect adaptations to plant acquired defensive compounds.

**CHEMICALS AFFECTING THE BEHAVIOR OF THE ASIAN LONGHORNED BEETLE, *ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE)**

Aijun Zhang<sup>\*\*</sup>, James E. Oliver, and Jeffery R. Aldrich

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The Asian Longhorned Beetle (ALB) is an invasive species that attacks, damages, and eventually kills a wide variety of healthy hardwood trees. It was first discovered in New York City in 1996 and then in Chicago in 1998. This insect threatens to cause billions of dollars in losses to forestry and the nursery industry. To date, detection of ALB infestation relies on visual inspection which is believed to detect at best only ~30% of the infested trees. An attractant to lure ALB's into traps is needed to detect and monitor this insect more efficiently. In a collaborative effort with APHIS scientists, we discovered that two male-specific beetle volatiles elicited strong gas chromatographic-electroantennographic detection (GC-EAD) responses from both sexes of adult ALB. The secretion consisted of a ~1:1 blend of functionalized dialkyl ethers, 4-(*n*-heptyloxy)butanal **1** and 4-(*n*-heptyloxy)butan-1-ol **2**. In preliminary behavioral tests this blend appeared to stimulate flight and walking in both sexes, and beetles were attracted to the synthetic beetle volatiles in an olfactometer. In contrast, in field tests conducted in China in July 1999 and July-August 2000, traps designed for flying insects and baited with **1** and/or **2** failed to catch ALB of either sex.

**CHEMICAL COMMUNICATION IN *ENDOCLITA EXCRESCENS* (LEPIDOPTERA: HEPIALIDAE)**

Tadakazu Nakashima

Forestry and Forest Products Research Institute, Japan

A hepialid moths in Japan, *Endoclista excrescens*, bores into stems and/or trunks of hosts, more than 110 species of herbal and woody plants. Male moths fly at dusk, less than 20 lux, and form leks from mid summer to early autumn. Females fly into a lek, even if they fly from upwind. This means that they are visual (or audio) receptors. If a female finds an appropriate flying male, she hovers beside it, then the pair flies away from the lek and mate.

It is said that the male sex pheromones play an important role in mating behavior of some hepialid moths, *Hepialus hecta*, in Europe, and *H. californicus*, in North America.

Hexane extract of brush organ on male hind legs induced an electrographic detection response only in female antenna. The chemical structure of the active principle was identified as 1,3,8-trimethyl-2,9-dioxabicyclo[3.3.1]non-7-ene from the MS and NMR data.

The results of structure analyses and mating behavior analysis will be discussed.

## DELAYED MATING AND ITS SIGNIFICANCE IN MATING DISRUPTION TREATMENTS

R.A. Vickers

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Numerous laboratory-based studies have demonstrated the deleterious effects that delayed mating can have on the fecundity and the fertility of female moths. Whilst the potential of these effects to contribute to the overall impact of mating disruption treatments has been recognized in theoretical studies, for the most part they have been ignored as a factor when evaluating in the field a technique whose primary goal is to prevent mating.

One explanation for this may be that gathering evidence of delayed mating and measuring its impact is not as easily achieved in the field as it is in the laboratory. A means of sampling females without bias towards mating status and age is required, as is a means of determining female age. Laboratory studies must also be conducted to determine the potential impact of delayed mating on fertility and fecundity to enable interpretation of the field data.

This paper explores the potential contribution of delayed mating to population suppression and explores ways in which its impact might be measured where mating disruption is being used as a control tool.

## TANNIN TOXICITY TO MINUTE ANIMALS

Toru Yamasaki\*, Masashi Sato, Tomoaki Mori, Ahmed S.A. Mohamed,  
Kyoko Fujii and Junko Tsukioka

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Faculty of Agriculture, Kagawa University

Tannin impacts on phytophagous insects and their physiological adaptations have been investigated during the past three decades on the supposition that tannin may have been primarily noxious to the insects. Tests on rats or mice have proven subcutaneously injected tannic acid and orally given punicalagin are hepatotoxic. It has been also reported that an orally applied tannin material showed no evidence of toxicity. The results, however, have been based on administration of tannic materials or only a few compounds. We report here on toxicity of ellagi-, gallo- and condensed tannins (isolates and fractions) to minute animals – the free-living hermaphroditic nematode *Caenorhabditis elegans* and the brine shrimp *Artemia salina*.

Ellagi-, gallo- and condensed tannins besides related compounds were isolated by open column chromatography over Sephadex LH-20, Diaion HP20, MCI gel CHP 20P and/or Toyopearl HW-40F and spectroscopically identified. Some other gallotannins were enzymatically prepared.

Peracetates of fractions of ellagi- and polymeric condensed tannins were subjected to gel permeation chromatography. The L1-, the young adult- and the egg-bearing adult stages of the wild-type strain of *C. elegans* var. Bristol (N2) were synchronously induced from a monoxenic culture and submitted to toxicity testing. Individual experimental worms were examined for their survival, motility, development and/or reproduction. Bright-field photomicrographs (400x) were taken. Lethality testing on newly hatched nauplii of *A. salina* was also performed. The nematocidal activities of the gallo- and the condensed tannins correlated with degrees of galloylation and polymerization, respectively. The test of the ellagitannins produced the results suggestive of an activity-structure relationship. The three tannin classes were also active in the brine shrimp test. In terms of defensive properties of plants against herbivores, the results may indicate a prototypical case in ecological time, because both animal species do not encounter tannin in their normal milieus and cannot adapt to tannin.

A5

**MATING BEHAVIOR IN A LOCAL BLISTER BEETLE, *EPICAUTA HIRTICORNIS*  
(COLEOPTERA: MELOIDAE)**

Yien-Shing Chow

National Museum of Natural Science  
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The blister beetle, *Epicauta hirticornis* Haag-Rutenberg, shows unique pre-copulating behavior. When males dorsally mount females, their antennae continuously wave to female antennae; often the male's right or left antenna grasps on the corresponding female antennae, thereby forming a double helix structure. The frequencies of establishing right- or left-handed antenna spiral complexes are equal. If the female's left antenna cannot be grasped, the male will form a spiral with female's right antenna, and vice versa. When both male and female antennae are entwined, they vibrate at a frequency of 7-12 c/s. This vibration is similar to the precopulatory sexual display of wing fluttering by male moths. The blister beetle's connection with cantharidin is also discussed.

**THE DIVERSITY OF CHEMICAL COMMUNICATION SYSTEMS BETWEEN THE  
RESISTANT AND SUSCEPTIBLE STRAINS OF COTTON BOLLWORM,  
*HELI COVERPA ARMIGERA*.**

Jia-Wei Du\* and Jian Wu

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Communication system of insects can be influenced by many factors, including chemical insecticides. Up to now, no comparative studies on the pheromone communication systems of the resistant and susceptible insect strains had been done, though some studies showed temporary behavior changes with sub-lethal dose treatments of chemical insecticides. Recently we have systematically studied the pheromone communication systems of resistant and susceptible strains of cotton bollworm *H.armigera* through GLC analysis of pheromone titer and ratio as well as male orientation behavior in the wind tunnel.

The results showed obvious differences in pheromone communication systems between resistant and susceptible strains of *H.armigera*. The sex pheromone titer of the resistant female was much higher than that of the susceptible female. It seems the resistant female released a large amount of sex pheromone and more chance to attract males than the susceptible females. Furthermore, the resistant male of *H.armigera* preferred resistant female to susceptible female in a wind tunnel. But, resistant moths could still communicate with susceptible ones and mate with each other since there were no significant differences in female sex pheromone components and their ratios, releasing rhythm of female sex pheromone, calling rhythm, and calling rate of females.

From our preliminary research, it seems the resistant strain of *H.armigera* has stronger competition ability than the susceptible strain in the chemical communication of mate findings. This is the most difficult problem in “insect resistance management.”

**ARE THE PROBING STIMULANTS A HOST RECOGNITION SUBSTANCE FOR  
RICE LEAFHOPPERS AND PLANTHOPPERS? –ISOLATION AND  
IDENTIFICATION OF THE PROBING STIMULANT FROM THE RICE PLANT FOR  
*NEPHOTETTIX CINCTICEPS* UHLER**

Chul-Sa Kim\*, Masami Takemura, Francis Adjei-Afriyie,  
Shin-ichi Tebayashi and Michio Horiike

Faculty of Agriculture, Kochi University  
Nankoku, 783-8502 Japan

The green rice leafhopper (*Nephotettix cincticeps* Uhler) as well as the three planthoppers, *Nilaparvata lugens*, *Sogatella furcifera* and *Laodelphax striatellus*, are serious rice pests in Asia. These sucking insects show characteristic probing behavior. Prior to sucking the sap, they insert their stylets into the parenchymal tissues of the rice plant to reach the sucking site. Probing behavior is well known to be controlled by plant chemicals. The structures of these plant chemicals have partially been elucidated for the three planthoppers. Since the probing stimulant for *N. cincticeps* is yet to be isolated and identified, this study was thus undertaken.

*N. cincticeps* frequently showed probing behavior when feeding on a 5% crude rice plant extract plus a 2% sucrose solution. Many branched stylet sheaths were observed on a parafilm membrane as a result of the response shown by the insects for 24hr. On the other hand, when given only a 2% sucrose solution as a control, they did not leave any probing sheaths on the membrane but only probing marks. These results clearly revealed the presence of the probing stimulant in the rice plant.

According to the bioassay guidance, only one active compound was isolated as a probing stimulant for *N. cincticeps* from ODS 40% methanol/H<sub>2</sub>O eluate by using a reverse-phase HPLC and identified as tricetin 5-O-glucoside through spectroscopic analyses. This flavonoid is known to be widely distributed in the gramineae plant family.

Monophagous *N. lugens* requires eight special flavonoid C-glycosides as its probing stimulant, and the oligophagous *S. furcifera* or *L. striatellus* require plural flavonoid glucosides including the above-mentioned common flavonoid O-glucoside. While polyphagous *N. cincticeps* requires only one common flavonoid O-glucoside in the gramineae plants.

These results and reports may show that their host range bear some relationship with the quality and quantity of the probing stimulant. It seems that a probing stimulant is a host recognition substance in a wider sense for the rice leafhoppers and planthoppers.



## NUPTIAL SECRETION OF THE MALE GERMAN COCKROACH: PHEROMONAL ROLE OF LIPID COMPONENTS IN COURTSHIP

Soichi Kugimiya<sup>1\*</sup>, Ritsuo Nishida<sup>1</sup>, Yasumasa Kuwahara<sup>1</sup> and Masayuki Sakuma<sup>2</sup>

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In sequential courtship behavior of the German cockroach, *Blattella germanica*, a male secretes a pheromonal substance from the abdominal tergal glands. The glandular secretion functions as a “nuptial gift,” arresting a female into an appropriate position for the male to make a genital connection while she is feeding on the fluid. Thus, the male courtship pheromone in this species also acts as a feeding-stimulant for the female.

Active components in the nuptial secretion were examined by a feeding bioassay using polyethylene glycol (PEG) as a medium, in which the test samples were mixed. Methanolic extracts of the male 8th tergal glands (TG-8) strongly elicited the feeding response in the virgin females. The feeding stimulant activity was found in two fractions, consisting mainly of lipids and oligosaccharides, respectively. In the oligosaccharide fraction, active components have been identified previously as maltooligosaccharides and oligoglucosyl trehaloses. The activity of this fraction was enhanced by adding the lipid fraction, indicating a significant contribution of polar lipid fraction to the activity. We report here a series of lipid components synergistically acting with the oligosaccharides components in the TG-8.

The lipid fraction was composed mainly of phosphatidylcholine and phosphatidylethanolamine, with fatty acid composition similar to that of the male whole body (saturated and unsaturated fatty acids with 16 and 18 carbon atoms). On the other hand, another active component in combination with oligosaccharides was found in a less polar fraction and identified as cholesterol.

A median effective concentration [EC<sub>50</sub>: male equivalent (m.e.)/40μg PEG spot] of the TG-8 methanolic crude extract was 0.0044 m.e. An authentic oligosaccharide blend (maltose + maltotriose) showed a relatively weak activity [EC<sub>50</sub>: 0.83 m.e.]. Although authentic cholesterol and an authentic phospholipid blend (1,2-dioleoylphosphatidylcholine + 1,2-dioleoylphosphatidylethanolamine) showed little activity [EC<sub>50</sub>: >1.0 m.e.], the activity was strongly enhanced to the level of 0.045 m.e. and 0.0070 m.e., respectively, when mixed with the oligosaccharide blend. These results indicate that the nuptial feeding behavior of the female cockroach is elicited by a synergistic action among phospholipids, cholesterol and oligosaccharides in the male tergal secretion.

**COMPARATIVE ATTRACTION OF THREE  
*BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE) COMPLEX  
SIBLING SPECIES TO METHYL EUGENOL**

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Universiti Sains Malaysia  
11800 Minden  
Penang, Malaysia

The use of methyl eugenol (3,4-dimethoxyallyl benzene) has been attributed to the success of the implementation of male annihilation and sterile male techniques in the area-wide control programmes of the Oriental fruit fly, *Bactrocera dorsalis*.

However, despite some successes achieved in the eradication of these pests, the control for some of the *B. dorsalis* sibling species have proved to be difficult. This is evident in the control of *B. papayae* in Queensland, Australia and that of *B. carambolae* in the upper South American countries. We believe that the success of the area-wide control programmes lies not in the use of methyl eugenol alone but the amount applied.

Investigation into the attraction of these three sibling species (*B. dorsalis*, *B. papayae* and *B. carambolae*) revealed that there is significant differences in the sensitivity of male attraction to methyl eugenol.

The comparative attraction of the three sibling species to methyl eugenol and their implication in area-wide control programme are further discussed.

## SYNTHESIS OF JH-BIOSYNTHESIS INHIBITORS AND PHYTOSIDEROPHORES

Takeshi Kitahara

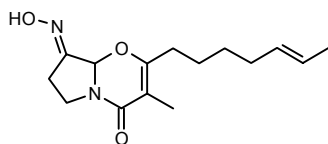
Department of Applied Biological Chemistry  
The University of Tokyo, Japan

Expeditious Synthesis of several natural products and their analogs with remarkable bioactivities related to plant chemical ecology will be presented.

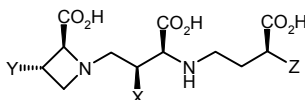
Brevioxime (1) is a juvenile hormone (JH) biosynthesis inhibitor isolated from *Penicillium brevicompactum*. Concise synthesis of both enantiomers of 1 and related analogs to determine the absolute configuration of natural product and to investigate SAR will be discussed.

Deoxymugineic acid (DMA, 2) is a phytosiderophore excreted from roots of graminaceous plants, such as barley, wheat, rice etc. under iron-deficient condition, for example, when they are exposed to alkaline soil. Expeditious Synthesis of 2 and its more potent analogs will be discussed.

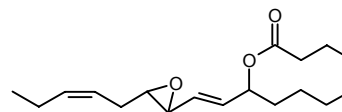
Mueggelone (3) is a toxin isolated from pure-water plant, most probably playing an important role in inhibiting the development of herbivorous fishes. The first and efficient synthesis of all the possible four stereoisomers of 3 and the dertermination of the absolute configuration will also be discussed, if the schedule permits.



Brevioxime (1)



2'-Deoxymugineic acid (2)



Mueggelone (3)

**ALLENIC HYDROCARBONS FROM AUSTRALIAN (MELOLONTHINE)  
SCARAB BEETLES**

Mary T. Fletcher<sup>1</sup>, Matthew J. McGrath<sup>1</sup>, Wilfried A. König<sup>2</sup>, Christopher J. Moore<sup>3</sup>, Bronwen W. Cribb<sup>4</sup>, Peter G. Allsopp<sup>5</sup> and William Kitching<sup>1\*</sup>

<sup>1</sup> Department of Chemistry, University of Queensland, Brisbane, Australia

<sup>2</sup> Institut für Organische Chemie, Universität Hamburg, Germany

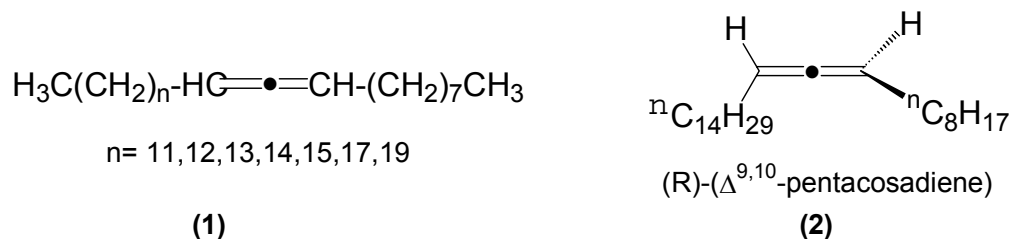
<sup>3</sup> Department of Primary Industries, Yerongpilly, Brisbane, Australia

<sup>4</sup> Department of Zoology and Entomology, University of Queensland, Brisbane, Australia

<sup>5</sup> Bureau of Sugar Experiment Stations, Bundaberg, Australia

Melolonthine scarabs are pests of fields-crops and pastures and now described are studies of the chemistry of certain sugarcane scarabs from north-eastern Australia.

The most interesting finding is the high level of **allenic hydrocarbons** in the cuticular hydrocarbons of a number of species. These allenes, previously unknown as a molecular class from insects, are represented by **(1)** below and include both odd-and even carbon numbered systems.



The structures of these allenes have been confirmed by synthesis, chromatographic and spectral comparisons and co-elution studies. For the cases of n = 11 ( $\Delta^{9,10}$ -tricosadiene) and n = 13 ( $\Delta^{9,10}$ -pentacosadiene) **(2)**, a predominating (R)-chirality (86% and 89%, respectively) has been demonstrated by enantioselective synthesis and gas chromatographic comparisons (cyclodextrin phase).

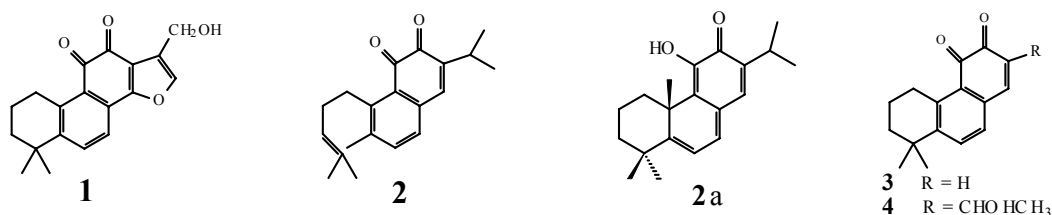
The biological role of these compounds is being investigated.

## SYNTHESIS OF SOME INSECT PHEROMONES

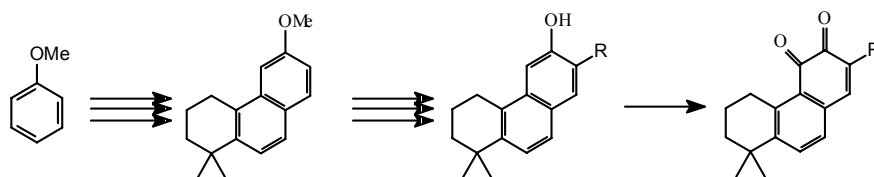
Shigefumi Kuwahara\*, Izuru Nagashima and Jiro Ishikawa

Laboratory of Applied Bioorganic Chemistry  
 Graduate School of Agricultural Science  
 Tohoku University

Our recent studies on the synthesis of some insect pheromones will be presented. Especially, we would like to focus on the synthesis of pheromones with sesquiterpene frameworks, including the sex pheromones isolated from a stink bug (*Tynacantha marginata* Dallas), the southern green stink bug (*Nezara viridula* L.), and the smoky brown cockroach (*Periplaneta fuliginosa* Serville). In the first example, we achieved the synthesis of both enantiomers of the pheromone possessing a new type of tricyclic sesquiterpene structure, which enabled us to determine the absolute configuration of the pheromone. In the second example, we accomplished a short-step synthesis of the pheromone with a bisabolane-type structure. In the final example, improvement of the synthesis of periplanone-D and revision of its physical properties were made.



Scheme 1



Scheme 2

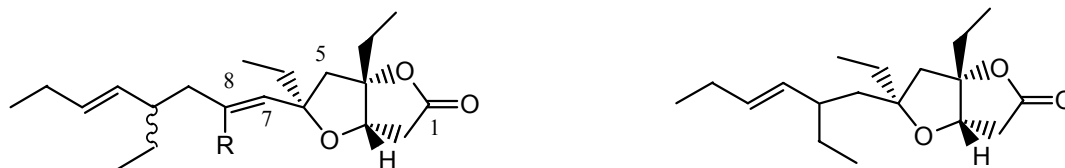
In conclusion, using anisole as the starting material, we synthesized two analogues of tanshinone such as 8,8-dimethyl - 5, 6, 7, 8-tetrahydrophenanthrene -3,4-dione **3** and 8,8-dimethyl-2-(1-hydroxy ethyl)-5, 6, 7, 8-tetrahydrophenanthrene-3,4-dione **4**. Compounds **3** and **4** exhibited high activity against leukemia P-388 cell lines. Chemical syntheses and biological investigations of a series of structural analogues of the title compounds are in progress.

**EFFICIENT SYNTHESIS OF THE PLAKORTONES,  
BICYCLIC LACTONES FROM THE SPONGE *PLAKORTIS HALICHONDRIOIDES***

Patricia Hayes\*, Gregory C. Paddon-Jones, Natasha L. Hungerford and William Kitching

Department of Chemistry, University of Queensland  
Brisbane, 4072 AUSTRALIA

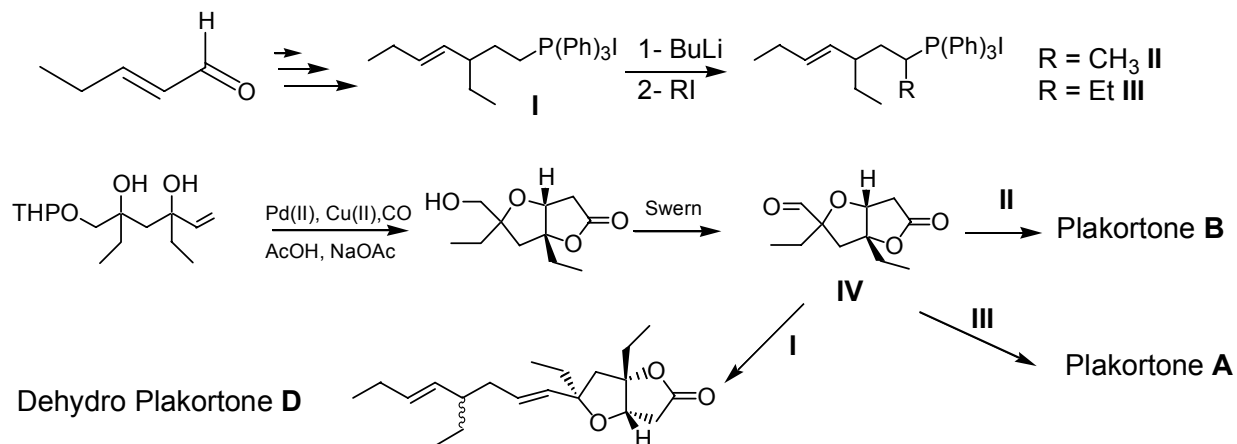
The plakortones (**A-D**) were isolated in 1996 from a sponge collected in Jamaica, *Plakortis halichondrioides*, and more recently from a specimen of *Plakortis simplex* (*Homosclerophorida plakinidae*) (Caribbean sea, 1999) for the plakortones (**E-F**). Their activity as micromolar activators of  $\text{Ca}^{2+}$  pumping in cardiac muscle *sarcoplasmic reticulum* (SR) is relevant to correction of relaxation abnormalities and so these plakortone metabolites are of pharmacological interest.



**A** (R = CH<sub>3</sub>), **B** (R = Et), **C** (7,8 dihydro, R = CH<sub>3</sub>),  
**D** (7,8 dihydro, R = H), **E** (7,8 dihydro, R = Et)

**F**

Our approach to the plakortone cores was based on a palladium (II)-catalysed hydroxy-cyclization-carbonylation-lactonization sequence in a “one pot” conversion of appropriate enediols (yields > 80%). This and elongation of the side chain by reaction of the aldehyde **IV** with suitable Wittig salts **I**, **II**, or **III** will be discussed. These procedures provide efficient access to the plakortones **A**, **B**, and dehydro plakortone **D**.



**NEW FUNCTION AND SYNTHESIS OF  $\beta$ -ACARIDIAL AND IDENTIFICATION OF THE NEW MONOTERPENE FROM *CALOGLYPHUS POLYPHYLLAE***

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Kyoto University

From a pair of opisthonotal glands, *Caloglyphus* (= *Sancasania*) *polyphyllae* Zakhvatkin discharges a characteristic set of volatiles, i.e. four monoterpenes and seven hydrocarbons. Among them,  $\beta$ -acaridial [2(*E*)-(4-methyl-3-pentenylidene)-butanedial] which has been known to act as the female sex pheromone with antifungal activity was recently demonstrated to act as the aggregation pheromone for nonfeeding and nonmating mites. Feeding mites, however, exhibit sexually aroused behavior instead of a tendency to cluster, when exposed to  $\beta$ -acaridial. This is the first example of the compound demonstrating two pheromonal functions depending upon circumstances faced by the mites.

Incidentally  $\beta$ -acaridial is distributed widely in astigmatid mites and has also been identified as the alarm pheromone of *Tyrophagus longior*. The biological activities of  $\beta$ -acaridial are still speculative in many mite species. The effective method of  $\beta$ -acaridial synthesis is not available and the conventional method must rely upon environmentally hazardous reagents and the yield is low. Therefore, we tried to establish a simple and novel synthetic route with high yield.  $\beta$ -Acaridial was synthesized by a Wittig reaction of 4-methyl-3-pentenyltriphenyl phosphonium bromide with 1,4-di(*tert*-butyldimethylsilyl)oxy-2-oxobutane (derived from the protection of two primary hydroxy groups of 1,2,4-butanetriol with *tert*-butyldimethylchlorosilane, and subsequent oxidation of secondary hydroxy group with PDC), and by deprotection of the hydroxy groups, and subsequently the Swern DMSO oxidation. Instead of Swern oxidation, the corresponding  $\beta$ -(*E*)-acaridiol was oxidized with PDC to give a new monoterpene  $\beta$ -acariolal [2(*E*)-(4-methyl-3-pentenylidene)-4-hydroxybutanal]. This new monoterpene was detected and identified as a secretory component of *Caloglyphus polyphyllae* from GC/MS spectral data of natural and synthetic compounds. From literature search, no identification of  $\beta$ -acariolal has been reported from synthetic and natural sources.  $\beta$ -acariolal is the third novel product identified from excretion of the *C. polyphyllae* opisthonotal gland. Although the biological function of the monoterpene is currently obscure, the species is significant from both the natural products chemistry and chemical ecology viewpoints.

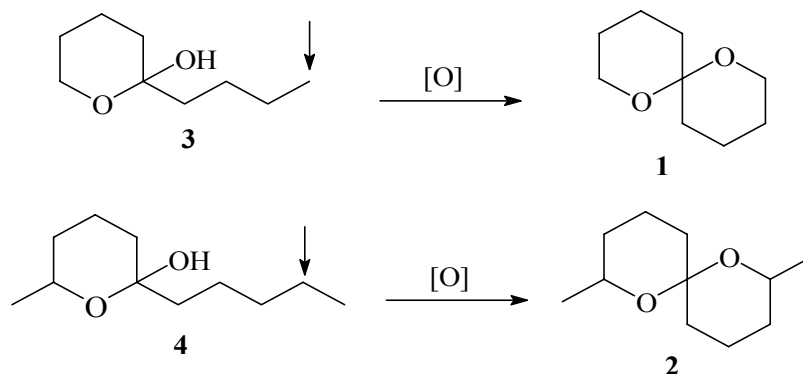
**INVESTIGATIONS INTO THE BIOSYNTHESIS OF SPIROACETALS  
IN *BACTROCERA CACUMINATA* AND *B. CUCUMIS*:  
MAPPING [<sup>18</sup>O]-OXYGEN INCORPORATION FROM DIOXYGEN**

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and William Kitching

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Brisbane, Australia 4072

*Bactrocera cacuminata* and *B. cucumis* are two species of fruit fly found along the coastal regions of eastern Australia. Glandular secretions from these species are rich in spiroacetals. The predominant volatile component produced by *B. cacuminata* is 1,7-dioxaspiro[5.5]undecane (**1**) and in *B. cucumis* the related spiroacetal, 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**2**) is the major component.

These spiroacetals have been postulated to arise from oxidation of the corresponding tetrahydropyransols **3** and **4** (Scheme 1), and previous feeding experiments utilising deuterated precursors has supported these proposals.



Scheme 1

The present study investigates the origin of the ring oxygens in spiroacetals **1** and **2** using fruit flies exposed to an atmosphere enriched in [<sup>18</sup>O]-oxygen. The results from *B. cucumis* support the mechanism shown in Scheme 1, in that one oxygen of **2** incorporated [<sup>18</sup>O]-oxygen and hence arises from molecular oxygen, whilst the other, presumably that of the tetrahydropyransol **4**, arises from another source. Interestingly in *B. cacuminata*, both oxygens in **1** incorporated [<sup>18</sup>O]-oxygen and thus must both arise from oxidations involving molecular oxygen.



## POSTERS

B7

### RESEARCHES ON THE FORMATION, TRANSLOCATION, AND ACCUMULATION OF ROTENONE FROM *DERRIS ELLIPTICA* BENTH

Phan Phuoc Hien<sup>1\*</sup>, Nguyen Cong Hao<sup>1</sup>, Mai Dinh Tri<sup>1</sup>,  
Nguyen Cuu Thi Huong Giang<sup>1</sup>.

<sup>1</sup>Institute of Tropical Biology (ITB)  
Vietnam National Center for Natural Science and Technology

The initial researches on the formation, translocation, and accumulation of rotenone in *Derris elliptica* Benth plant by the RP-HPLC analysis show that: Rotenone content has the co-variation with their precursors-phenylalanine and methionine from the young leaf to the old one. In this stage, the biosynthesis of phenylalanine, methionine and rotenone has the co-variation with the photosynthetic rate. Whereas, from the old leaf to the stem and the root are reverse. Actually, phenylalanine and methionine decrease by about 50% from the old leaf to the stem while rotenone content increases many times and reaches a very high level in the root. Experiment by cutting out the bark in order to block off the transportation of solutes in the phloem, demonstrates that: After biosynthesizing at the leaf, rotenone moves downward to the under organs by the phloem and eventually accumulates in the root. Micrurgy to observe structure of the phloem and xylem and explain the mechanism of "load" at the phloem cell source and to "unload" at the xylem cell sink in the Derris root was also presented in this paper. These results may be applied to increase Derris root yield and rotenone content for farmers in the field.

## ANTHOCYANINS OF *CARISSA CARANDAS*

Syamsinar Osman, Shaída Fariza Sulaiman<sup>1</sup> and  
Mohamed Zulkali Mohamed Daud<sup>2\*</sup>

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<sup>2</sup>School of Chemical Engineering, Transkrian Campus  
Universiti Sains Malaysia, Malaysia

This work was an attempt to extract anthocyanins from ripe berries of *Carissa carandas*. The maroon coloured berries were depitted and the mesocarps were treated with acidic ethanol solution during the extraction procedure. The extraction method employed was similar to those used by Fuleki and Francis (1968). As a comparison, anthocyanins of known sources, fresh black grapes and morning glory flowers, were extracted and used as reference pigments.

Qualitative studies of the crude anthocyanin extracts obtained, were carried out. These encompassed spectral and chromatographic studies.

The berries were found to contain anthocyanins, and the results of the chromatographic studies suggested that they were delphinidin and cyanidin. However, petunidin may also be present. This work has demonstrated the potential of *Carissa carandas* as an exploitable source of anthocyanins. Further studies are required to put this plant in a wider and more significant perspective with respect to national and global interest in agro-based industries.

## $\beta$ -NITROSTYRENE: NATURAL PRODUCT AS DEFENSE SECRETION OF MILLIPEDE, *EUCONDYLODESMUS ELEGANS*

Hisashi Omura<sup>1\*</sup>, Yasumasa Kuwahara<sup>1</sup> and Tsutomu Tanabe<sup>2</sup>

<sup>1</sup>Graduated School of Agriculture, Kyoto University  
<sup>2</sup>Tokushima Prefectural Museum

*Eucondylodesmus elegans* Miyosi (Polydesmida: Doratodesmidae) is a small millipede found among litters of deciduous broad-leaved trees, and has so far been described as endemic species in eastern region of Japan. However, we recently collected the species in Kyoto about 500 km west from the known habitat, suggesting its distribution is much wider. The millipede possesses a pair of gland orifice where odoriferous droplets are discharged in response to disturbance. We thus attempted to reveal the chemical composition and biological activity of the secretion.

The secretion was extracted by soaking the species in benzene (10 $\mu$ l) for 3 minutes. A part of the extract (3  $\mu$ l) was subjected to GC-MS analysis, which gave two peaks with the M<sup>+</sup> ion at  $m/z$  149 and the base ion at  $m/z$  77. The major component was elucidated to be E- $\beta$ -nitrostyrene

(1-nitro-2-phenylethylene), and the minor component to be Z- $\beta$ -nitrostyrene by GC-MS and NMR. In addition, the synthetic standard (E/Z ratio: 56/1) prepared from benzaldehyde and nitromethane in methanol-aqueous sodium hydroxide, gave identical GC-MS and NMR results as those from the millipede components. Average contents of  $\beta$ -nitrostyrene per millipede were  $1.86 \pm 1.42 \mu\text{g}$  (E/Z ratio: 157/1) for males and  $2.94 \pm 1.66 \mu\text{g}$  (E/Z ratio: 154/1) for females.

Repellent activities as the defense substance was demonstrated against foraging ants *Pristomyrmex pungens* Mayr (Formicidae) in the field by using granular sugar (3.2 x 2.7 x 1.3 mm) impregnated with the synthetic  $\beta$ -nitrostyrene at dose of more than 0.71  $\mu\text{g}$ . In addition, noticeable anti-microbial effects have been reported on this compound. Therefore, this nitroalkene of *E. elegans* was concluded as a defense substance against ants and noxious soil microbes.

Incorporation of seven deuterium atoms into  $\beta$ -nitrostyrene ( $M^+$  ion at  $m/z$  156,  $m/z$  7 increase from the original) was demonstrated by GC-MS analysis of the defense secretion, after feeding millipedes with litters treated with  $\alpha, \beta, \beta, 2, 3, 4, 5, 6-d_8$ -L-phenylalanine for five days. The amino acid has been known as the precursor of mandelonitrile biosynthesis, to produce hydrogen cyanide and benzaldehyde which were widely distributed as common defense secretions in Polydesmida. It is, therefore, evident that L-phenylalanine is an essential substance for chemical defense of polydesmid millipedes.

## INSECTS LINKED TO ELECTRONICS MAKE POWERFUL SENSORS

Jan N.C. Van der Pers

SYNTECH / VDP Laboratories, The Netherlands

The chemical senses of insects are characterized by an extremely high sensitivity and selectivity and are key factors for the insect's behavior. A variety of electrophysiological and behavioral bio-assays have been developed to use these features for scientific and practical applications. At present, the sensory information can be recorded at three levels: 1) at the peripheral sensory level using Electroantennography (EAG) and Single Sensillum Recording (SSR); 2) in the central nervous system by means of electrophysiological recording in brain tissue; 3) at the output level by observing and recording the insect's behavior.

EAG and SSR alone and coupled to high resolution gas chromatography (GC-EAD and GC-SSR) are indispensable laboratory techniques for the detection and identification of behavior-modifying chemicals. Recent advances in microelectronics and the application of signal processing with computers facilitated further development of the EAG and SSR from a restricted laboratory technique to portable EAG and SSR devices. Other advances can be seen in devices for simultaneous recording of EAG signals from multiple preparations and EAG sensors combined with miniature anemometers. Recordings from chemosensory responses in the brain are, however, still exclusively laboratory methods aimed at providing more information about the signal processing in the insect's brain. Both the peripheral recording by EAG and SSR and recording in the brain reflect partly processed information, whereas the insect's behavioral response is the conclusion of total processing. Therefore, recording at the behavioral level may provide essentially different and probably more relevant information for the researcher.

Traditionally, behavior responses are studied by direct observation or video recording and subsequent analysis. Both techniques are time-consuming and may be biased by the observer. Direct coupling of the insect to an electronic device designed to respond specifically to characteristic behavior would be of great advantage. A selection of novel devices are presented for recording overall activity, partial movements and locomotion behavior. These devices are based on optical and microwave sensors combined with electronic signal processors. Their possible applications in chemoreception research will be discussed.

**ODOR SOURCE LOCATION USING A DISCRIMINATING INSECT  
ANTENNAL BIOSENSOR ARRAY COUPLED WITH A WIND-DIRECTION  
INDICATOR**

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Ames, Iowa 50011, U.S.A

Insect antennae offer a sensitive way to detect the presence of odor plumes in the field, and to monitor their fine structure both in the laboratory and field, as first demonstrated by Baker and Haynes (1989). Interest in using insect antennal biosensors to quantify ambient concentrations of odor in the field and relate them to effective levels of disruption of pheromone communication has grown in recent years as more successful commercial mating disruption products have come on the market (Sauer et al., 1992; Suckling et al., 1994; Suckling and Angerelli 1996). The Controlled Biological Systems Program of the Defense Advanced Research Projects Agency (DARPA) has funded projects aimed at detecting and locating sources of anthropogenic compounds, such as those emitted by unexploded landmines or by toxin manufacturing facilities. Our laboratory was funded by DARPA to design a discriminating antennal biosensor array having high sensitivity to compounds of interest from the groups listed above. We have designed such a biosensor and measured its sensitivity to a variety of anthropogenic compounds such as 2,4-DNT, 2,6-DNT, and 3,4-DNT, common contaminants of the soil around unexploded landmines. Recently we have found a way to couple our antennal biosensor to a wind-direction sensor and allow an operator to steer with high reliability towards an odor source and locate it within a short period of time from tens of meters away.

**ELECTROANTENNOGRAPHIC RESPONSES OF THE ORIENTAL TOBACCO  
BUDWORM, *HELICOVERPA ASSULTA*, TO PLANT VOLATILE COMPOUNDS**

Kyung Saeng Boo\* and Seung Joon Ahn

Graduate School of Agricultural Biotechnology  
Seoul Natl. Univ., Korea

Electroantennograms (EAGs) from *Helicoverpa assulta* were recorded in response to host plant extracts and some synthetic volatiles. Linalool, hexanal, acetophenone, 1-hexanol, butyric acid, eugenol and terpineol elicited relatively large EAG responses among 29 synthetic volatile compounds tested. In general, females' EAGs were greater than those observed from male moths. When the relative EAGs of mated and unmated adults were compared, the unmated female elicited greater EAG responses than the mated, but the mated male showed larger responses than the unmated. In response to primary alcohols, aldehydes and acetates with a range of between C<sub>1</sub> and C<sub>10</sub> carbon chain-length, EAGs were significantly greater for a

particular carbon chain-length(s), with responsiveness to primary alcohols peaking at C<sub>4</sub> and C<sub>5</sub>, aldehydes at C<sub>8</sub> and C<sub>9</sub> and acetates at C<sub>6</sub>. And the responsiveness with a ranking of alcohols > acetates > aldehydes was recorded in a range of between C<sub>1</sub> and C<sub>6</sub>, but over C<sub>7</sub>, with aldehydes > acetate > alcohols. The leaf extracts of *Capsicum annum*, one of major host-plants, evoked significantly larger responses than other parts of the plant. These electrophysiological data are expected to help in finding kairomones attractive to *H. assulta* and explaining our latest observations that female showed more active calling behavior and produced a large amount of sex pheromone in the presence of its host-plant, *C. annum*.

C4

## STRENGTHS AND WEAKNESSES OF FIELD ELECTROANTENNOGRAMS AS BIOSENSORS

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Mating disruption of moth pests has achieved several notable successes, but information on the sensory environment of the insect has been generally lacking until relatively recently. Field electroantennogram (EAG) systems have been developed, to detect both the mean and variance in pheromone concentrations. Field EAG systems offer measurements with higher temporal resolution than other current methods. The increasing portability of EAG systems allows repetitive measurements over a short time, and measurements at different sites or treatments. Information processing remains relatively slow, with approximately two days of data analysis for each day in the field, in our experience. Estimates of mean atmospheric concentrations have broadly agreed with expectations, although not in all cases. The use of a proprietary field EAG device by researchers inexperienced in the subtleties of electrophysiology carries risks of misinterpretation, and other measures of efficacy remain important. For studies of plume structure, field EAGs can offer information about how the disruptant pheromone dose is received by the target insects, and highlights the influence of key variables. The high sensitivity of the antenna to pheromones enables information to be gained concerning the three-dimensional distribution of pheromone in mating disruption trials, which is otherwise not obtainable.

The major drawbacks of the approach are the need for dose response and other underpinning information, the non-linearity of the detector outside a certain concentration range, the potential in some species for a lack of sensitivity due to interaction of pheromone and host-plant detection, the variance between individual antennae which requires reasonable replication, and the effort required in data processing. For other applications of field EAGs as biosensors, the antennal specificity for certain odourants such as pheromones, or lack of specificity and lower sensitivity to others odourants (e.g. general plant volatiles) can also be limiting.

**AUTOMATIC BEHAVIORAL MONITORING THROUGH TRAINING WITH  
ARTIFICIAL NEURAL NETWORKS TO DETECT CHARACTERISTIC PATTERNS  
OF RESPONSE BEHAVIOR OF AQUATIC INSECTS AFTER SUBLETHAL  
TREATMENTS OF INSECTICIDE IN SEMI-NATURAL CONDITIONS**

Hyun-Min Kang<sup>1</sup>, Inn-Sil Kwak, Mi-Young Song, Sung-Kyu Lee<sup>2</sup>  
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A multilayer perception network was used to train the locomotive tracks of aquatic insects after being treated with an organic phosphate insecticide, diazinon. The fourth instar larvae of *Chironomus* sp. were placed in an observation cage (6cm X 7cm X 2.5cm) at temperature of 18<sup>0</sup>C (light condition(10LL: 14DD)).

The activity and the locomotive tracks of each test insect, pre- and post- treated with sublethal concentration (0.01 ppm) of diazinon, were individually observed for four days (1 day before treatment, 3 days after treatment) by using an auto remote sensing system. Irregular locomotive behavior and the characteristic "ventilation" movements occurred more frequently to the organisms treated with the insecticide. The backpropagation algorithm was implemented to the multilayer perceptron network to train the input data of the locomotive tracks of the organisms. The patterns of behavioral response were accordingly extracted through the training process, and the network was able to detect the characteristic patterns of treatment when new data for the locomotive tracks were provided to the network. This computational patterning on the locomotive tracks could be an alternative for "in situ" biomonitoring tool for detecting the presence of toxic chemicals in the environment.

**BASIC RESEARCH IN INSECT COMMUNICATION ECOLOGY  
ENHANCING THE POTENTIAL FOR MANAGEMENT OF TARGET INSECTS**

Gries, G.\*, Takacs, S., Mullan, J., Babor, D., Olfert, O., Kaminski, L.-A.,  
Khaskin, G., and Gries, R.

The talk will present selected examples of basic research in insect communication ecology that greatly enhances the potential for management of target insects. Examples will include research on the cabbage looper, *Trichoplusia ni*, in greenhouses, webbing clothes moth, *Tineola bisselliella*, and house fly, *Musca domestica*, in urban settings, and the orange wheat blossom midge, *Sitodiplosis mosellana*, in prairie agriculture.

**SEX PHEROMONES OF THE STINK BUGS *CHLOROCHROA LIGATA*, *C. SAYI* AND  
*C. UHLERI* (HEMIPTERA: PENTATOMIDAE)**

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Feeding by phytophagous stink bugs on agricultural crops causes premature abortion or deformation of seeds, fruits, and nuts, and also transmits disease. Current monitoring methods for stink bugs include beating tray or sweepnet sampling, and visual inspection of crops for damage, all of which are labor intensive and time consuming. Traps baited with attractant pheromones may provide a sensitive and specific alternative method for monitoring stink bugs.

Attractant pheromones for three pentatomid bugs in the genus *Chlorochroa* were investigated, and a total of five potential pheromone compounds that were produced only by sexually mature male bugs were identified. *C. ligata* and *C. uhleri* males appear to produce very similar blends of compounds, with methyl (*E*)-6-2,3-dihydrofarnesoate as the major component, and methyl (2*E*,6*E*)-farnesoate and methyl (*E*)-5-2,6,10-trimethyl-5,9-undecadienoate as minor components. Methyl geranate is the major male-specific compound from *C. sayi* with small amounts of methyl citronellate and methyl (*E*)-6-2,3-dihydrofarnesoate also being produced.

Production of the male-specific compounds and mating frequency peaked at around 2-3 hours before dark, indicating that bioassays should be conducted during the evening hours. A vertical Y-tube olfactometer was used for laboratory bioassays. Field bioassays were conducted by hanging a pheromone source on a plant. Each day, two to three hours before dark, the numbers of bugs on pheromone-baited plants were compared with the numbers found on plants baited with solvent controls. Results of laboratory and field bioassays, using insect extracts or reconstructed blends of synthetic chemicals, will be presented.



## QUANTIFICATION AND UTILISATION OF INSECT GROWTH PARAMETER IN BIOASSAY OF INSECT GROWTH INHIBITOR

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Studies of effects of allelochemicals or other insect growth regulators on development of insects are not easy using parameters currently employed. From measurement of the growth-inhibiting effect of crude extract of soursop seed (*Annona muricata*), extract of neem seed (*Azadirachta indica*) and crude leaf extract of lantana (*Lantana camara*) on the *Heliothis armigera* larvae, growth index (GI) and Relative Growth Index (RGI) calculations are demonstrated in this paper.

## SUPERIOR INSECT CONTROLS WITH PLANT DERIVED ATTRACTANT BAITES

Philipp Kirsch

IPM Technologies USA

“The United States demand for plant-derived chemicals is projected to increase more than 7% per year to \$2.9 billion in 2005. Primarily driven by plant-based pharmaceuticals and gains in the beverage market, bulk botanical extracts found in nutraceuticals will also contribute to this growth. Botanical extracts are expected to experience the fastest growth, nearly 11% annually.” (May 2001, www.Happi.com).

While the above analysis does not even consider applications in pest management, insects are the dominant herbivore, and the chemical basis of insect-plant interactions presents a golden opportunity for development of new products. The global insecticide industry, valued at \$6.93 billion in 1998 (\$1.56 billion in the US in 1997), is undergoing significant change in response to environmental and consumer pressures against the spraying of pesticides. Government regulation is removing many compounds from the market, restricting the use of existing compounds, and delaying permit approval of novel chemistry. Pharmaceutical giants are divesting their pesticide divisions, and the market is consolidating. The market for conventional insecticides has fallen from \$8.749 billion in 1995, to \$US8.07 billion in 1997. At the same time, growing at about 20% annually over the past decade, organic produce sales have reached \$6 billion in 2000 in the United States. Suppliers are responding to an increasing demand from consumers for healthful foods.

IPM Technologies ([www.ipmtech.com](http://www.ipmtech.com)) is focussed on the development of innovative non-polluting semiochemical products for agricultural, forestry and public health pest management.

The company offers two competitive product lines: (i.) monitoring systems (traps and lures), and (ii.) attract and kill (A&K) technology - Last Call - an attractant/insecticide bait matrix for effective, selective and residue-free control of agricultural, forestry and horticultural pests. Recent trials demonstrate the efficacy of plant based extracts (PBEs, food/floral/fermentation odors) in attracting codling moth, Australian cotton bollworm, cabbage looper, Mediterranean fruit fly and Colorado potato beetle to the Last Call matrix. It is clear that this formulation has considerable utility in controlled release of a wide range of semiochemical attractants for a wide range of insect targets, and that plant-derived A&K baits would offer considerable promise for global pest management applications.

D5

**COMPARISON OF REPRODUCTIVE CHARACTERISTICS BETWEEN  
RICE-FEEDING AND WATER-OATS-FEEDING POPULATIONS  
IN *CHILO SUPPRESSALIS* (LEPIDOPTERA: CRAMBIDAE)**

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The striped rice borer, *Chilo suppressalis*, is known in Japan to feed on rice plants, *Oryza sativa*, and on related aquatic weeds, water-oats, *Zizania latifolia*. Although the body size of the rice population is much smaller than that of the water-oats population, these populations have been regarded as a single species, since no other morphological differences are shown between the populations, and each population can feed on the different host plants and can easily be hybridized with each other under the laboratory conditions. Recently, however, a clear difference in the mating time has been shown between the two populations and the reproductive isolating mechanism has become of interest. We have therefore compared various characteristics related with their reproductive behaviors to know the relationship between the two populations.

Clear differences were present between the rice and water-oats populations during female calling, male orientation to females and copulation. Roughly speaking, these behaviors were shown during much earlier time of scotophase in the rice population than in the water-oats population, although some overlapping was observed. These differences in the mating behaviors seemed to be determined primarily by the difference in the time of female calling behavior. The peak of mating activity was observed 1 day after emergence in the rice population, whereas in the water-oats population on the day of emergence.

Analyses of the female sex pheromone glands revealed no differences in the pheromone composition, Z11-16:Ald, Z13-18:Ald and Z9-16:Ald, between the populations. However, small but significant differences were detected in the blend ratios of the 3 components; 76:15:9 in the rice population and 65:32:4 in the water-oats population. The temporal changes in the pheromone titers in the gland during scotophase reflected the time of calling. Rice population females produced much greater amount of sex pheromone than water-oats population females,

despite the much smaller body weight. Similarly, rice population males produced significantly greater EAG responses to the synthetic pheromone components than water-oats population males.

Hybridization between the populations produced F1 and F2 generations. The mating of the F1 moths occurred during the intermediate time between the two populations. Interestingly, the time of mating of the F2 moths was shown during the same period as in the F1 moths, indicating that the time of mating is probably controlled under a polygenic system.

A consideration of the present results can lead to the conclusion that there is a reproductive isolating mechanism based mainly on the difference in the mating time between the rice and water-oats populations in *Chilo suppressalis*. This may necessitate some revision in the management strategy for this insect as a rice pest.

D6

## THE PROGRESS ON THE DEVELOPMENT OF NEEM PRODUCTS AS INSECTICIDES

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Since 1986, the development of phytochemical pesticide was initiated by IUC Life Sciences ITB. It was decided to adopt Neem (*Azadirachta indica* A. Juss) as the topic for the main research program. The first step in this program was the survey and monitoring of neem trees in Java, Bali and Lombok, Indonesia, which covered mapping, phenology, seeds production, azadirachtin content, and usage by local societies. It was apparent that the distribution of neem trees covers a large arid land in East Java, Bali, Lombok, and the rest of the Lesser Sunda Islands. Ripened fruits and seeds are available from December to February each year and followed by seedling in the surrounding trees. The second step covered the work on the phytochemistry and bioassay on insects, non-target invertebrates, and vertebrates. The third step is formulation, which consisted several activities. The formulation of neem seed extract was registered to the Pesticides Committee in order to receive the commercialization permit for the product. The development of small-scale formulation-plants was started in 1994 in our institute. The source of neem seeds in a large quantity came from several organizations from East Java, Bali and Lombok. The formulation equipment consisted of seed crusher, macerator, distillator and freeze drier that were made locally workshop. The socialization for the product acceptance was held by means of seminars, workshops, and demonstration plots. At present neem seed extract as an insecticide is available from our institute, and is currently used by tea growers.

**CAN PHEROMONE TRAPS HELP IN BIOLOGICAL CONTROL  
OF INSECTS AND WEEDS ?**

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Pheromone traps have been used for monitoring pests for many years, but their use in biological control is more recent. Pheromones have been identified and used for monitoring two Lepidopterous biological control agents introduced into New Zealand for biological control of gorse, a major weed species. In the case of *Cydia succedana* (Tortricidae) and *Agonopterix ulicetella* (Oecophoridae), traps baited with a sex attractant were developed as a new tool for monitoring the establishment, phenology, and efficacy of the agents. The prospects for using sex attractants in traps as an aid for monitoring populations of weed biological control agents are excellent. The traps have also been used to determine the optimal size of founding populations to ensure establishment of the control agents. Pheromone traps have also been used to monitor the phenology, abundance and distribution of an insect biological control agent (*Ascogaster quadridentata* (Braconidae), an egg parasitoid of codling moth, *Cydia pomonella*). The emergence of the parasitoid was shown by outdoor emergence cages and pheromone trapping to be slightly ahead of the male moth flight period, but essentially synchronous with the female moth emergence. A survey of orchards in four regions of New Zealand indicated considerable regional variation in the presence and abundance of the parasitoid on orchards with little or no pesticide use. Pheromone trapping of the parasitoid appears to be less directly useful in management of the insect pest, compared with the cases involving biological control of weeds. The possible reasons for this will be discussed.

**EFFECT OF RED PALM WEEVIL MASS TRAPPING WITH SYNTHETIC  
PHEROMONE  
IN TRADITIONAL IRANIAN DATE PALM GROVES**

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The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* is one of the most serious palm pests in Asia. It has been reported from Iran for 10 years. Quarantine and insecticides have been applied to control it. As the actual impact of synthetic pheromone in RPW control has been

poorly documented, an experimental mass trapping was realised to evaluate the method. The compared effects of the trapping on the catches of, and the damage by, RPW were studied in 224-ha of traditional date palm groves at 4 trap densities: 0 (control), 0.5, 1 and 2 pherotraps/ha in Apr.-Dec. 1998. A precise mapping of the plots was initially realised to study the correlation between the plot structure and RPW infestation. Plastic buckets were used as traps with synthetic pheromone (5 mg/day) and date palm pieces as attractant. Palms were checked every 2 months for infestation (4 times) and sanitised when necessary. Five- to 20-year old palms were 20 times more infested than the other ones. Mazafati was 10 times more infested than the other varieties. Infested palms were clustered and accounted for 1.4 % of the 47,027 palms examined. 4298 RPWs (3 F:1 M) were caught in the traps (0.6 RPW/trap/week). The higher the trap density, the higher the total catches per plot but also the lesser catches per trap. More date palms were newly infested in the plots with 4 traps than in the controls (2.7 vs. 1.7), but the percent newly infested palms and the relative variation of infestation (during vs. before trapping) did not differ from the controls. As much new infestation was recorded in the controls as in the plots with 1 or 2 traps. The new damage was clustered and highly correlated to the trap location, following a negative loglinear relationship with the distance to the nearest trap. If one season pherotrapping could eliminate many RPWs, it was insufficient to trigger a reduction of the damage and increased the local risk of infestation when applied to highly susceptible plots. Maximising the number of RPW catches appeared not adapted to the treated area. We then recommend to perform a trapping with low trap densities and precisely taking into account of the plot susceptibility. A better compromise between the capture level and the risk of indirect damage has to be determined for a rational application of a pherotrapping that contributes to an efficient long term RPW control.

D9

## FIELD TRAPPING OF *CHRYSOPA COGNATA* (NEUROPTERA: CHRYSOPIDAE) WITH SOME SYNTHETIC CHEMICALS IN KOREA

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*Chrysopa cognata* is known as an important source in aphid biological control. *C. cognata* adults have been reported to be attracted, mainly through laboratory experiments, to (-)-(1*R*,4*aS*,7*S*,7*aR*)-nepetalactol and (+)-(4*aS*,7*S*,7*aR*)-nepetalactone, sex pheromone components of aphids. Here we summarize field trapping data of the chrysopid to the two chemicals and their synthetic analogues.

Nepetalactol was found to be much more attractive to the insect in field tests than nepetalactone, although nepetalactone was also attractive in Y-tube olfactometer tests and elicited high response in EAG tests, as in the case of nepetalactol. The neomatatabiol isomers synthesized, which are similar to nepetalactol in structure, also attracted male *C. cognata* in the field. *C. cognata* were caught in traps mainly during nights, especially from 7.30PM to 1.00AM in July and August in Suwon (a little south of 30 degree north latitude), Korea. Female chrysopids

were also reported to respond to the chemicals, but, surprisingly, only males were caught in traps throughout the trapping experiment. Males apparently chewed the rubber septum impregnated with the chemicals. Besides this species, a few individuals of *C. formosa* and *C. phyllochroma* were also caught in the traps baited with nepetalactol or nepetalactone and all of them were also males only.

D10

## ROTENONE BIO-ACTIVATOR CONTROLLING VEGETABLE INSECT PESTS AND PREDATORY FISH

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In Vietnam, until the late 80's the use of rotenone as an insecticide was rarely applied to plants in the North. During 1993-1994 we have started studying methods for analysing rotenone, qualitatively as well as quantitatively, in *Derris elliptica* Benth. All this while in the Mekong River Delta (MRD) Viet nam, the local farmers have extensively used rotenone as a fish poison for protecting shrimp against predatory fish in the fields. A few of them have effectively used rotenone as an insecticide for some vegetables crops. Based on the situation, after studying successfully the methods for extracting and isolating rotenone crystal from the root of *Derris elliptica* Benth in 1995, we have carried out manufacturing some kinds of preparation for use both in the killing of predatory fish and controlling of vegetable and rice insects. This paper reports the test results of rotenone preparations in the fields from 11/3/1995 to 12/01/1998 in the MRD as follows:

- Rotenone preparations produced by extraction using acetone and chloroform solvents had the effect twice as that from ethanol extraction, the latter was 3-5 times as potent as that from water extraction. Ethanol was used as a solvent largely due to its low cost and facile production. This preparation was labelled ROTND. ROTND 1.0-2.0 % had exterminated 65%-95% common insects of vegetables in MRD Viet nam. ROTND 200-400ppm ai. had the same effect as Decis (Deltamethrin) of 12.5ppm ai..
- For controlling fish in the field, only 1 ppm of ROTND was required to block the respiratory system of most fish among which trash fish can tolerate for 30 minutes while the predatory fishes of the lower class can tolerate for 180 minutes.
- For controlling rice insects, ROTND had the strongest effect after three days post spraying, 1-3 times as much as Bt, 66-71% of Karate and Trebon but the effect decreased to 75% 10 days post spraying, equivalent to Bt. This is a very good condition for fast rehabilitation of natural enemies especially spiders. ROTND have exterminated 65-68% of rice skippers, similar to Bt and 80% compared to Trebon, Karate three days post spraying. For brown plant hopper, ROTND had an effect on mortality 72.5 times as that of Bt at one day post spraying, and only 1.5 times after 10 days post spraying.

**THE POTENTIAL OF *CLAUSENA EXCAVATA* AND *CITRUS* SPP ESSENTIAL OILS AS AN ANTI-T LYMPHOBLASTIC LEUKAEMIA (CEM-SS CELL LINE)**

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Essential oils of plants from Rutaceae family e.g. *Clausena excavata* (“pokok cerek hitam”) and *Citrus* spp (“pokok limau”) were prepared scientifically by hydrodistillation process using fresh leaves and stem barks and then analysed using GC-MS. The main essential oil components from *C. excavata* (Jabi A) were safrole (62.5%) and terpinolene (18.5%), while *C. excavata* (Jabi B) gave safrole (59.8%) and terpinolene (23.5%) as major constituents. The essential oils from several species of local Malaysian Citrus have been investigated. Citronellal (83.3%) and citronellol (8.5%) were the major components of *Citrus hystrix* (“limau purut”). The major constituents of *C. aurantifolia* (“limau nipis”) were limonene, (Z)-citral and (E)-citral. The major constituents of the oils of *C. sinensis* (“limau manis”) were found to be linalool and  $\beta$ -elemene. Germacrene-D, elemol and  $\beta$ -eudesmol were the major constituents of the oil of *C. microcarpa*. The oil of *C. maxima* (“limau bali”) from Kedah contained significant amounts of sabinene,  $\gamma$ -terpinen and  $\beta$ -caryophyllene. However,  $\beta$ -phellandrene, *trans*- $\beta$ -ocimene and  $\beta$ -caryophyllene are major constituents of *C. maxima* from Johor. The other pure chemicals such as 1,8 dihydroxy-3-formyl-4-prenylcarbazole, 3-carbomethoxy-2-hydroxy-7-methoxycarbazole and Clausine-K gave very strong activities against CEM-SS cell lines (T-Lymphoblastic leukaemia) with IC<sub>50</sub> values of 2.1, 8.2 and 5.1  $\mu$ g per ml respectively. Crude hexane and ethyl acetate extracts from the stem barks of *Clausena excavata* gave significant cytotoxic effect on a CEM-SS cell line with IC<sub>50</sub> values of 3.27 – 2.47  $\mu$ g per ml. respectively. However, all essential oils of *C. excavata* and *Citrus* spp gave significant cytotoxic activities with IC<sub>50</sub> values around 2.19 – 3.69  $\mu$ g per ml for all plant material sources. The major chemical constituents of studied essential oils e.g. safrole, terpinolene, citronellal, citronellol and limonene have some useful aroma or fragrances. Besides that, those particular essential oils have also some potentials as an anti-cancer drug.

**CHEMICAL CONSTITUENTS AND BIOACTIVITY OF  
*AEGLE MARMELOS* (RUTACEAE)**

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*Aegle marmelos* is a small tree which belongs to the Rutaceae family, growing in the Indian Subcontinent and Southeast Asia. The plant is used in traditional medicine treating intermittent fever and intestinal ailments, in fertility control and for use afterbirth. The study involves the isolation and identification of chemical constituents from leaves, stem bark and roots of the plant. The plant samples were each extracted subsequently with petroleum ether, chloroform and methanol. The extracts were column chromatographed over silica gel, and the structure of the chemical constituents obtained were elucidated using spectroscopic techniques. Several alkaloids, coumarins and terpenoids including aegeline, skimmianine, marmin and auraptens were characterized. Antimicrobial activity and cytotoxic tests of the crude extracts and the pure compounds against CEM-SS leukemia cells were also carried out.

**CYTOTOXIC EFFECTS OF *PERISKIA CORRUGATA* EXTRACTS AGAINST  
DIFFERENT CLASSES OF CELL LINES**

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Preliminary *in-vitro* screening of the methanol extract from *Periskia corrugata* plant indicated cytotoxicity against six different cell lines, CaOV (Human ovarian carcinoma), Vero (normal monkey kidney epithelial cells), MDBK (normal bovine kidney epithelial cells), HepG2 (Human hepatocellular carcinoma), NCI-H23 (Human lung adenocarcinoma) and T-47D (Human breast ductal carcinoma). Cell survival was determined by the procedure using Methylene Blue staining as described by Yamazaki *et al.* (1986). EC<sub>50</sub> for all cell lines were reasonably below 30 µg/ml, indicating cytotoxicity and potential anticancer properties, as judged by the criterion set by the National Cancer Institute, USA (Geran *et al.*, 1972). T-47D and NCI-H23 appeared to be the most sensitive to this extract with EC<sub>50</sub> less than 20 and 2 µg/ml respectively. This preliminary study indicated that *Periskia corrugata* possesses a potent cytotoxic effect.



## NEW ACYLATED FLAVONOLS FROM *MIMOSA PIGRA*

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Four acylated flavonols *i.e.* kaempferol 3-*O*-cinnamyl sophoroside, quercetin 3-*O*-acetylgalactoside, quercetin 3-*O*-acetyl-arabinoside and myricetin 3-*O*-acetylxyloside have been isolated from the leaves of *Mimosa pigra*, a weedy species which is widely distributed in the tropics and subtropics. The finding suggested the evolutionary advancement of this species compared with other species of *Mimosa* which are considered a primitive genus and mostly endemic to the New World.

## NOVEL NEW ATTRACTANT AND FORMULATIONS FOR CONTROL AND MONITORING OF TEPHRITID FRUIT FLIES.

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\*IPM Technologies USA and Insect Science South Africa

A novel plant based attractant (EGO) shows activity for three species of *Ceratitis* fruit flies: Mediterranean fruit fly (Medfly, *C. capitata*), Natal fruit fly (Natal fly, *C. rosa*) and Marula fruit fly (Mango fruit fly, *C. cosyra*). In field trials in Hawaii, South Africa, and Kenya, EGO captured more Medfly than trimedlure, and attracted Natal fruit fly to traps. Obtained economically from natural sources, EGO was formulated with a toxicant in IPM Technologies proprietary Last Call paste matrix to control Marula fruit fly and medfly. Its multispecies activity presents a very useful new tool for regulatory agencies involved in monitoring and control activities. The Last Call matrix has been used successfully to dispense cuelure and methyl eugenol in field traps. Tephritid fruit fly attractants can be easily and precisely dispensed in the field for either control or monitoring purposes using highly portable, stable, and environmentally safe Last Call formulations.

In trapping trials measuring species response of three *Ceratitis* fruit flies to a novel alpha-copaene based natural attractant in Africa, all three species of *Ceratitis* were attracted to the new EGO bait formulations. The EGO bait is superior to commercial lures in South Africa.

Melonfly, oriental fruitfly, and other *Bactrocera* and *Dacus* species were trapped in trials measuring field responses to traps baited with Last Call formulations (20% methyl eugenol OR cuelure). Oriental fruitfly captures in Hawaii were equivalent to conventional wick-baited traps.

In trials measuring the efficacy of the Last Call EGO attract and kill formulation in controlling *Ceratitis* species in South African citrus, a 3 ha commercial citrus orchard was treated with Last Call (10% EGO) droplets at 3000 droplets/ha, and compared to an adjacent conventionally treated orchard on the same farm. Droplets were distributed evenly throughout the orchard at 2m height within the tree. Efficacy was equivalent to the standard insecticide program.

Alpha-copaene based natural extracts are promising competitive new semiochemical tools for management of *Ceratitis* flies. Last Call EGO is effective in controlling fruitflies in citrus orchards, and versatile in dispensing other known fly attractants.

**D16**

### **FIELD EVALUATION OF *BEAUVERIA BASSIANA* FOR THE CONTROL OF COCOA POD BORER IN SABAH, MALAYSIA.**

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A field evaluation of the effectiveness of an entomopathogen, *Beauveria bassiana* for the cocoa pod borer (CPB), *Conopomorpha cramerella*, was conducted in three cocoa estates in Lahad Datu (Sinrico Plantations) and Tawau (Ladang Koko Merah Hijau, Tawau Cocoa Estate). The three locations differed in the levels of severity of CPB of attacks. The entomopathogen applied at  $2.5 \times 10^5$  to  $6.5 \times 10^5$  spores/ml was compared to the standard estate insecticide treatment (pyrethroids and/or chlorpyrifos) and no treatment. The results of the field trials over a full crop season of 7 months indicate that in locations where the severity of CPB attack was high as in the two estates in Tawau, there was significant difference among the treatment in crop loss due to CPB although the overall rate of pod infestation was similar. Plots given the entomopathogen treatment showed higher percentage of recoverable beans by a minimum of 6 points over those untreated plots. In the location where the level of CPB attacks was less severe as at the trial site at Lahad Datu, negligible crop loss from CPB infestation was observed although the rate of infestation of pods may reach 70% at its most severe. In this location differences in recoverable beans among the plots treated with insecticide or the entomopathogen or untreated plots were marginal and non-significant. The results indicate that there is practical potential in the use of *B. bassiana* for the control of the cocoa pod borer.

***ANASTREPHA* SPP. KAIROMONES**H.N. Nigg<sup>1</sup>, S.E. Simpson<sup>2</sup>, R.A. Schumann<sup>1</sup> and Eric Jang<sup>3</sup><sup>1</sup>University of Florida<sup>2</sup>Florida Department of Agriculture and Consumer Services<sup>3</sup>USDA-ARS-PBARC, USA

Kairomones are one of the highly utilized technologies for fruit fly management. Although usually considered as plant products, kairomones are associated with all living organisms and fall under the broad categories of attractants, arrestants, and excitants. Kairomones for the fruit fly genus *Anastrepha* include all three categories. Management methods for *Anastrepha* which use kairomones will be discussed. Examples of kairomones that are attractants, arrestants and excitants will be presented and discussed by using *Anastrepha* species.

**ATTRACTANTS AND THE MATING SUCCESS  
OF MALE MEDITERRANEAN FRUIT FLIES**Todd E. Shelly  
USDA-APHIS

Spurred by previous work on the association between methyl eugenol and the mating success of male *Bactrocera dorsalis*, our laboratory has investigated the influence of  $\alpha$ -copaene-containing oils on the mating success of male *Ceratitidis capitata*. Specifically, we have monitored the mating performance of wild-like and mass-reared males in field-caged host trees following exposure to the aroma of ginger root oil. Mature, wild-like males exposed to this oil (20  $\mu$ l for six hours) and tested two days later obtain 81% of all matings compared to 19% non-exposed males. Immature males (one day old) exposed to the oil and tested four days later also have a pronounced mating advantage. Similarly, the mating competitiveness was greatly enhanced via exposure to ginger root oil. When competing against wild males, non-exposed, mass-reared males obtained only 26% of all matings with wild females. However, following chemical exposure mass-reared males obtained 75% of all matings. The practical implications of these results for the sterile insect technique are discussed.

More recently, we have been monitoring the influence of "hot spots" on the trunk and branches on the mating performance of *C. capitata* males. Field observations revealed that males sometimes aggregate along specific sections of guava branches, where they generally rest quietly with the body appressed to the bark surface. Females display no such aggregative tendency. Males exposed to such sites gain a clear mating advantage over non-exposed males when tested one day after exposure. Exposure to live leaves alone, dead leaves, or non-hot spot sections of branches does not boost mating performance. The chemical basis of 1) male attraction to hot spots and 2) the enhanced male mating success remain unstudied.

**KAIROMONE-BASED INSECT CONTROL:  
EFFECTIVE, SELECTIVE AND ENVIRONMENTALLY SECURE.**

Philipp Kirsch

IPM Technologies USA

How many minutes of any insect's life cycle are devoted to mating? What do we know about the many other semiochemicals mediating behaviour during the rest of its life? In the application of chemical ecology, many public and almost all commercial agricultural pest management product development programs have until recently focussed on deciphering and manipulating sex-pheromone controlled mating activity for pest control outcomes. Sex-pheromones have been identified for more than 1200 species of moths and hundreds of commercial pheromone products crowd today's marketplace.

Recent research suggests that exploitation of multiple behaviours (e.g. mating, feeding, oviposition, etc.), will result in more robust semiochemical-based control programs. When targeting male, female and even larval insect stages with kairomones, it becomes possible to selectively remove large numbers of individuals from a pest population. Chemical ecology prospecting is starting to focus on elucidating the chemical signals that mediate this wide range of non-sexual behaviour. IPM Technologies recently tested over 90 compounds in trapping surveys in different ecotomes in the Middle East, Central Europe and Eastern Asia.

Kairomones are already proven effective in pest management. Cucurbitacin baits and squash volatiles form the basis of semiochemical tactics targeting diabroticine beetles, and are in development for Colorado potato beetle. Several research teams are racing to identify optimum blends for tortricid and noctuid moths. Dried food odors are being used in management of stored product moth and beetle pests. Medical and veterinary entomologists have long relied on carbon dioxide for the sampling of haematophagous invertebrates, and the search for additional mammalian host odors is very active. Kairomones are enabling the development of new attract and kill strategies.

Attracticides, which use the concept of lure and kill, consist of an attractant and a killing agent. Their greatest commercial application has targeted Diptera, including tephritids, houseflies, blowflies and tsetse flies. IPM Technologies has developed a very robust, viscous, grease-like, liquid gel matrix, called Last Call or Sirene, that can accept diverse classes of semiochemicals. The company is actively developing novel A&K formulations, including highly effective kairomone-based monitoring and control products for management of *Ceratitis* fruit flies.

## CHEMICAL BASIS OF HOST RESISTANCE IN *TRIFOLIUM GLANDULIFERUM* TO REDLEGGED EARTH MITE

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Redlegged earth mite, *Halotydeus destructor* (Tucker) (Acarina: Penthalidae), is a major pest of pasture, crops and vegetables in Australia. The development of pasture plants resistant to *H. destructor* is a major priority of the national pasture legume breeding program. In previous studies, we have identified some chemical mechanisms of resistance in *Trifolium subterraneum*, the most commonly used pasture legume in Australia. An alternative pasture legume, *T. glanduliferum*, recently released in Australia has shown high levels of resistance to mites under laboratory and field conditions. Here, we report on the chemical basis of resistance of *T. glanduliferum* to redlegged earth mites.

The chemical mechanisms involved both volatile compounds and non-volatile compounds produced in leaves of *T. glanduliferum*. Volatile compounds, collected by trapping on Tenax TA adsorbent or hydrodistillation, showed strong feeding deterrence to redlegged earth mites. The major constituents in the volatiles, identified by GC-MS, are coumarin,  $\beta$ -ionone and  $\alpha$ -ionone as well as aldehydes and alcohols, together with some minor terpene constituents. In bioassays, redlegged earth mites were strongly deterred by these compounds. Coumarin and the ionones showed significant deterrence to mites at 70 ppm and 100 ppm, respectively. It is suggested that resistance is, in part, due to the presence of coumarin and the ionones.

Dichloromethane extracts from both leaves and flowers, and methanol extract from leaves, of *T. glanduliferum* showed significant deterrent activity in the bioassay. Using bioassay-guided fractionation, a number of bioactive components have been isolated from these extracts. Coumarin,  $\beta$ -ionone, phenylethanol, phytol,  $\beta$ -sitosterol and the pterocarpan medicarpin have been isolated from the dichloromethane extract. The separation of the methanol extract yielded the flavones kaempferol and quercetin, a new glycoside of kaempferol, as well as uracil, uridine and a mixture of triterpenoid saponins. The structures of these metabolites have been elucidated by spectroscopic methods.

All these compounds were tested for feeding deterrence. Of these, coumarin,  $\beta$ -ionone and  $\alpha$ -ionone were most deterrent to mites, showing strong antifeeding activity at 0.01%. Medicarpin (55%), linaool (76%), phenylethanol (91%), quercetin (23%) and uracil (36%) showed significant deterrence at 0.5% concentration. The level of coumarin in the leaves was 0.006%, within the range at which this compound remains active against these mites.

It is interesting to note that a greater variety of compounds appears to be involved in the resistance of *T. glanduliferum* to redlegged earth mite than in *T. subterraneum* in which deterrence to mites is conferred largely by constitutive isoflavones.

## POSTERS

E5

### ANTIMYCOTIC ACTIVITY OF *ALPINIA GALANGA* EXTRACT AGAINST *MICROSPORUM CANIS*: ULTRASTRUCTURAL CHANGES BY SCANNING ELECTRON MICROSCOPE

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*In vitro* studies were conducted on *Microsporum canis*, a dermatophyte pathogenic to human, using extract from *Alpinia galanga*. The extract was prepared from the dried powdered rhizomes using Soxhlet extraction with chloroform. The minimum inhibition concentration (MIC) after incubation at 28-30°C for 24 hours using broth dilution method was 370 µg/ml. The effects of the extract at the MIC value, after incubation for 24-72 hours in broth culture were then studied using scanning electron microscope. The extract caused alteration to the outer structure of the hyphae after 24 hours incubation. Hyphae appeared swollen and later collapsed, wrinkled, flattened and translucent. This indicates the potential value of extract from *A. galanga* as an antimycotic substance.

E6

### ANTIBACTERIAL AND PHOTOTOXIC ACTIVITIES OF THE *TRIPHASIA TRIFOLIA* EXTRACTS

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The antibacterial and phototoxic properties of the essential oil extracts and the *n*-hexane, chloroform and methanol fractions from various parts *i.e.* leaves, fruits and stems of *Triphasia trifolia* were investigated using agar diffusion method. The bacteria used in this study were *Escherichia coli* and *Proteus vulgaris* (gram negative) and *Staphylococcus aureus* and *Micrococcus luteus* (gram positive). The maximum activity was exhibited by 50mg/ml of the hexane fraction from the fruit extracts against *Staphylococcus aureus*. The minimum inhibitory concentrations of the extracts ranged between 5µg/ml to 10µg/ml. The most remarkable phototoxic effects were shown by the chloroform fractions. These fractions were also found only to exert phototoxic effect in the presence of long-wave length ultra-violet light against *P. vulgaris*.

## STRATEGIES FOR IMPROVEMENT OF CROP ALLELOPATHY WITH TRANSGENES

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Allelopathy, the ability of certain plant species to chemically repress the growth of other plants in their surroundings, has received a significant amount of attention by the scientific community. However, the use of allelopathic crops to reduce input of and dependence on synthetic herbicides in agricultural settings has not been developed into an effective tool for farmers. Traditional breeding methods have focused on crop lines having superior yields and increased tolerance to insects and diseases. This selection process seems to have produced commercial varieties with relatively poor allelopathic potentials, although 'ancestral' biotypes may have had some useful allelopathic traits. Genetic engineering has been recently used to render crops resistant to insects, pathogens, and herbicides. While similar biotechnological tools can be used to generate crops with high expression of allelopathic traits, enabling a plant to produce allelochemicals may be relatively more complex because it most likely involves the introduction or manipulation of multiple genes. Manipulation of the allelopathic potentials of sorghum (*Sorghum bicolor*) and rye (*Secale cereale*) is at different stages of development and illustrates the various difficulties involved in this endeavor. The biosynthesis of allelopathic hydroxamic acids, such as DIBOA, in rye and corn (*Zea mays*) is fairly well characterized, and the key enzymes, as well as the genes encoding them, are already known. However, field studies have shown that weed control by rye grown as a cover crop fluctuates because the amount of DIBOA released in the soil varies with time and environmental conditions. The DIBOA/rye model system will be presented as an example for the strategies used to enhance and regulate the production of an allelochemical already present in a crop. Sorghum is known to produce and release the phytotoxic *p*-benzoquinone sorgoleone that acts by inhibiting photosynthetic electron transfer. The sorgoleone/Sorghum model system will be discussed as an example of the elucidation of the biosynthetic pathway of an allelochemical in a crop species and inserting this pathway in another crop. The potential problems associated with either strategy, including the development of tissue-specific promoters, autotoxicity, metabolic imbalances, and proper movement of the allelopathic compound to the rhizosphere will be presented.

## ALLELOPATHY IN AGRICULTURE – THE AUSTRALIAN EXPERIENCE

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The role of allelopathy in agricultural systems in Australia has only recently been recognised. The pre-occupation with increased yields in new crop varieties has resulted in such varieties having variable and generally poor competitive abilities and allelopathic capability. The dependence on herbicides for weed control has also resulted in a narrow focus for management of weeds but the development of significant herbicide resistance has required a refocus to other options including natural mechanisms. The development of conservation farming systems where crop dry matter residues (i.e. straw) are retained for soil surface cover and organic matter generation has raised the issue of allelopathic interactions between the crop residues and the emerging crop seedlings.

This paper provides three examples of the impact of allelopathy in Australian farming and pasture systems. Firstly, one of Australia's common weeds, silvergrass (*Vulpia spp*), has the capacity through its carryover dry matter residues to interfere with the regeneration of annual crop and pasture seedlings. Secondly, carryover of dry matter residues of pastures and crops also impacts on the establishment of the new season's desirable species. Thirdly, the interaction between crop seedlings and weeds at establishment provides an interesting study into the potential for incorporating allelopathic capability into new varieties.

The prospects for the development of allelopathic varieties, the development of natural herbicides and the need to consider allelopathy in management are discussed in the paper.

## ALLELOPATHIC POTENTIAL OF *MACARANGA TANARIUS* L.

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*Macaranga tanarius* distributes widely in the abandoned lowland of Taiwan and a substantial amount of *M. tanarius* fallen leaves accumulated on the floor. A unique pattern of weed exclusion underneath the stand is often found and is thought to be an allelopathic phenomenon. To elucidate the allelopathic potential of *M. tanarius*, a series of experiments, such as field observation and measurements, greenhouse experiments, phytotoxicity determination, and isolation and identification of responsible allelopathic compounds were conducted. The observation and measurement of understory species in the *M. tanarius* stand and its adjacent



grassland area have tentatively concluded that the allelopathic pattern in fields was unlikely due to physical competition for light, soil moisture, or nutrients. *Biden pilosa* and *Alocasia macrorrhiza* were commonly found in the grassland and *M. tanarius* adjacent area, but *B. pilosa* was absent underneath *M. tanarius*. Density-dependent phytotoxicity analysis of lettuce growing in the soil mixed with the powder of *M. tanarius* fallen leaves showed one significant deviation from expected yield-density relationship. The result revealed that the lettuce plants were suppressed pronouncedly as the density of lettuce plants was low. The fact implies that the phytotoxins produced during the decomposition of the fallen leaves in soil certainly inhibited the growth of lettuce seedlings. Two kinds of weed seedlings, *B. pilosa*, and *Leucaena leucocephala*, were also significantly suppressed growing in soil mixed with the powder of *M. tanarius* fallen leaves. Furthermore, a series of aqueous extracts of fallen leaves were bioassayed against lettuce and *B. pilosa*, and the bioassay results exhibited a significant suppression of their radicle growth. Aqueous extracts of *M. tanarius* fallen leaves were partitioned with ethyl ether, and allelopathic compounds isolated were identified by means of column chromatography, TLC, HPLC, MS and NMR. Compounds identified from the fallen leaves included nymphaeol-A (1), quercetin (4), abscisic acid (ABA) (5), blumenol A (6), blumenol B (7), reseoside II (8), tanariflavanone A (9) and tanariflavanone B (10). Of them, ABA was the major growth inhibitor in the fallen leaves. At concentration below 20 ppm ABA remarkably suppressed the growth of lettuce, while at 40 ppm it inhibited significantly the growth of two kinds of weeds including *Miscanthus floridulus* and *B. pilosa*. The remaining seven compounds at a concentration of 200 ppm showed 10-30% inhibition on lettuce germination. The amount of ABA in *M. tanarius* fallen leaves was about 3000-5000 ng g<sup>-1</sup> dry weight, which was significantly higher than that of previous literature reported. The findings conclude that the invasion of *M. tanarius* into its adjacent grassland vegetation is due primarily to an allelopathic effect of its fallen leaves.

F4

## GENETIC CONTROL OF ALLELOPATHY IN RICE

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In 1996, a program at the International Rice Research Institute was started with the specific objective to locate the genes involved in allelopathy. For this purpose, hundreds of rice varieties were tested for their allelopathic potential using a bioassay called the relay seeding technique. On the basis of this screening, several varieties were chosen for their contrasted allelopathic behavior and recombinant inbred line populations were developed. Two populations were selected for further studies to reveal the genetic control of allelopathy in rice.

An already existing population initially, developed for the study of blast resistance, was chosen for the first mapping study. The recombinant inbred line population consisted of 142 recombinant inbred lines derived from a cross between cultivar IAC 165 (*japonica* upland variety) and cultivar CO 39 (*indica* irrigated variety). The relay seeding technique showed that IAC 165 was strong and consistent allelopathic against the test weed species *Echinochloa crus-galli* using the relay

seeding technique, whereas CO 39 was weakly allelopathic. QTL mapping was performed using the phenotyping results from the relay seeding technique.

To verify these initial results on the genetic control of allelopathy in rice and to assess how they are influenced by a change in genetic background, the study was extended to a new population. This population was developed specifically for the study of allelopathy with highly contrasting cultivars as the parents.

The population consisted of 150 recombinant inbred lines produced from a cross between the *indica* cultivar AC 1423 and the *aus* cultivar Aus 196. AC 1423 showed strong and consistent allelopathic activity against *E. crus-galli* using the relay seeding technique, whereas Aus 196 was non-allelopathic. Further phenotyping of the population was done in a greenhouse screening also using *E. crus-galli* as a test species. QTL mapping was performed on the phenotyping results from both the relay seeding technique and the greenhouse screening.

Comparison between the two mapped populations and discussion on the progress to reach our goal: to develop rice varieties with enhanced allelopathic potential, will be done.

**INSECT PHENOL OXIDASE INHIBITORS FROM PLANTS**

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Insect phenol oxidase (PO) (EC 1.14.18.1) is the key enzyme in the biochemical cascade of B-sclerotization, quinone tanning and melanin biosynthesis, which occupies a major role in insect development and immunity, such as cuticle sclerotization, wound healing, and defense against foreign pathogens, and thus provides a new target for pest insect control by inhibiting this key enzyme. So far, we have isolated and purified PO from blowfly (*Sacrophaga neobelliaris*) pupae by chromatography methods, and characterized its basic features, such as kinetics parameters, optimal assay conditions, pH and temperature effects on activity of the enzyme. A method for large scale isolation and purification of the enzyme for testing inhibitors against the enzyme has been established. A number of PO inhibitors have been isolated from plants. They show strong inhibitory activity against insect PO and are compatible with those against mushroom tyrosinase. The above results indicate that copper chelators can be used as potential lead inhibitors for future studies. We also tried to build a molecular model for insect PO, which will be helpful in explaining the interaction between PO and its inhibitors and the structure activity relationship (SAR) of those inhibitors.

Two common flavonols, quercetin and kaempferol, isolated from the dried flower of *Heteroteca inuloides* CASS (Compositae), a Mexican medicinal plant, are examples. Flavonols are copper chelators as long as the 3-hydroxyl group is free. The inhibition mechanism analyzed by Lineweaver-Burk plots shows that flavonols are competitive inhibitors. In the time course of the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) catalyzed by the enzyme in the presence of different quercetin concentration, the rate decreased with increasing time until a straight line was approached. The inhibition of tyrosinase by quercetin is a slow and reversible reaction with remaining fractional enzyme activity. The microscopic rate constants have been determined for the reaction of quercetin with the enzyme.

**OVIPOSITION AND HOST SHIFT IN DANAID BUTTERFLIES MEDIATED BY PLANT CHEMICALS**

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Most danaid butterflies are tropical or subtropical insects feeding on a limited range of plants belonging to Asclepiadaceae, Apocynaceae or Moraceae. For example, *Idea leuconoe*, a

primitive Old World danaid, specializes on an apocynaceous plant, *Parsonsia laevigata*, in many districts; *Ideopsis similis* and *I. Juventa* on *Tylophora* plants (Asclepiadaceae); *Parantica sita* and *P. aglea* on several plants in the genera *Tylophora*, *Cynanchum* and *Marsdenia* (Asclepiadaceae); and *Euploea mulciber* on plants belonging to Moraceae and Apocynaceae.

Recent progress in studies on chemical interactions between herbivorous insects and plants has shed light on phytochemical mediators involved in oviposition behavior of a number of lepidopterans. It is well established that ovipositing females of butterflies rely, to a large extent, on chemotactile stimuli evoked by plant secondary metabolites as cues to assess the suitability of potential hosts they land on.

We have previously reported that females of *I. leuconoe* lay eggs in response to peculiar macrocyclic pyrrolizidine alkaloids (PAs) specifically present in its host plant, *P. laevigata*. *I. similis* utilizes several phenanthroindolizidine alkaloids (PIAs) as crucial host-recognition chemicals present in *Tylophora* plants. A mixture of certain PIAs strongly stimulated oviposition by the butterfly. *I. juventa* was also induced to oviposit by these PIAs.

Investigations on oviposition stimulants for *P. sita* present in *M. tomentosa* revealed that conduritol F 2-*O*-glucoside was a key substance involved in stimulation of oviposition. However, its content in the plant was very small, and co-occurring conduritols A and F synergistically enhanced the stimulatory activity of conduritol F 2-*O*-glucoside. *P. sita* and *P. aglea* also lay eggs on *Tylophora*, *Cynanchum* and *Asclepias* plants. Some of these plants were found to contain other conduritol F glucoside isomers, and some, but not all, females of *P. sita* positively responded to PIAs. Taking these findings into consideration, a possible pathway of host shift of these danaid butterflies will be discussed.

G3

**IMPROVED SEX APPEAL, ANTIPREDATION AND POLLINATION - COMPELLING INCENTIVES IN THE *BACTROCERA* FRUIT FLY AND *BULBOPHYLLUM* ORCHID RELATIONSHIP.**

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Male fruit flies of many *Bactrocera* (pest and non-pest) species are attracted to certain plant attractants. They are broadly categorised into two groups, depending on their attraction to either methyl eugenol (ME) or raspberry ketone (RK), henceforth, referred to as ME-sensitive species (e.g. *Ba. carambolae*, *Ba. papayae*, and *Ba. umbrosa*) and RK-sensitive species (e.g. *Ba. albistrigata*, *Ba. caudata*, *Ba. cucurbitae*, and *Ba. tau*).

Male melon fly, *Ba. cucurbitae*, feeds on and sequesters RK which is subsequently released from its rectal gland as a component of its sex pheromone that doubles as an allomone against house lizard. The same sequence of behavior is observed when it is attracted to zingerone. However, *Ba. papayae* is attracted strongly to and feeds compulsively on ME, a sex pheromone

precursor, which is converted to other phenylpropanoids (ME analogues) in the crop. The ME analogues are then transported to the rectal gland and eventually released as sex pheromone. Furthermore, they are able to act as the fly's allomone in deterring vertebrate predator. ME-fed males are obviously more attractive to females and more successful in mating when compared with males of the same age group not exposed to ME. *Ba. papayae* males are attracted to and feed on zingerone which is converted to zingerol (attractive to conspecific females) for storage in and subsequently released from the rectal gland.

Many orchid species release floral scent as synomone to attract insect pollinators. Several species of *Bulbophyllum* (characterised by a moveable, hinged-see-saw labellum [lip] - a mechanism that helps topple an attracted fly into the floral column cavity during pollination), with non-resurpinate flowers, have evolved specifically to attract male fruit flies as pollinators. Flowers of *Bu. ecornutum* and *Bu. macranthum* release RK to attract RK-sensitive *Bactrocera* species. On the other hand, *Bu. cheiri* flowers secrete ME, as the major component, to attract the ME-sensitive species. Interestingly, flowers of *Bu. patens* attract both the RK- and ME-sensitive *Bactrocera* species by secreting zingerone (a relatively weak fruit fly attractant). The beneficial, evolutionary, and co-evolutionary aspects of the fruit fly-wild orchid relationship will be discussed.

G4

## MECHANISMS OF POLLINATOR ATTRACTION IN SEXUALLY DECEPTIVE ORCHIDS

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Flowers of sexually deceptive orchids mimic the sex pheromones of their insect pollinator species. Pollination occurs when the males attempt to copulate with the orchid flowers. Pollination is usually species specific and the floral odor is primarily responsible for attracting the pollinators. We investigated floral volatiles of orchids as well as the sex pheromones and mating behavior of their respective pollinator species in the Australian orchid genus *Chiloglottis*, which is pollinated by Thynnine wasps (Hymenoptera: Tiphidae), and the European genus *Ophrys*, pollinated by solitary bees (Hymenoptera: Apoidea). We examined semiochemicals and their detection by the pollinators using gas chromatography with electroantennographic detection (GC-EAD). Gas chromatography-mass spectrometry (GC-MS) was used to identify odor compounds and behavioral tests in the field to investigate reactions of males to odor samples and synthetic odor compounds. Behavioral tests with flowers of *Chiloglottis* species demonstrated that pollinators are attracted over long distances to labella as well as sepals of the flowers. In GC-EAD experiments, 1-3 peaks, found both in labella and sepals extracts, proved to be electrophysiologically active in the pollinators' olfactory neurons. Flowers of *C. trapeziformis* contained the same active compound as was found in the females' sex pheromone of the pollinator, *Neozeleboria cryptoides*. In *C. trilabra* and *C. reflexa* we found an overlap in

active compounds: both species emit two identical active compounds, but *C. reflexa* emits one additional, distinct compound, which could be responsible for the species-specific pollinator attraction. Since as few as one additional compound may lead to the attraction of a new and exclusive pollinator species, reproductive isolation driven by odor changes could be a rather quick process. In *Ophrys sphegodes*, as many as 14 alkanes and alkenes have been found to be electrophysiologically active and were shown to elicit copulation attempts in the pollinator bee species, *Andrena nigroaenea*. The same compounds also constitute the bees' sex pheromone. These compounds are low in volatility and act only over short distances. They are common constituents of plant and insect cuticular waxes, and only their specific pattern is thought to mediate behavioral activity in the male bees. This research is financially supported by the FWF Austria, the American Orchid Society, and the Australian Orchid Foundation.

G5

**CO-EVOLUTION OF PLANT AND INSECT: THE ORIENTAL ORCHID (*CYMBIDIUM FLORIBUNDUM* LINDL.) CONTROLS THE SOCIAL BEHAVIOR OF THE ASIAN HONEY BEES ?**

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The Japanese honeybee (*Apis cerana japonica* Rad) and the introduced European honeybee (*Apis mellifera* L.) share the same habitat in Japan. Very little is known about the biology of *A. c. japonica*. The red and white flower varieties of the oriental orchid, "Kinn-ryou henn" (*Cymbidium floribundum* Lindl.: Cf), has been observed to attract workers, drones, queens even the entire swarming colony of *A. c. japonica*. However, the orchid does not attract *A. mellifera*. This is due to the fact that the flower scent mimics the Nasonov and mandibular glands pheromones of *A. c. japonica*. Both Nasonov gland and mandibular gland extracts induced aggregation behavior in both bee species, but the GC profiles of the extract from *A. mellifera* and *A. c. japonica* were significantly different. It is interesting that a plant like *C. floribundum* can affect the social behavior of *A. c. japonica*. The experiments using other Asian honeybee such as *Apis cerana cerana* are underway.

We report here for the first time that: (1) The same species of Cf is found in China in 1999. (2) Flower aroma of Chinese Cf were analyzed by GC/MS and these results were compared between Japanese Cf and the Chinese one. (3) In search of keys to socialities and diversity in insects, the components of pheromone glands (Nasonov and mandibular gland) of honey bees in Asia (*A. cerana japonica*, *A. c. cerana*, *A. c.*, *Apis mellifera* etc.) were analyzed by GC/MS. In this paper, we summarize our recent findings on chemical ecology of the flower scent and semiochemicals of the Asian honey bees.

## MULTIPLE SEMIOCHEMICAL ROLES OF *neo*-CLERODANE DITERPENES IN PLANT-INSECT INTERACTIONS

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A number of *neo*-clerodane diterpenoids produced by the plant families Verbenaceae and Labiatae have been shown to act as potent antifeedants against various phytophagous insects. Clerodendrins (e.g. A and B) contained in *Clerodendron trichotomum* Thunb. (Verbenaceae) deter feeding of some lepidopterous larvae (e.g. *Spodoptera* species) and exhibit toxicity to other insect species (e.g. silkworm moth larvae). The bitter-tasting nature of these compounds seems primarily to play a role as a chemical barrier (**plant allomones**) against herbivores.

On the contrary, male and female adults of the turnip sawfly, *Athalia rosae ruficornis* (Jacovlev) (a specialist on the Brassicaceae, Tenthredinidae: Hymenoptera) frequently visit a shrub of *C. trichotomum* and strongly stimulated to feed by clerodendrins (e.g. B and D), imbued in the glandular trichomes, on the leaf surface (a **kairomonal role**). The adults fed selectively on the trichomes and sequestered a series of the compounds (clerodendrin D, ajugachin A, athaliadiol) in the body tissues, and would hence taste bitter to their predators. Such insects effectively deterred predatory lizards and birds, indicating an **allomonal role** of the acquired compounds from plants. The sawflies showed the same pharmacophagous affinity towards *Ajuga* plants (Labiatae) that also contain related *neo*-clerodane diterpenoids in the glandular trichomes. Females of *A. rosae ruficornis* fed either on *Clerodendron* or *Ajuga* leaves, or ingested intact clerodendrins B or D were subsequently more successful in mating than unfed females, suggesting a **sex-pheromonal role** of the sequestered compounds in the courtship. Thus, females seem to obtain an advantage in mating success through the acquisition of the defensive principles from these non-host plants.

The pharmacophagous association of the turnip sawflies with the specific diterpenes appeared to be parallel to that of danaine butterflies with pyrrolizidine alkaloids and *Bactrocera* fruit flies with methyl eugenol. In this association, the multiple semiochemical roles (as kairomones, allomones and sex/aggregation pheromones) of the plant secondary compounds are involved in their ecologically adaptive mechanisms. However, it should be noted that in the case of the turnip sawfly the males select females with good chemical defense, while in the latter two cases the females select males that have acquired a potent chemical defense. These examples illustrate a unique phenomenon of sexual and natural selection via pharmacophagy.

## A PATH TO RESISTANCE AS WELL AS BIOFUMIGATION?

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Isothiocyanates, released from damaged or degrading canola (*Brassica napus*) tissues, are toxic to a great range of soil borne organisms. The toxicity of soil amended with root tissue to the root lesion nematode (*Pratylenchus neglectus*) is closely linked to the root levels of a single isothiocyanate precursor, 2-phenylethyl (2-PE) glucosinolate. The higher the levels of 2-PE glucosinolate in a 0.75% root tissue amendment, the fewer live nematodes could be retrieved from amended soil after 72 hours incubation. Amendment with purified 2-PE isothiocyanate showed comparable results.

Despite the fact that canola roots, when damaged, are toxic to the root lesion nematode, the crop is observed to be susceptible to *P. neglectus* in the field. This is curious, as one would imagine that the damage caused by the nematode would result in significant isothiocyanate release. Studies were undertaken to more closely examine the relationship between root glucosinolates and the resistance of the crop to *P. neglectus*.

A high root level of 2-PE glucosinolate does appear to reduce plant susceptibility to *P. neglectus*. Over one quarter (27%) of the plants containing below the median 2-PE glucosinolate level were more susceptible than the control (Machete wheat), some supporting nematode populations up to four times greater. No plants containing above the median 2-PE glucosinolate level exhibited susceptibility greater than the control. Canola varieties whose roots uniformly produce above the critical level of 2-PE should exhibit reduced susceptibility, as the population is less likely to contain these highly susceptible individuals.

It is important to note that many plants containing low 2-PE levels did exhibit strong resistance to the nematode. Clearly, other mechanisms of nematode resistance (non-glucosinolate) existed within plants in the population. Work is currently underway to determine the nature and heritability of this variation.

Plentiful variation in root 2-PE levels was observed within current varieties, and breeding studies have uncovered a single gene (*Pe*) of major effect, the dominant allele providing high 2-PE levels. Pure breeding S4 populations for the 'High' (*Pe*) and 'Low' (*pe*) phenotypes have been selected from the Spring canola line, Dunkeld. In the glasshouse, such lines do show significant differences in their susceptibility to *P. neglectus*, and the toxicity of their root tissues as they degrade in the soil. These lines are currently under field evaluation within the Southern Australian cereal belt.



## BEHAVIORAL RESPONSES OF COTTON BOLLWORM AND THE ASIAN CORN BORER TO VOLATILES OF TRANSGENIC BT COTTON

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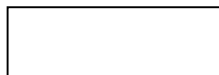
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Since 1996, Bt cotton has been planted in China on a large scale. The use of transgenic crops in China has been proven to reduce the cost, time and efforts; and it could reduce the use of insecticides in crops protection from lepidopteran insects. However, large scale plantation of transgenic crops in cotton production, has raised concerns over the safety and ecological impact of plant trials. In plant-insect interactions, plant chemical volatiles play crucial roles in mediating host searching and ovipositional behaviors in many insects; and change of plant volatiles in concentration and / or components might alter host finding and / or ovipositional behaviors and thus the biodiversity of insect pests and natural enemies in agroecosystems. We therefore investigated if there is any change in volatile profiles of transgenic cotton, and if so, if there is any impact of this change on behavior of some important pest and beneficial insects. We report here the behavioral responses of two important cotton insect pests, cotton bollworm, *Helioverpa armigera* (Hübner), and the Asian corn borer, *Ostrinia furncalis* (Gueneé), to volatiles of transgenic Bt cotton.

Volatiles from transgenic Bt cotton (GK-97) plants and its parental (regular) plants were collected and analyzed with GC-MS. GC-EAD and ovipositional experiments were conducted with cotton bollworms and the Asian corn borers. GC-MS analysis showed that there were basically no differences in volatile profiles between transgenic Bt and the regular plants. Cotton bollworm antennae responded to seven compounds ( $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene,  $\beta$ -ocimene, 4,8-dimethyl-1,3(E)7-nonatriene, (Z)3-hexenyl acetate and benzaldehyde). There were also two compounds not detected by FID, probably due to their extremely low concentrations, but were responsible for EAD peaks in volatiles from both transgenic Bt and regular cotton. No significant differences between egg numbers of either cotton bollworm or the Asian corn borer were found on transgenic Bt cotton and on regular cotton. The results indicated that exotic gene insertion into cotton plants does not alter the volatile composition of the plants and change the ovipositional behavior of the cotton bollworm and the Asian corn borer.



## POSTERS

G9

### CYTOTOXICITY EVALUATION OF THE HERBAL *PHYLLANTHUS* EXTRACTS USING BRINE SHRIMP LETHALITY BIOASSAY

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Methanolic extracts from six *Phyllanthus* species were screened for their toxicity using the Brine shrimp (*Artemia salina*) lethality bioassay. The results showed that all the extracts were relatively non-toxic to brine shrimps ( $LC_{50} > 1\text{mg/ml}$ ). *P. debilis* exhibited the highest  $LC_{50}$  with 1.56 mg/ml for acute toxicity. The lowest acute toxicity was shown by *P. pulcher* with  $LC_{50}$  more than 5.00mg/ml. However, *P. pulcher* showed a very high chronic toxicity value with  $LC_{50}$  1.65 mg/ml. The highest chronic toxicity with  $LC_{50}$  of 1.17mg/ml was shown by *P. acidus* although its acute toxicity was low compared to that of *P. debilis* and *P. emblica*.

G10

### OVIPOSITION STIMULANTS AND DETERRENTS CONTROLLING HOST PREFERENCE IN TWO RUTACEAE-FEEDING SWALLOWTAIL BUTTERFLIES

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A number of swallowtail butterfly species in the genus *Papilio* feed exclusively on the plant family Rutaceae. Their host selection within the Rutaceae is strongly controlled by specific secondary metabolites contained in their hosts. In this paper, the chemical bases of the host preference in two Rutaceae-feeding *Papilio* species toward a single rutaceous plant, *Orixa japonica*, are contrasted in the context of oviposition stimulants and deterrents.

(a) **Oviposition stimulant of *Papilio bianor*.** *P. bianor* Cramer utilizes *O. japonica* as one of the major hosts. Two oviposition stimulants of *P. bianor* were isolated from the water-soluble fraction of *O. japonica* extracts and identified as (-)-4-(*E*)-caffeoyl-L-threonic acid (**1**) and (-)-2-*C*-methyl-D-erythrono-1,4-lactone (**2**). Compound **1** was found to be active alone and there was no significant synergistic effect on other active fractions. On the other hand, compound **2** was inactive alone, but elicited oviposition behavior when mixed with other unidentified

components. These results indicate the intricate oviposition stimulant system of *P. bianor* consisting of multiple factors acting both synergistically and non-synergistically.

(b) **Oviposition deterrents of *P. xuthus*.** *P. xuthus* L. feeds on various rutaceous plants but always rejects *O. japonica*. Methanolic extracts of *O. japonica* strongly deterred oviposition of *P. xuthus*. Two deterrent compounds were isolated from the aqueous fraction and identified as 5-{{2-O-( $\beta$ -D-apiofuranosyl)- $\beta$ -D-glucopyranosyl]oxy}-2-hydroxybenzoic acid and a bisyringoyl ester of an aldaric acid (stereochemistry undetermined). These compounds deterred not only oviposition but also larval feeding, which appeared to represent the congruent rejection of chemicals in the unfavourable plant both at oviposition and larval feeding in this species.

Both oviposition stimulants and deterrents in the host and non-host plants are perceived by tarsal chemoreceptors on the forelegs of female butterflies. It is of great interest to know the chemical basis of host preference among these closely related species in association with their chemosensory mechanisms.

G11

## PROBING STIMULANTS OF *MEGOURA CRASSICAUDA* FROM THE NARROWLEAF VETCH ( *VICIA ANGUSTIFOLIA* )

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Feeding behavior of aphids is known to be regulated by host plant chemistry in the sequential steps by detecting (1) specific plant volatile or surface chemicals during approaching and at landing, (2) inter- or intracellular chemicals during the process of probing, and (3) nutrients and secondary chemical contents of phloem sap during settling and sucking. However, phytochemical nature affecting the aphid feeding has been studied only in a few cases. We report here the characterization of specific host plant chemicals responsible for stimulating probing behavior (step 2) in *Megoura crassicauda* Mordvilko.

*M. crassicauda* is a pest of broad bean (*Vicia faba*) and other bean plants especially in the genus *Vicia* (Fabaceae). When the aphids were fed artificially with an aqueous solution (defatted with hexane) of a crude extract of the narrowleaf vetch (*V. angustifolia*) through parafilm membrane (1 g leaf equivalent / ml), they positively ingested the solution and left numerous proteinous stylet sheaths detected by staining the film with basic fuchsin. The number of stylet sheaths was significantly greater than those formed using distilled water as control. Probing stimulant activity was thus determined by counting the number of the stylet sheaths remaining on the film.

The probing stimulants were found to be moderately soluble in water, and the main activity was recovered from a 60% methanol-water eluate when fractionated by C<sub>18</sub>-reverse phase column chromatography. Two major active compounds were isolated by successive high pressure liquid chromatography. Although each of them exhibited only weak activity when used alone (1 g leaf equivalent / ml), a mixture of the two compounds restored the original activity of the crude

extract. Alternatively, each compound independently exhibited significant activity at higher concentrations (e.g. 2 g leaf equivalent / ml). These results indicated that the probing stimulant activity elicited by these two components was additive rather than synergistic. Spectrometric analyses of the stimulants suggested that both were closely related *p*-coumaroyl esters of flavonoids. The structural elucidation and bioassay for actual behavioral / physiological roles of these compounds are in progress.

G12

**CHANGES OF VOLATILES FROM DROUGHT STRESSED ASH-LEAF MAPLE (*ACER NEGUNDO* LINN.) AND THEIR OLFACTORY RESPONSES TO ASIAN LONGHORNED BEETLE (ALB, *ANOPLOPHORA GLABRIPENNIS* MOTSCH.)**

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The volatile organic compounds emitted from 3-year-old ash-leaf maple seedlings (*Acer negundo* L.) under drought stress were identified and reported. The response of Asian longhorned beetle (ALB, *Anoplophora glabripennis* Motsch.) to the volatiles induced by drought stress was examined with GC-EAD, and with field bioassay using traps and synthetic compounds. Gas chromatographic (GC) analysis of volatiles with flame ionization detector (FID) and electroantennographic detector (EAD) revealed that six compounds elicited strong antennal responses. 2-Pentanol gave the strongest antennal response, followed by 1-pentanol, 1-hexenol, *cis*-3-hexen-1-ol, 2-butoxy-ethanol and 1-butanol. Trap catches of beetles were examined with various combinations of test chemicals in various places. Interestingly, only a few beetles were caught in traps baited with 1-butanol, 1-pentanol and 2-pentanol respectively. However, the attractiveness to ALB adults was greatly enhanced in field when all the 3 alcohols are mixed together.

Trees growing under water stress are damaged more seriously than unstressed trees by ALB. Our field trap test confirmed that volatiles from stressed ash-leaf maple could attract ALB adults. However, in this preliminary study we have only tested partial compounds induced by drought stress. Further field tests on other drought-stress induced compounds should be conducted further. Additionally, it is evident from these results that volatiles released from plants under water stress play a key role in ALB behavior, but volatile compounds from plants are also influenced by many other factors, e.g. light, temperature, nutrients and physical damage.

G13

## PHENOLIC CHANGES IN TREE LEAVES EXPOSED TO NATURAL AND SYNTHESIZED WOUND-INDUCED VOLATILES

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The resistance of plants towards insect and pathogen may involve cell wall strengthening and phytoalexin synthesis. Phenolics play a key role in these reactions. Phenolics encompass a wide range of structural classes and physiological functions. Previous studies have demonstrated that total phenolic contents in leaf extracts elevated when trees were damaged or exposed to wound-induced volatiles and phenylalanine ammonia-lyase (PAL) gene activated after wound. Few reports have specified the particular phenolic compounds. In this research, concentrations of ten phenolic compounds were monitored by high-pressure liquid chromatography (HPLC). Some of them are orthodihydroxy phenolic compounds and tanins which had been shown as strong inhibitors of exterior enzymes. Others are phenylpropanoids which were components of plant cell wall while others are key intermediates in the biosynthesis pathway.

Some phenolics increased after damage, others decreased or showed little changes. In this work, the concentrations of phenolics in healthy tree leaves had been assayed as controls. The wound samples were extracted from wounded leaves; the systemic samples were extracted from intact leaves on the wounded trees; the communication samples were extracted from leaves on healthy plants placed in the same air-tight enclosure with the wounded trees. The phenolic change pattern in wounded leaves was different from systemic leaves, which indicated that there were diverse signal cascades involved in their inducements. The wound-induced volatiles triggered phenolics increase in healthy trees, but had not reached the level as wounded leaves or systemic leaves did.

Two tree species were employed in this experiment. Hankow willow (*Salix matsudan*) is known to be more resistant to insect attack than Poplar opera 8277 (*Populus simonii* × *P. pyramibalis* c.v.). Consistent with their putative character, the concentrations of orthodihydroxy phenols were increased in Hankow willow, but not increased in Poplar opera 8277 when trees were wounded and exposed to wound-induced volatiles.

To determine which one of the wound-induced volatiles is responsible for this inter-tree communication, six wound-induced compounds detected in previous work had been bedewed at 0.4 μmol/L on a cotton-tipped dowel which was put in a air-tight enclosure near healthy plants but did not touch the leaves. Some of them can induce phenolic change but none of the synthetic compounds could mimic the bouquet of natural volatiles.

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## ALTERNATIVE DISPENSING TECHNOLOGIES FOR PHEROMONE MATING DISRUPTION OF CODLING MOTH IN POME FRUIT AND NUT CROPS

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Two dispensing technologies present promising alternatives to conventional hand-applied pheromone dispensers currently in wide usage in California, USA. Pheromone mating disruption of codling moth is currently being used on approximately 50% of the pome fruit orchards in the western United States north of Sacramento. While these programs have produced economically acceptable control of less than 1% damage on average, the relatively high cost and logistic difficulties associated with hand application has encouraged the development of alternative dispensing technologies. Two of these technologies include aerosol dispensers that emit high levels of pheromone from few point sources in the orchard as well microencapsulated sprayable formulations. The relative area of influence for the aerosol emitters was estimated indirectly using pheromone trap suppression as an indirect indicator. Uniform distributions of codling moth within an orchard were achieved by releases of sterile, marked codling moth within a uniform grid. Similarly, grids of recapture grids were established over the orchards. Indirect imaging of the areas of influence suggested that a single point source was capable of suppressing traps up to 600 meters downwind of the emitter, whereas lateral spread of 100-150 meters was also observed with downwind movement. Virtually no lateral spread was observed from emitters placed along the upwind edges of orchards. Using similar approaches, residual effects downwind from the emitters lasted for several days after the unit was no longer functioning. Fruit damage was evaluated in 2001 using a similar approach of mapping relative to the trap and release grids.

Impressive results for trap suppression were also observed for several formulations of sprayable microencapsulated pheromones for codling moth. Single applications were capable of suppressing traps for more than 60 days using products by 3M or Consep. Rate responses between trap suppression and application rate were extremely flat using replicated plots of approximately 1 ha per plot. The potential cost savings and ease of application may provide strong incentives to switch to these alternatives if damage suppression proves efficacious.

## ATTRACT AND KILL TECHNOLOGY: A “KNOCK-OUT” IN APPLICATION OF PHEROMONES FOR PEST MANAGEMENT

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IPM Technologies USA

Monitoring traps, mating disruption, and attract and kill baits are the principal methods that have been developed to exploit insect pheromone behaviours for pest management objectives. As with any management tool, the operational use of pheromones must be considered within the context of an integrated pest management system. The successful operational deployment of mating disruption tactics almost always requires judicious application of supplementary insecticides, as predicted by Pickett (1991) in that “pheromones alone will..... be insufficiently robust for the purposes of most agricultural production systems”.

A more recent approach, Attract and Kill (A&K), has been developed. This technique uses a semiochemical, such as the sex pheromone or a feeding attractant, in combination with an insecticide. Thus, when adults contact the lure they succumb to the toxic effect of the insecticide. A&K systems are more powerful than other semiochemical mediated control strategies such as mating disruption in that male moths are incapacitated and removed from the ecosystem. Dead males cannot mate. Yet, this approach has the obvious advantage of limiting any potential negative ecological effects of the insecticide, as only those insects coming to the lure will be affected.

Commercialization of the A&K approach has been undertaken by IPM Technologies Inc. who have global rights to a proprietary and patented matrix combining insecticide and attractant in a UV sensitive carrier material. Marketed as Sirene and Last-Call in Europe and the U.S., respectively, this technology was granted US EPA registration in 1998 and California registration in 1999. The robust matrix can accept, protect and release a wide variety of chemicals (acetates, alcohols, aldehydes) so it has the potential to be deployed against many pest species in diverse ecosystems.

Pheromone-based Last Call products have been commercialized against codling moth in apples and pears, potato tuber moth in solanaceous vegetables, false codling moth in citrus, litchii and other subtropical fruits, and pink bollworm and boll weevil in cotton. Experimental trials show promise against pine shoot borers (*Eucosma* and *Rhyacionia* in pine plantations), pecan nut casebearer in pecans, leafrollers in deciduous fruit and vines, loopers and fruitworms in vegetables, diamondback moth in crucifers and douglas fir cone gall midge in fir seed orchards.

## PRACTICAL USE OF PHEROMONE MATING DISRUPTION

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We have developed and sold pheromone dispensers for mating disruption over 17 years in many countries. This method has been highly successful in the field. However, in some cases, our collaborators have not been successful. Here, we will explain some successful cases and analyze the reasons for success. The analyses of these cases show us the important points in using this method. Mating disruption is useful and cost effective when farmers use pheromones as the main means of IPM.

The important points are as follows:

1. Choose pests that live in or covered by plant tissue during the larval stage  
For example, *Synanthedon Hector Butler*
2. Choose a good dispenser design  
Uniform release; long life
3. Apply a suitable quantity of active ingredient  
Sufficient concentration of pheromone in the air
4. Apply at an appropriate time  
Late application is a major reason for failure
5. Protection of natural enemies  
The main idea of using mating disruption in IPM
6. Treat large areas  
To avoid edge effects

The cost of pheromone dispensers is rather expensive when compared in isolation with the cost of pesticides used against a target pest. However, when using mating disruption in an IPM program, natural enemies are able to work effectively. This means that farmers can reduce the use of acaricides and insecticides which would be required for secondary pests which occur in the absence of natural enemies. As a result, the cost of the total plant protection program is cheaper when compared with the conventional system. This point is important in practical farming.



**INTRA- AND INTER-SPECIFIC ATTRACTION, MATING BEHAVIOUR AND  
HYBRIDIZATION BETWEEN *BACTROCERA CARAMBOLAE* AND *B. PAPAYAE*.**

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Fruit flies, *Bactrocera carambolae* and *B. papayae* are two economically important species infesting many commercially grown fruits in Malaysia, Indonesia and Thailand. The responses of *B. carambolae* and *B. papayae* females to conspecific and heterospecific males were studied in a wind tunnel during courtship period. During peak courtship period, *B. carambolae* female attraction to heterospecific male was not significantly different from conspecific male. However, *B. papayae* female demonstrated a higher attraction to conspecific than heterospecific males. These sibling species exhibited a similar sequence of courtship behaviour and possessed an overlapping courtship period. However, *B. papayae* male preceeded *B. carambolae* in sexual excitation at a higher light intensity and spent longer time in courting the females. When engaged in courtship, *B. papayae* male were observed to be more aggressive than *B. carambolae* males. Likewise, *B. papayae* female was also observed to be more responsive to male calling than *B. carambolae* female. Most of the intra- and inter-specific copulation lasted for more than 9 hours. Biological parameters, such as egg production, immature development and adult sex ratio of the intra- and inter-specific mating of *B. carambolae* and *B. papayae* were obtained and compared. While *B. papayae* matured *ca.* 2 weeks earlier than *B. carambolae*, sexual maturation rate of hybrids of the reciprocal crosses was between that of the parents. Morphology of the hybrids ranged from typical parental characteristics to intermediate characteristics between the two species. Similar results were also obtained for hybrid male and female genitalia length when compared with the parental species. Pheromonal analysis revealed that hybrid possesses a more complicated combination of pheromonal components compared with those of their parents.

## DETECTION OF AROMATIC COMPOUNDS FROM THE CELL SUSPENSION CULTURES AND THE *IN VITRO* PLANTLETS OF *CYMBOPOGON NARDUS*

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*Cymbopogon nardus* (serai wangi) could be mass propagated via *in vitro* culture method by culturing the axillary buds in proliferating medium consisting of Murashige and Skoog medium salts (MS) supplemented with 0.3 mg/L benzyladenine (BA) and 0.1 mg/L indole-3-butyric acid (IBA). An average of 4.7 shoots were produced from each axillary bud within four weeks. These *in vitro* plantlets were found to contain 33% citronellal, 47.7% geraniol and 19.1% citronellol as compared to the mother plants which contained 49% citronellal, 30% geraniol and 14.2% citronellol. The cell suspension cultures of *Cymbopogon nardus* were prepared by culturing 0.5 g callus tissues into 20 ml MS liquid medium containing 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and an increased wet weight of  $0.23 \pm 0.11$  g of cells were obtained after 15 days of culture. Geraniol and citronellol were the main aromatic compounds in the cell suspension cultures of *Cymbopogon nardus*.

## IMPROVED SEMIOCHEMICALS FOR FRUIT FLY DETECTION AND CONTROL

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Semiochemicals are the basis for most tephritid fruit fly detection and delimitation programs, and in some cases used in control and eradication of introduced exotic pest species. Most notable are the male attractants methyl eugenol and cue lure which are powerful lures for several *Bactrocera* species throughout South East Asia and the Pacific. Trimedlure, the attractant for Mediterranean fruit fly is less attractive on a relative scale than either of the lures for the *Bactrocera* species. Although the precise role that these semiochemicals play in the ecology of the species has not been fully elucidated, several new interesting discoveries have furthered our understanding of the possible role these chemicals play in intraspecific behaviors.

In collaboration with chemists at the USDA-ARS, Insects Affecting Insect Behavior Laboratory in Beltsville, MD, we have been evaluating two improved semiochemicals which appear to be more attractive than their predecessors. Minus B1 Ceralure is a stereospecific enantiomer of Ceralure, an iodo derivative of Trimedlure. This compound appears to be significantly more attractive than Trimedlure in field tests using released laboratory-reared as well as wild-type flies. An additional benefit is that attraction last longer than with Trimedlure and significantly

less material is needed to obtain an equivalent attractiveness. We have also tested a formate derivative of cue-lure which appears to be more active than cue-lure. The formate captures more male melon flies than cue-lure and is thus a good candidate for detection and possible control of melon fly. While synthesis of the formate appears to be achievable, the synthesis of the minus B1 Ceralure is difficult at this time. These semiochemicals may further improve detection of these species and could lead the way to their use in control programs.

H7

**ASSESSMENT OF USING SEX PHEROMONE FOR CONTROL OF THE  
CARAMBOLA FRUIT BORER, *EUCOSMA NOTANTHES* MEYRICK, AT  
CARAMBOLA ORCHARDS**

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The carambola fruit borer (CFB), *Eucosma notanthes* Meyrick, is the key pest attacking carambola fruits in Taiwan. Assessment of using sex pheromone for monitoring, mass trapping, and mating disruption of CFB was studied in the orchards. Monitoring tests were conducted at Yuan-lin and Cho-lan areas from 1990 to 1992. CFB occurrence in these two areas was similar, showing lower population density from April to June. The population density in Cho-lan was significantly lower than that in Yuan-lin. Mass trapping was conducted at Guo-xing, Nan-tou county from 1993 to 1997. CFB density declined to 1.0-4.5 insects/trap/week when 40-80 traps/ha were used. CFB population density was reduced by 75.4-89.4% compared to untreated orchards, and the fruit damage was only 1.9%. There was no difference in the effectiveness of mating disruptants formulated with Z8-12:Ac from different sources. In order to control CFB by mating disruption, a 5- hectare orchard at Cho-lan was treated with 53.28 g/ha of Z8-12:Ac disruptants for 5 months during 1996-97. Percent inhibition of male attraction reached 100%. The mating inhibition in the cage experiment and carambola orchards was 89.4 and 97.45%, respectively. The fruit damage was reduced by 71% as compared to conventional chemical control.

**TRAPPING AND MATING DISRUPTION OF THE DIAMONDBACK MOTH,  
*PLUTELLA XYLOSTELLA* (L.) USING SEX PHEROMONES**

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Studies were done to evaluate the trapping efficiency and the feasibility of mating disruption using sex pheromones of the diamondback moth (DBM), *Plutella xylostella* (L.). In the first study, the trapping efficiency of six commercially-available pheromone trap designs, viz, the Halex Trap I (wing type, white color); the Halex Trap II (wing type, yellow color); the BCS Trap I (cross-line wing type, white color); the BCS Trap II (delta type, white color); the IPS Trap (delta type, yellow color) and the Hoechst Trap (delta type, green color) were evaluated in cabbage fields during the dry and wet season at three different localities in Cameron Highlands. Each trap, hung about 60 cm above the ground, had a rubber septum-based lure impregnated with the Halex DBM sex pheromone blend. Weekly records of trapped DBM and parasitoids were made and the lure and the sticky bottom component of the trap were replaced as necessary. In the second study, four commercial pheromone lures were evaluated for their effectiveness to attract DBM in cabbage fields during the dry and wet season in Kea Farm, Cameron Highlands. The procedures to set up the traps and data collection were as described previously. In the third study, the mating disruption of DBM using sex pheromones was conducted in two commercial cabbage fields of 0.3 ha each. One of the fields was treated with the Konaga-Con type rope dispenser which contained the DBM sex pheromone at a concentration of 25g a.i. per 100m. Three hundred and fifty meters of the rope dispenser was suspended 50cm above the ground, over the cabbage canopy at 10m intervals to cover the whole area. The male DBM adults trapped were recorded weekly from five pheromone traps placed in the treated and untreated fields. In addition, weekly counts of DBM larvae and pupae on 120 cabbage plants were made.

In the first study, the number of DBM caught in all traps at all the locations was higher in the dry season than those in the wet season. This suggested that rainfall could be one of the major mortality factor for DBM. In the drier months, all the traps were efficient in capturing male DBM adults. In the wet months, when populations were lower, the Halex I, Halex II, IPS and BCS I trapped higher numbers of male DBM. It was interesting to note that all the traps also attracted *Diadegma semiclausum*, and that the two yellow traps i.e. Halex II and IPS significantly trapped higher numbers of *D. semiclausum* than the white traps. In the second study, the mean number of DBM captured per trap in the dry season was also higher than in the wet season and catches in the former decreased as follows: Hoechst (361.88) > BCS (351.75) > Halex (169.87) and IPS (16.12). In the wet season, the values were: Hoechst (55.38) > BCS (36.50) > Halex (21.75) and IPS (7.50). This showed that the Hoechst and BCS pheromones were relatively efficient in attracting male DBM. In mating disruption studies, the number of DBM adults was greatly reduced in the treated cabbage field as compared to the untreated 'check' field. At the termination of the study i.e. 45 days after mating disruption treatment, only 4.54 DBM adult males were recorded in the pheromone trap in the treated field compared to 92.61 DBM adults in the untreated field. There was also a 76 per cent reduction in the combined

larvae and pupae populations in the treated field. The study suggested that the mating disruption technique could be used as a tool in the integrated pest management program of DBM.

H9

**SEX PHEROMONE COMPONENTS OF THE JAPANESE PERSIMMON MOTH,  
STATHMOPODA MASINISSA  
(LEPIDOPTERA: STATHMOPODIDAE).**

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The Japanese persimmon moth, *Stathmopoda masinissa*, is one of the pests which damages Japanese persimmon fruits, and its sex pheromone has not yet been identified. We will report here a part of the identification of sex pheromone of this species.

One to four-day-old virgin females show a calling posture about 8-11h after the beginning of the scotophase. Males show a mating behavior during the corresponding time. We extracted sex pheromone gland extract about 9 after the beginning of the scotophase from 1 to 4-day-old virgin females showing a calling posture upon the extraction.

The pheromone gland extract prepared from 1,500 virgin females showed three distinct EAG-active components (A, B and C) in the GC-EAD analysis. GC-MS data indicated that component A is a C16 aldehyde with two conjugated double bonds and components B and C are its acetate and alcohol derivatives.

The GC-MS data of component A indicated an unusual base peak at  $m/z$  84, so we estimated the double bond position of the component A and synthesized the corresponding compounds with the 4,6-diene function.

The GC-MS data of the synthetic standards with an E4, Z6 configuration matched well with those of the three natural pheromone components. We will synthesize other isomers and evaluate the attractiveness of each synthetics to the wild male moths.

**SEX PHEROMONE COMPOSITION OF THE SUMMER FRUIT TORTRIX,  
*ADOXOPHYES ORANA* IN KOREA**

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The summer fruit tortrix, *Adoxophyes orana* has been claimed as the major leafroller on apple trees in Korea. Most *A. orana* mated before lights-on under the photoperiod of 16L:8D and the concentration of sex pheromone components peaked at the mating period although the major components were detected throughout the day. GC-MS and GC-EAD analysis of its sex pheromone gland extracts revealed the two main known compounds, (Z)-9-tetradecenyl acetate (Z9-14:Ac) and (Z)-11-tetradecenyl acetate (Z11-14:Ac). In EAG assay, Z11-14:Ac and Z9-14:Ac elicited significant antennal responses in male moths, but no other compounds, which have been previously reported for species of *Adoxophyes*, did. GC analysis showed that *A. orana* sex pheromone is composed of Z11-14:Ac and Z9-14:Ac with the ratio of 95:5. In field trapping tests conducted at apple and peach orchards in Korea, *A. orana* males were attracted mainly to 95:5 blend, before the dawn.

**POSTERS**

***IN VITRO* PROPAGATION OF *SPILANTHES ACMELLA*, A BIO-INSECTICIDE HERB**

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*Spilanthes acmella* was found to contain chemical compounds with insecticidal properties. These plants were successfully micropropagated using axillary buds as explants. Multiple shoots were formed within five weeks when the aseptic axillary buds were cultured in Murashige and Skoog medium (MS) supplemented with 2.0 mg/L N<sup>6</sup>-benzyl adenine (BA). The addition of indole-3-butyric acid (2.0 mg/L) had no significant effect on the multiple shoots formation of these plants. This study indicated that a MS medium supplemented with 0.5 mg/L BA was sufficient for the production of rooted multiple shoots of *Spilanthes acmella*. Repeated subculturing of the *in vitro* individual shoot in the same medium increased the formation of multiple shoots by three fold.

**DETECTION OF JUVENILE HORMONE III FROM *IN VITRO* *CYPERUS AROMATICUS***

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An *in vitro* culture system was developed for *Cyperus aromaticus* using buds from the rhizomes as explants. The main problem encountered in the *in vitro* culture of *Cyperus aromaticus* was contamination of cultures mainly by fungus and bacteria. Aseptic explants could be obtained by surface sterilization with solution contain 1.0 g/l mercuric chloride plus 300 mg/l sodium dichloroisocyanurate. To maintain an aseptic culture, the buds were cultured in solid Murashige and Skoog (MS) medium supplemented with 1.0 g/l potassium permanganate and 100 mg/l kanamycin for one week before transferring to the proliferate medium. MS medium supplemented with 0.5 mg/l indole-3-butyric acid (IBA) and 1.0 mg/l 6-benzyladenine (BA) was found to be the best medium for the production of multiple shoots. Rooting of the *in vitro* shoots was induced in basic MS medium. Detection of Juvenile hormone III from the *in vitro* plantlets was compared with that obtained from its natural habitat.

**IMPROVED NEW MALE ATTRACTANT FOR MELON FLY**

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There is always a need for improvements of attractants used in monitoring and control programs of fruit flies. The current male attractant for melon fly is Cue-lure, an acetate derivative of raspberry ketone. Because of the limitations of Cue-lure as a result of its low volatility, some research has been conducted on other analogs of raspberry ketone. This research reports the results of a compound, which was shown to be 1.5 to 2 times more attractive to male melon flies than Cue-lure. The development of an improved melon fly attractant will provide a better tool for control of melon fly populations.

## USE OF PHEROMONE MATING DISRUPTION AS A TACTIC FOR INSECTICIDE RESISTANCE MANAGEMENT IN PEAR AND APPLE

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The evolution of insecticide resistance in pests of deciduous tree fruits, apple and pear specifically, has long confounded the development of stable integrated pest management programs. Resistance has occurred in codling moth, the key pest of apple and pear, to most classes of insecticides used for its control. Additionally, insecticide resistance has occurred in tortricid leafrollers, also important economic pests of pome fruits. Resistance has occurred in these pests not only to the older insecticide classes, such as organophosphates and pyrethroids, but also to new insecticides with novel modes of action. These newer insecticides include growth regulators with varied activities such as chitinase inhibition, juvenile hormone analogs, and ecdysone agonism.

The development of mating disruption for codling moth and leafrollers has provided a new tactic for resistance management in the western US. A project implementing large-scale mating disruption for codling moth was initiated in 1993 in California pear. This project examined both the feasibility of mating disruption as an IPM tactic, and resistance management for organophosphate resistant populations of codling moth. The Codling Moth Areawide Management Project, funded by USDA-ARS, implemented mating disruption in Washington, Oregon, and California in 1995. These pilot projects were successful in leading to widespread implementation of mating disruption, such that by 2000 more than half of the apple and pear acreage in Washington were using this tactic.

Widespread implementation of codling moth mating disruption allows for unique resistance management. Along with overall reduction in codling moth densities, organophosphate resistance has decreased significantly. Reduced pest pressure has permitted use of chemical control tactics such as IGR's that are more environmentally-benign but relatively less effective than organophosphates. Reduction in broad-spectrum insecticide use has also provided more opportunities for biological control.

Mating disruption for leafrollers is currently under development. One large-scale implementation project, initiated in 1997, has been successful in apple. As with codling moth, leafroller mating disruption provides unique opportunities for developing long-term, stable IPM systems. Synthesis of mating disruption for multiple pests, and integration into resistance management and IPM systems, provides challenges and opportunities in the future.



**CONTROLLING TREE FRUIT PESTS WITH LAST CALL:  
INNOVATIVE ATTRACT AND KILL TECHNOLOGY.**

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IPM Technologies USA and Insect Science South Africa

Last Call is a novel pheromone/insecticide bait matrix that exploits the attract & kill concept to interrupt the life cycle at the adult moth stage. The product is applied in drops which imitate a female moth based on an equivalent release of sex pheromone in purity and dose. Attracted to this female decoy, the male tries to mate with Last Call and inevitably comes into contact with a lethal dose of insecticide. Following 10 years of development against codling moth (CM) in Switzerland and South Africa, and US EPA registration in 1998, product evaluation trials were established in commercial apple and pear orchards in 1999 in California, Oregon, Washington and British Columbia. This ready to use, highly viscous slow release formulation of Last Call CM, containing 0.16% pheromone (EE-8,10-12OH) and 6% permethrin, is applied to wood in the tree canopy at 1200 droplets/acre (3000/ha), with a uniform spacing throughout the treated orchard. Fields are retreated at 5 weeks.

In the 1999 trials, the cumulative total codling moth trap catches were considerably reduced in Last Call CM treated plots compared with conventional plots (707 males in 132 traps in Last Call vs 4466 males in 87 traps in conventional); and almost equally suppressed in comparison to disruption plots. Fruit damage was reduced in Last Call CM treated plots compared with both conventional and mating disruption plots (0.35% average in Last Call vs 1.75% in conventional plots).

Other trials show considerable promise for multi-species formulations targeting codling moth and leafrollers in a combination product, and for other tree fruit species including false codling moth, California red scale and Mediterranean fruit fly on citrus.

Last Call CM successfully controlled codling moth under diverse climates and geographic conditions (California to British Columbia), in orchards with high population pressures, uneven slopes and canopies, small size and irregular shape. The waterproof grease is effective for 5-7 weeks under overhead irrigation systems. Isolation is of minor importance as long as neighboring orchards are conventionally treated. The Last Call matrix has considerable utility for development of selective, effective and residue-free tools targeting tree fruit.

**LAST CALL ATTRACT AND KILL TECHNOLOGY  
SHOWS PROMISE IN VEGETABLE PEST MANAGEMENT.**

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Last Call, a novel pheromone/kairomone + insecticide gel, offers considerable utility in management of insect pests, using the attract & kill concept to interrupt the life cycle at the adult moth stage. The product is applied in drops imitating either a female moth (sex pheromone as bait) based on an equivalent release of sex pheromone in purity and dose, or the host plant (host volatiles as bait). Attracted to the bait, the adult insect inevitably comes into contact with a lethal dose of insecticide. Pheromone- and kairomone-based formulations were tested against vegetable pests in Florida, South Africa, Canada and Australia.

Commercial efficacy was proven in management of potato tuber moth on tomato in Southern Africa with a ready to use, highly viscous slow release formulation containing 0.16% pheromone (PTM I & PTM II) and 6% permethrin, dispensed at 100g/ha or 200g/ha onto the lower half of the plant as precise 50 ul droplets, for a total of 2000 or 4000 evenly spaced droplets/ha. Retreatment interval was 28 days. Moth flight was measured with traps and damage was measured by sampling leaves and fruit within Last Call treated and insecticide comparison plots. Pheromone trap capture was most suppressed, and fruit protection was highest in plots treated with the higher dose rate of 4000 droplets/ha or 200g/ha. Beneficials were not affected by the Last Call treatments. Compared with the conventional insecticide program, Last Call PTM was a superior control tactic.

Other species: Soybean and Cabbage Looper male and female moths were attracted to floral volatiles formulated into Last Call. Beet armyworm trap captures were reduced up to 100% over 19 days in small plots treated at 1450 droplets/acre. Diamondback Moth: dead moths were easily found on leaves in cabbage plots treated with Last Call formulated with pheromone. Male and female *H. armigera* moths were attracted to kairomone-baited Last Call droplets and a sex pheromone baited formulation was almost as attractive to male moths as standard laminate lures.

This work, as well as recent research success with Colorado Potato Beetle, and *Ceratitis* fruit fly formulations, indicates that attracticidal tactics will be important components of future pest management programs.

## THE EFFECT OF BLEACHING ON METABOLITE CHEMISTRY IN SOFT CORALS

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Biochemical changes in soft corals (*Lobophytum compactum* and *Sinularia flexibilis*) which had been affected by bleaching were investigated in an attempt to understand how molecular changes are linked to biological responses observed in the wake of bleaching events.

Variation in the levels of the photo-protective mycosporine-like amino acids (MAAs) in soft corals provides evidence that MAA tissue concentrations are positively correlated with seasonal cycles in solar irradiance and sea-surface temperatures. The role of MAAs as photoprotectants is corroborated by observations that MAA concentrations peaked in summer when exposure to solar radiation and sea temperatures are greatest, and in female colonies immediately prior to spawning.

High water temperatures and increased radiance were synergistic in causing the bleaching process. Corals initially respond to bleaching with short-lived changes in the levels and/or composition of their terpenoid secondary metabolites, possibly as a strategy to prevent fouling by opportunistic microorganisms.

Although (surviving) corals from a bleaching event recovered their zooxanthellae levels within 4 months, protein, lipid, MAA, and carotenoid levels were reduced in coral tissues for at least 8 months. This had a pronounced effect on reproductive output, with both polyp fecundity and mean egg diameter being directly correlated with severity of bleaching.

To demonstrate the role of zooxanthellae in production of coral terpenoid defenses, asymbiotic larvae were reared and infected with zooxanthellae which had been cultured from disparate marine sources. Symbiotic associations were established for more than half of the treatment groups, but growth rates of polyps were dependent on the source of the zooxanthellae strain. Polyps from all treatment groups which established symbiosis exhibited the terpenoid chemistry of the parents (detected by mass spectrometry), rather than that of the host from which the zooxanthellae had been isolated. Asymbiotic polyps also exhibited the terpenoid chemistry of the parents, confirming that zooxanthellae are not responsible for terpene production. The concentration of terpenoids was however dependent on the success of the symbiotic association, being below detection levels for 25-50% of individuals in some treatment groups. It appears that successful symbiosis is essential before the polyp can expend the energy required to maintain high levels of terpenoid chemical defenses.

## UNSTABLE AND MASKED METABOLITES OF MARINE ORGANISMS: THEIR ECOLOGICAL IMPLICATION

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Secondary metabolites having toxic, feeding-deterrent, or antimicrobial properties can be regarded as defensive substances. In some cases metabolites are not apparently toxic or feeding-deterrent as they are, but they may be easily converted to active products by enzymatic action or exposure to an atmosphere (light, oxygen, and/or moisture). A typical example is a cyanogenic glycoside such as amygdalin known from seeds of the plant family Rosaceae, which liberates hydrogen cyanide by the action of a glycosidase. Such compound is a masked toxin in which a toxic component is occluded by covalent bond in a molecule.

Polygodial, a sesquiterpene possessing 1,4-dialdehyde function, is a potent antifeedant discovered first from a terrestrial plant and later from a marine mollusk, a nudibranch. In general, compounds containing 1,4-dialdehyde functionality have feeding-deterrent properties. A number of compounds having a 1,4-diacetoxybutadiene (bis-enol acetate), a protected form of the 1,4-dialdehyde, have been reported from green algal families Caulerpaceae and Udoteaceae. An enol acetate can be easily hydrolyzed to form an aldehyde. The algal metabolites are feeding-deterrent and toxic to fish.

A major constituent of the red alga *Portieria hornemanni* collected in Okinawa was 2-(1-chloro-2-hydroxyethyl)-4,4-dimethylcyclohexa-2,5-dione. This highly unstable compound decomposes spontaneously to 4,5-dimethylbenzofuran and liberates hydrogen chloride (HCl). It could be considered as a reservoir of the strong acid HCl. When a predator attempts to graze the alga, the compound could instantly be decomposed to generate HCl and the cytotoxic dimethylbenzofuran in the mouth of the predator, discouraging its further grazing. There are a number of unstable or masked marine metabolites having ecological implication. Additional examples will be presented.

**LARVAL SETTLEMENT AND METAMORPHOSIS OF *HYDROIDES ELEGANS* IN  
RESPONSE TO CHEMICAL CUES: A MODEL SYSTEM FOR STUDYING CHEMICAL  
ECOLOGY OF MARINE INVERTEBRATE LARVAE**

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*Hydroides elegans* is a gregarious tube-building marine tubeworm that occurs as major marine biofouling organism in tropical and subtropical waters. This species often jams seawater pipelines, fish cages and nets, and colonizes on ship hulls. Larval settlement and metamorphosis of *H. elegans* have been studied extensively by my group and others in the world with the aim of finding effective ways to control the colonization of this species. It is clear that larval settlement and metamorphosis require chemical cues which can be originated from conspecific adults, sympatric organisms, and biofilms. With aid of rigorous bioassay-guided isolation and purification procedures, we have identified the potential inhibitors and inducers from marine organisms. More importantly, our study elucidates a very complicate relationship among chemical cues, diatoms and bacteria in biofilms, and the larval settlement and metamorphosis. Chemical cues can either directly act on larval receptor system (s) or modify bacterial community structure of biofilm which subsequently release the cues to trigger the chemical receptors of larvae leading to settlement and metamorphosis. For some bacterial species, the settlement induction activity is associated with the bacterial cell surfaces. Exopolymers from selected bacterial strains can induce incomplete metamorphosis while highly volatile compounds accumulated by the sponge-like property of exopolymers may contribute to the completion of settlement and metamorphosis. The case of *H. elegans* thus, makes a model system that well illustrates the complexity of chemical signaling involved in larval settlement and metamorphosis in marine invertebrates.

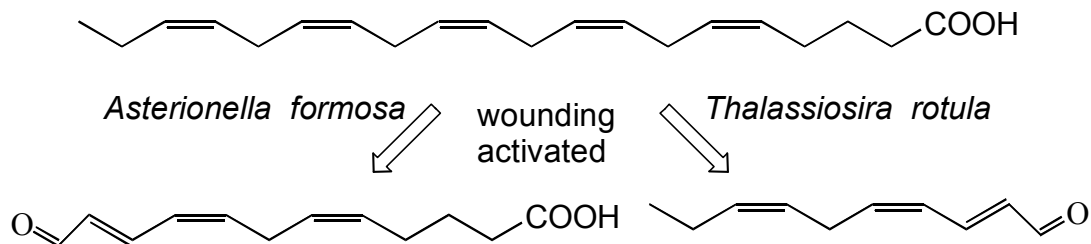
**WOUND-ACTIVATED CHEMICAL DEFENSE IN PHYTOPLANKTON**

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Diatoms are highly successful unicellular algae occurring in ocean and fresh water phytoplankton, as well as in biofilms on solid substrates. They are exceedingly abundant and among the most important primary sources sustaining the marine food chain. Despite this, little is known about their chemical defence. Two of the few reported defensive secondary metabolites are the aldehydes decadienal and decatrienal from the diatom *Thalassiosira rotula*, which reduce the hatching success of grazing copepods. This observed activity explains the

paradox that herbivorous copepods are less successful feeding on diatoms, although they are considered as high quality food.



Surprisingly, conjugated unsaturated aldehydes are not produced from C18-fatty acids as was shown for higher plants. The algae exclusively rely on eicosanoids (C20-fatty acids) as direct precursors for their defensive principles. Detailed mechanistic investigation of the lipoxygenase-mediated biosynthesis revealed that not only the reported volatile aldehydes are produced as chemical defence. Moreover also the widespread release of hydrocarbons is indicative of the production of highly reactive aldehydes. Thus, biosynthesis of C8- and C11-hydrocarbons is correlated with the production of aggressive oxygenated shorter chain fatty acids.

The biochemical mechanism to produce these  $\alpha,\beta,\gamma,\delta$ -unsaturated dienals is efficiently activated seconds after cell disruption and leads to high local concentrations of defensive metabolites only on demand. The strategy of rapid transformation of eicosanoic fatty acids after cell damage allows certain diatoms to build up a remarkably high local concentration of the defensive metabolites. In the diatoms investigated, cellular resources are invested in the production of fatty acids. These are activated only upon demand for chemical defence. The production of costly constitutive defensive secondary metabolites can thus be minimised and the risk of self-toxicity is simultaneously reduced.

## ANTIFUNGAL PROPERTIES OF SINGAPORE GORGONIANS: A PRELIMINARY STUDY

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Gorgonians possess a huge array of secondary metabolites for various functions, many of which are not known. One of these functions is antifungal. This study investigates if gorgonians from reefs in Singapore can defend themselves against the settlement and invasion of fungi. Crude extracts from 10 species of gorgonians from 3 families, Ellisellidae, Subergorgiidae and Plexauridae were screened against 9 species of fungi previously isolated from gorgonians.

Minimum Inhibitory Concentration (MIC) experiments were carried out in 96-microwell plates using extract concentrations ranging from 1.5mg/ml to 24.0mg/ml. It was found that extract from *Euplexaura cf pinnata* inhibits the growth of *Gliomastix cerealis* and *Acremonium furcatum*. Extract from *Echinogorgia* sp. C inhibits the growth of *Aspergillus foetidus* var. *pallidus* and *Trichoderma pseudokoningii*. *Echinogorgia* sp. A extract inhibits the growth of *Cladosporium sphaerospermum* and *Penicillium citrinum*. Preliminary screening showed that most of the antifungal activities were exhibited by gorgonians from the family Plexauridae. MICs range from 12.0 mg/ml to 24.0 mg/ml. However, these concentrations required to inhibit fungal growth are much higher than the natural concentrations of extracts found in gorgonian tissues.

**THE ABUNDANCE OF BIOACTIVE SPONGES IN THE SPERMONDE  
ARCHIPELAGO.**

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Sessile marine invertebrates are sources for a wide variety of substances with bioactive properties. Many of these substances have a potential as pharmaceuticals or as biochemical tools for a variety of useful applications. Of all marine invertebrate taxa, sponges are doubtless the most diverse in both numbers and types of compounds. It is thought that sponge bioactivity is enhanced in tropical environments, such as coral reefs. It is predicted that the species-rich Indonesian reefs harbour the highest diversity in sponge chemical compounds. As the supply-matter is a major problem, a growing interest for alternative ways to obtain these compounds is rising. In the near future, an attempt will be made to set up a sponge mariculture in the Spermonde Archipelago, SW Sulawesi, which is presently one of the best marine explored regions of Indonesia. Presently, a descriptive-correlative inventory is made of sponge bioactivity and the factors that are hypothesised to act as important regulators. These results will eventually contribute in choosing a habitat where sponge growth and production of the bioactive substances is optimal.

**INDUCTION OF LARVAL METAMORPHOSIS OF THE RED SEA URCHIN,  
*PSEUDOCENTROTUS DEPRESSUS*, BY THE MICROBIAL FILMS**

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Among echinoderms, the microbial film is known to induce larval metamorphosis of sand dollar, starfish, sea cucumber and sea urchin. In fisheries of Japan, periphytic diatoms film are cultured on a corrugated plate ('nami-ita') for the induction of larval metamorphosis in the mass production of red sea urchin juveniles. This study aimed to verify the role of periphytic diatoms and bacteria of the microbial film in larval metamorphosis of red sea urchin. We had analysed the inducing activity of (1) natural microbial film in the sea, (2) diatom-based film in the 'nami-ita' tank and (3) laboratory cultured diatom-based film. The characteristic of the chemical cues for larval metamorphosis was investigated using the laboratory cultured diatom-based film.

Formation of natural film was done by immersing slide glasses in the sea (1-25 days) from autumn to winter. Simultaneously, slide glasses were also immersed in the 'nami-ita' tank for the formation of diatom-based film. For the larval assay, one piece of filmed slide glass was put inside a 200-ml beaker with 100-ml fresh sea water, and 30 competent larvae were introduced for 24 hours. Laboratory cultured diatom-based film was prepared by propagating 'nami-ita' diatom film into a 200-ml beaker for 5 days. The density of diatom and bacteria was determined before each assay. The characteristic of the chemical cues was investigated by treating the cultured films with heat, EtOH and HCl.

In natural film, the metamorphosis percentage of sea urchin increased gradually until 25-day-old films. The relationship between the diatom density of natural film was difficult to determine due to the accumulation of detritus and sessile organisms. Only in winter, periphytic diatoms dominated the natural film. In the diatom-based film, the metamorphosis percentage increased linearly as the diatom density increased from  $10^4$ - $10^7$  cells/cm<sup>2</sup>. For the cultured film, the mean metamorphosis was 90%, and this finding proved that it is possible to conduct larval metamorphosis of sea urchin by microbial film under laboratory condition. The results of the heat and chemical treatment showed that the chemical cues seem to be an unstable substance which was destroyed by heat at 40<sup>0</sup>C, 10% EtOH and 0.1N HCl.



**INDUCTION OF LARVAL ATTACHMENT AND METAMORPHOSIS IN *HYDROIDES ELEGANS* BY MARINE BACTERIAL METABOLITES**

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The life cycle of most benthic marine invertebrates includes a dispersed pelagic larval stage. The commencement of the benthic life is preceded by larval attachment and metamorphosis (in the following referred to as settlement) into sedentary juveniles. Larval attachment to marine substrata is a non-random process, which is guided by physical, biological and chemical cues.

Marine natural biofilms - a complex consortium of bacteria, diatoms, fungi and adsorbed organic matter - play important regulatory roles in the substratum selection and the settlement induction of marine invertebrate larvae. So far, a variety of marine bacterial strains have been identified to either inhibit or induce larval settlement in a broad range of invertebrate taxa. Examples for the elucidation of the interaction of bacterial chemical signaling and larval behavior are rare.

Here, we present recent results of our endeavor to study larval settlement of the marine polychaete *Hydroides elegans*. Larval attachment and metamorphosis of this species is effectively mediated by natural marine biofilms. A variety of bacteria have been isolated from such films and investigated for their efficacy to induce larval settlement. The biological activity of these strains is clearly associated with their cell surface, a matrix of exopolymeric compounds dominated by polysaccharides and proteins. However, the larval settlement response is not induced by these exopolymers. Crude samples containing a variety of bacterial metabolites were obtained from bacterial suspension cultures by liquid-liquid extraction and partly characterized by GC-MS. A novel bioassay was developed to demonstrate the effect of nonpolar, low-molecular weight compounds as semiochemicals for larval settlement induction in *H. elegans*. Adsorbed on thin carbon wafers, these samples evoke a similar larval settlement induction effect as natural biofilms.

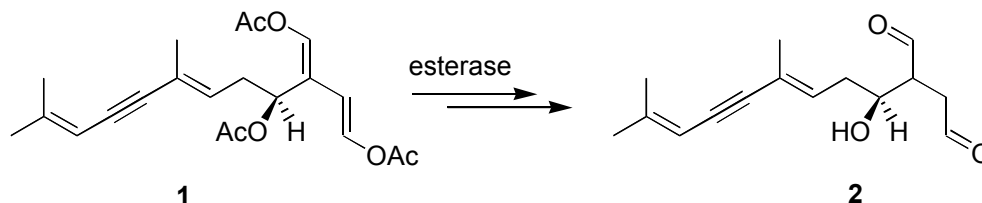
**Rapid wound-activated transformation of the green algal defensive metabolite caulerpenyne**

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Since its accidental introduction into the Mediterranean Sea in 1984, the tropical green macro-alga *Caulerpa taxifolia* has spread tremendously, covering more than 46 km<sup>2</sup> by the end of 1997. As a bioinvasive species it represents a threat to temperate marine ecosystems. Its success in conquering new habitats is based on high temperature and substrate tolerance, and on fast growth, which enables it to overgrow previously ecologically diverse parts of the sea. The most important key to its success, however is its efficient chemical defence towards potential predators such as sea urchins. It is believed that it is almost exclusively based on the acetylenic sesquiterpene caulerpenyne (**1**) that can account for up to 1.3 % of the algal fresh weight.

We show that this invasive green macro-alga reacts upon wounding with rapid transformation of its main defensive metabolite caulerpenyne (**1**). The three acetate groups of **1** are enzymatically cleaved within minutes after mechanical damage.



Transformation of the 1,4-bis-enol acetate moiety of caulerpenyne results into labile 1,4-dialdehydes after tautomerisation. Normal phase HPLC/APCI-MS measurements of freshly prepared algal extracts allow characterization of these reactive products, which are of the oxytoxin family. Final structural elucidation was achieved after trapping of the aldehydes with 2,4-dinitrophenylhydrazine. The role of wound-induced transformation in activated chemical defence of *C. taxifolia* is suggested.

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**SEX PHEROMONE FOR MONITORING THE PINE SAWFLY, *DIPRION JINGYUANENSIS* XIAO ET ZHANG (*HYMENOPTERA: DIPRIONIDAE*)**

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Sex pheromone is an important and useful tool for monitoring insect pests. The sex pheromone of *Diprion jingyuanensis* was identified and its artificial components were proven to be reliable in the field trap bioassay. In this study, synthesized pheromone was used to monitor the flight peak and the population density of the species. Between the two dosages, 400µg/trap and 800µg/trap tested so far, the higher dosage demonstrated clearer flight peak although the flight patterns at the two dosages were the same. In a day, the response time of male to the synthesized pheromonal compounds ranged from 9:00am to 4:00pm with a peak at 1:00pm. Regarding to trap positions, traps deployed at the middle of the slope captured significantly much more males than those at top and foot of the slope. Within a tree, traps located at the south side captured more males than those arranged at the north side. The first flight peak of *D. jingyuanensis* came in the middle of June in all three years, whereas in 1997 and 1998, a second flight peak was also recorded in middle July. Therefore, the monitoring period should be from late May to late July and early August. For populations located at a higher altitude (1400m) and a lower altitude (1100m), the flight peak was found to be the same. Comparison between kinds of traps, more males were caught using Lund-I trap than Unitrap. The former, which needs continuous change of sticky board, is suitable for monitoring flight peak, and the latter is recommended for monitoring population density. With the dosage above 800µg/ trap, the synthesized pheromone can detect the sawfly at very low population density, which is not detectable by other means.

**FLORAL ATTRACTANT AND SEX PHEROMONE FOR A CRYPTOMERIA BORER, *ANAGLYPTUS SUBFASCIATUS* (COLEOPTERA: CERAMBYCIDAE)**

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*Anaglyptus subfasciatus* (Coleoptera: Cerambycidae) is one of the most harmful insect pests of the Japanese cedar, *Cryptomeria japonica* (Linn. fil.) D. Don, and the Japanese cypress, *Chamaecyparis obtusa* (Sieb. et Zucc.), the most abundant tree species of forest plantations in Japan. Females of the beetles lay their eggs on the dead twigs of the cedar or the cypress and

the larvae bore into the twigs and migrate further into the tree trunk. Feeding by the larvae on sapwood followed by fungal infection causes discoloration and decay of the wood, resulting in a decrease of the commercial value of timbers. Since *A. subfasciatus* males and females were known to congregate on several species of flowers for feeding, floral scents and their structural similarities have been screened both in the laboratory and in the field. Methyl phenylacetate has been demonstrated as the most attractive and highly specific to *A. subfasciatus* and has been used as the most reliable floral attractant for *A. subfasciatus* in practice. *A. subfasciatus* females were attracted to males in a wind tunnel and that a male-specific cuticular structure in the pronotum seems to be an organ of pheromone excretion. Furthermore, the male-released sex pheromone constituents of the beetle are identified as a 7:1 blend of (*R*)-3-hydroxy-2-hexanone and (*R*)-3-hydroxy-2-octanone. We have observed that the beetles were attracted to the trap baited with floral attractant alone, but they walked around the tree trunks or logs near the trap. Therefore we tested whether a combination of male sex pheromone constituents and the floral attractant improved pinpoint location of the beetles and increased the trap catches. The trap catches of the female beetles were increased by a combination of male sex pheromone constituents and the floral attractant.

J3

**THE SEX PHEROMONE OF LEAF ROLLER, *CELYPHA PSEUDOLAXICOLA* LIU  
(TORTRICIDAE: LEPIDOPTERA) AND ITS FIELD APPLICATION**

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The leaf roller, *Celypha pseudolexicola* Liu is a serious pest of the Chinese traditional ornamental tree *Pseudolarix kaempferi* (Lindl.) Gord. In order to pursue the suitable management methods without side effects on environment, its sex pheromone identification and field application were conducted. The highest percentage of female's calling appears on the fourth hour in the scotophase under a 14:10 photoperiod, it reached as high as 85%. The existence of five components in the sex pheromone gland extracts of *C. pseudolexicola* were confirmed by polar and non-polar GC separation. By means of micro-chemical reactions, gas chromatography-mass spectrometry, and comparison with their retention times and mass spectra with those of the authentic samples, the five components were identified as 12:OH, Z10-12:OH, 12:OAc, Z9-12:OAc, and Z10-12:OAc at the ratio of 6.8:5.4:24.6:1.0:22.3. In the wind tunnel behavioural bioassay, the results indicated that the single component, Z10-12:OAc could elicit males to produce complete behavioural response of orientation, upward flight, and landing on the lure sources. It was also found that the dosage of the component significantly affected on the male orientation behaviour. At the lower dosage (300 ng), higher percentage of males showed orientation behaviour, whereas at the higher dosage (5000 ng), orientation percentage was

significantly reduced. The above results have now been confirmed in field tests of mass trapping and mating disruption. It showed great potential in the practical application.

J4

**SEX PHEROMONE OF THE CARPENTERWORM, *HOLCOCERUS INSULARIS*  
(LEPIDOPTERA: COSSIDAE): IDENTIFICATION AND FIELD TRIALS**

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The carpenterworm moth, *Holcocerus insularis* Staudinger (Lepidoptera: Cossidae) is a serious forest pest widely distributed in China and ex-USSR. In China, the larvae inflict destructive damage to deciduous trees, such as ash, ginkgo and Chinese scholartree by tunneling into their trunks. The trunks of damaged trees are full of wounds and holes that eventually can cause the death of trees.

The female carpenterworm moths produce a sex pheromone to attract the males for mating. Extracts of the sex pheromone obtained from abdominal tips were isolated, analyzed and identified by thin-layer chromatography (TLC), electroantennogram (EAG), gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and field tests. GC and GC-MS analyses indicated that the extracts contained (Z)-3-tetradecenyl acetate (Z3-14:Ac), (E)-3-tetradecen-1-ol (E3-14:OH), and (Z)-3-tetradecen-1-ol (Z3-14:OH) at a ratio of 51:39:10. The average amounts of Z3-14:Ac, E3-14:OH and Z3-14:OH in a single sex pheromone gland of calling moth were  $7.29 \pm 2.72$  ng,  $5.72 \pm 2.43$  ng and  $1.44 \pm 0.56$  ng, respectively. EAG responses to the identified compounds and the female extract showed that the male antennae produced the strongest responses to Z3-14:Ac, and moderate to E3-14:OH and Z3-14:OH. Field tests were carried out around Beijing in year 2000, and the result showed that Z3-14:Ac was essential for the attraction of the males. On the contrary, E3-14:OH and Z3-14:OH had hardly any attraction to the males, and with the addition of Z3-14:Ac did not show any antagonistic or synergistic effect. Therefore, Z3-14:Ac is an essential active component of the sex pheromone from female carpenterworm moth, *Holcocerus insularis*, and this is first time that Z3-14:Ac was identified as an active component of lepidopteran sex pheromones. The effectiveness of Z3-14:Ac as a sex attractant for the male carpenterworms provides useful procedures to survey and control this pest.

**ANALYSIS OF VOLATILES FROM *MONOCHAMUS ALTERNATUS* HOPE  
(COLEOPTERA: CERAMBYCIDAE)**

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Behavioral bioassay in Y-shaped olfactometer, silica gel column chromatography and gas chromatography-mass spectrometry analysis were used to identify the volatiles collected from *Monochamus alternatus* (Coleoptera:Cerambycidae). Earlier studies showed that the female beetle is attracted and approaches a motionless male beetle calling on the pine trees at night. This indicates the presence of male sex pheromone emitted by the male beetle. This study was conducted from May 1998 until March 1999 at Kyoto Institute of Technology, Kyoto, Japan. Beetles were collected from Shiga, Fukuoka, Mie and Miyage prefectures. Emergence date of the beetles were recorded. Individual beetles were reared in plastic containers containing current and 1 year old pine twigs. Beetles were fed for 3 weeks for maturation feeding. Only unmated beetles which had undergone maturation feeding were used in the bioassay observations. In this study, the volatiles of individual beetle was entrapped on Tenax TA for 18 hours starting from 17:00 until 10:00 the next morning. Ether was used to extract the volatiles and concentrated. Hexane was then added to the aliquot. The crude extract was separated using mixtures of ether:hexane in different percentages. Crude extract and fractions were bioassayed in the Y-shaped olfactometer. Behavioral bioassay conducted in this study showed that the females were attracted to the live male, male ether extract and 100% hexane eluate after silica gel column chromatography. Crude extract and fractions were injected into gas chromatography-mass spectrometer. This study aimed to (a) identify the volatiles emitted from *Monochamus alternatus* and (b) to compare differences in the chemical components found in both male and female volatiles collected from *M. alternatus*. Gas chromatography mass-spectrometry analysis of 100% hexane fractions of male volatiles indicated the presence of compounds with spectra similar to the monoterpenes, myrcene and 3-carene and to the sesquiterpenes alpha-farnesene, alpha-caryophyllene, alpha-cubebene and ylangene. Sesquiterpenes were not found in the female fractions.

**BIOPESTICIDAL EFFECTS OF SENTANG, *AZADIRACHTA EXCELSA* (JACK),  
EXTRACTS ON SOME MAJOR INSECT PESTS OF VEGETABLES IN MALAYSIA**

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The potential insecticidal and antifeedant effects of the extracts of the wood and other plant parts of the lowland forest tree, *Azadirachta excelsa*, commonly known as sentang, were evaluated against some of the major pests of vegetables. The preparation of both the aqueous

and methanol extracts was done using the methods described by Ng *et al.* Egg hatchability of the diamondback moth, *Plutella xylostella*, was not significantly affected by both the aqueous (hereafter, Aq) and methanolic (hereafter, Me) extracts of sentang. High mortality was obtained using the Me extract against the larvae of *P. xylostella* and *Spodoptera litura*. However, the antifeedant effect on both the latter species was low. A significantly reduced oviposition on leaves by *P. xylostella* was also observed using the Me extract but not those treated with the aqueous extract. Under field conditions, the 2 percent methanolic extract-treated cabbage plants had relatively lower numbers of *P. xylostella* and *Hellula undalis* in the field compared to the microbial insecticide, *Bacillus thuringiensis* var. *aizawai* (Xentari<sup>R</sup>), neem, *A. indica* (Neemix 4.5%<sup>R</sup>) and a need/pine oil formulation (Bionature<sup>R</sup>). A high insecticidal activity on *P. xylostella* larvae was still seen on the Me extract-treated cabbage leaves up to 7 days post-spray. Against flea beetles, *Phyllotreta* spp., the 2 percent Aq extract gave repellent and antifeedant effects compared to a ketone-based neem (*A. indica*) product and an insecticide, profenofos (Selecron<sup>R</sup>). In contrast, the 2 percent Me extract showed a relatively higher number of *Phyllotreta* compared to the other treatments tested. Relatively higher aphid-infested plants were also obtained in Me extract-treated cabbage plants. In tomato, both the Aq and Me extracts used at 2 percent gave 60 and 74 mortality of *Thrips taiwanus*, respectively, compared to imidachlorpid (Confidor<sup>R</sup>) which gave 100 percent mortality. In laboratory studies, high larval mortality of 86 percent was obtained against the lowland leafminer, *Liriomyza sativae*, using the 2 percent Me extract whereas only about 22 percent larval mortality was obtained using 2 percent Aq extract. Both extracts gave 100 percent pupal mortality of *L. sativae*. A low mortality of 20 percent was obtained against the second-instar larva of *Epilachna indica*, a major pest of brinjal, compared to the insecticide abamectin (Agrimec<sup>R</sup>) which gave 93 percent mortality.

## POSTERS

J7

### ATTRACTANTS FOR THE LARGER PINE SHOOT BEETLE, *TOMICUS PINIPERDA*

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An optimized, patented lure has been developed for the larger pine shoot beetle, *Tomicus piniperda*. Five compounds: alpha-pinene, alpha-pinene oxide, nonanal, myrtenol, and trans-verbenol were tested singly and in binary, tertiary, and quaternary combinations as lures in 12-unit multi-funnel traps. This study demonstrates that at least tertiary combinations of these semiochemicals are required to assure maximum trap captures.

To identify optimal blends and release rates of semiochemicals for trapping *T. piniperda* during reproductive flight in the spring, beetles were trapped in 12-unit multi-funnel traps (Phero Tech, Vancouver, Canada) baited with alpha-pinene alone or in binary, tertiary or quaternary combinations with alpha-pinene oxide, nonanal, myrtenol, and trans-verbenol. Alpha-pinene was released at a constant rate of 300 mg/24h and the remaining semiochemicals were released at low (0.1-0.8 mg/24h) and high (0.8-5 mg/24h) rates.

Trap catches of *T. piniperda* were increased by 175-433% in traps baited with at least binary combinations of alpha-pinene and alpha-pinene oxide, nonanal, myrtenol, and trans-verbenol when compared to catches in traps baited with alpha-pinene alone. Trap captures with tertiary and quaternary semiochemical combinations were more effective and consistent from year to year than captures with binary combinations. Alpha-pinene oxide, nonanal, myrtenol, and trans-verbenol combined with alpha-pinene attracted more beetles when released from dispensing lures at the high dose.

Effective *T. piniperda* lures require at least tertiary combinations of alpha-pinene and alpha-pinene oxide, nonanal, myrtenol, and trans-verbenol. Quaternary combinations of the above mentioned semiochemicals are recommended. All semiochemicals should be released at high rates. Patented lures containing quaternary or quintuple semiochemical combinations for monitoring *T. piniperda* are commercially available from IPM Technologies.

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.



## INTERCEPT PANEL TRAP A NOVEL TRAP FOR MONITORING FOREST COLEOPTERA.

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A novel trap, the Intercept Panel Trap, is now available for monitoring forest Coleoptera. The trap is made from corrugated plastic. It is light weight, water proof, and durable. Trap efficacy in capturing several forest Coleoptera was measured in field trials in comparison to the Phero Tech (Vancouver, British Columbia) 12-unit Multi Funnel Trap. Field trials targeted Spruce Beetle (*Dendroctonus rufipennis*), Douglas Fir Beetle (*D. pseudotsugae*), Western Pine Beetle (*D. brevicomis*), Western Balsam Bark Beetle (*Dryocetes confusus*), Pine Bark Beetles (*Ips* spp.), Larger Pine Shoot Beetle (*Tomicus piniperda*) and Pine Sawyers (*Monochamus* spp), Asian Longhorn Beetle (*Anaplophora glabripennis*), Buprestid beetles, Wood Wasps and several other exotic forest pests.

Field performance of Intercept Panel Trap was compared to Multi Funnel Trap baited with species-specific pheromone lures in replicated, paired trials. Trap catches of Douglas Fir Beetle and Western Balsam Bark Beetle were significantly higher in the Panel Trap. There was no significant difference between Panel and Funnel Traps in captures of Western Pine Beetle, Larger Pine Shoot Beetle and Cerambycid beetles. Captures of Spruce Beetle and Buprestid beetles were significantly higher in the Funnel traps compared with the Panel Trap. Panel Traps capture fewer non-target beneficial predators compared to Funnel Traps. The Intercept Panel Trap captured a substantial number of Cerambycid and Buprestid beetles, and Siricid wood wasps. Trap captures of Mountain Pine Beetle, *Ips* spp., exotics and Wood Wasps have not been processed and are not available at this time

In proof of concept trials targeting surveillance of exotic forest pests in Oregon, the Intercept Funnel Trap captured substantially more insect species than the multi-funnel trap.

The Intercept Panel Trap is an effective tool for monitoring Scolytids, Cerambycids, Buprestids, and other forest Coleoptera. Panel Traps were very robust under rigorous field conditions, light-weight to carry, weather and water proof, and easy to install. Panel traps disassemble rapidly, can be stored flat, and use less storage space than Funnel traps. The Intercept Panel Trap is now commercially available.

Data on captures of Douglas Fir Beetle, Spruce Beetle, Western Pine Beetle, and Western Balsam Bark Beetle is supplied courtesy of Dr. D. Ross. Data on captures of Pine Shoot Beetle, Cerambycidae, and Buprestidae is supplied courtesy of Dr. P. deGroot, Canadian Forest Service, Sault Ste. Marie, ON.

**ATTRACT AND KILL TECHNOLOGY FOR MANAGEMENT OF  
EUROPEAN PINE SHOOT MOTH, *RHYACIONIA BUOLIANA*, AND  
WESTERN PINE SHOOT BORER, *EUCOSMA SONOMANA*.**

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An attract and kill bait matrix was deployed for management of European pine shoot moth, *Rhyacionia buoliana*, and Western pine shoot borer, *Eucosma sonomana* in pine plantations and tree nurseries. Due to the cryptic larval habits of these shoot borers, conventionally sprayed insecticides are not very effective. A&K technology very selectively removes male moths of the target species from the ecosystem with negligible impact on non-target organisms. Baits combine the selectivity of pheromone (only 0.21 g/ha, compared to 3.5-20 g/ha for mating disruption) with rapid toxicity of insecticides (only 7.92 g/ha, compared to 500-800 g/ha for conventional sprays). This bait retains the insecticide within a hydrophobic matrix that precludes run-off or drift, thus preventing ecosystem contamination and damage.

Last Call formulations, 0.016% and 0.16% pheromone content (w/w) were applied as follows: *R. buoliana* - 50 droplets/tree on small groups of trees or 1200 droplets/acre on plantations; *E. sonomana* - at a rate of 600 and 1200 droplets/acre, respectively. Moth populations were monitored with A-traps baited with rubber septa pheromone lures. Damage by *E. sonomana* was estimated at the end of the growth season. Trap catches of *R. buoliana* were reduced 56-95% and 83-100% on plots treated with 0.016% and 0.16% formulation (respectively) when compared to untreated plots. Trap catches of *E. sonomana* were reduced 76-90% and 90-93% on plots treated with 0.016% and 0.16% formulation (respectively) when compared to untreated plots. Damage by *E. sonomana* in two plantations was significantly reduced on plots treated with either Last Call formulation while moth populations did not change in the untreated plots. Damage on Last Call treated plots in the third plantation was not significantly reduced while moth populations significantly increased in the untreated plot.

Trap catches of both moth species did not differ significantly between traps baited with 5-week old and fresh Last Call droplets. Chemical analysis shows the Last Call formulation retains and protects the integrity of both *R. buoliana* and *E. sonomana* pheromone for at least 42 days.

Last Call technology is a very effective, alternative method for management of *R. buoliana* and *E. sonomana*.

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

## USE OF INSECT GROWTH REGULATORS FOR POPULATION CONTROL OF SUBTERRANEAN TERMITES

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Two classes of insect growth regulators, juvenoids and chitin synthesis inhibitors, have been tested against termites. Earlier on it was suggested that juvenoid-induced production of excessive presoldiers and soldiers would overburden the colony leading to its demise, but studies have yet to produce positive results. One obstacle could be the species specificity of juvenoids that are more effective against species with lower natural soldier proportion. Of the chitin synthesis inhibitors that have been tested, hexaflumuron demonstrated its effects against a wide range of economically important species of subterranean termites, but species specificity problem was shown in other CSI such as diflubenzuron. Numerous field studies with hexaflumuron baits documented the elimination of colonies of subterranean termites. The commercial bait product that contains hexaflumuron, the Sentricon® Colony Elimination System, is currently being used for population control of *Reticulitermes*, *Coptotermes* and *Heterotermes* species in the United States.

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

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

## HOMING OF THE GERMAN COCKROACHES TO A VIRTUAL PHEROMONE SOURCE

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The orientation behaviour of the German cockroach male towards its aggregation attractant pheromone source was investigated on a digital servo-sphere apparatus. A test animal walked freely on the top of a large sphere, whose rotation was controlled by a digital feedback system so that the insect was kept at the same spatial position. The apparatus was combined with an actuator that generated odorous cues to create a virtual sensory field, which enabled us to investigate how the cockroach manoeuvred in response to the cues in a fully automated experiment.

A series of experiments conducted in a T-maze olfactometer has already revealed that the individual components of the pheromone, as well as the crude mixture, induces the odour-modulated upwind anemotaxis to the cockroach: the cockroach goes upwind when the flowing air is laden with the pheromone odour. As expected, a male on the sphere walked upwind along the narrow stream of air only when it was odoured. When a male in laminar airflow was exposed to odorous and clean air in turn, it walked straight upwind in odorous air, but it turned a lot or even kept stationary without odour in the air. In the orientation, males alternatively stood and ran showing a bimodal behavioural pattern. If the odour release was limited while a male was standing, it moved upwind though its track meandered. In contrast, it showed no directional preference within the odorous airflow during its run, though the track was relatively straight. These results imply that the males, when they are exposed to the odour, direct and launch themselves upwind when standing, and walk straight as long as they sense the odour.

The above idea was examined in a cylindrical wind tunnel with eight solenoid-driven valves along its wall. When a male stops, a system calculates its relative position to a virtual odour source defined (5,5) m from the origin, and then opens the nearest valve to release odorous air along the direction towards the source. Males successfully homed in on the source to demonstrate the above orientation mechanism, which may be applicable to the control of this insect pest.

## DEVELOPMENT OF ATTRACTANTS FROM NATURAL PRODUCTS FOR CONTROLLING FRUIT FLIES

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Although the vinegar fruit fly, *Drosophila melanogaster*, has never been considered to be a serious pest, they are a nuisance in households and their presence in commercial food handling areas has economic considerations. The olfactory system of the vinegar fruit fly has been reviewed that it contains three major types of olfactory organs (*Sensilla trichodea*, *S. basiconica* and *S. coeloconics*) located at the third antennal segment and the maxillary palp. This presentation will tell the story of “How these fruit flies are attractive to fermenting fruits, and what key chemical components trigger this attraction”. The study was initiated by observing the attractiveness of fruit flies towards different fermenting fruits in the wind tunnel and the local grocery store. Among the six different fruits tested, fermenting mango evoked the best attraction. Using solid phase microextraction (SPME) and tenax trapping techniques together with combined gas chromatography-electroantennography (GC-EAD), we found that there were several volatile compounds from the extracts that elicited significant EAD peaks. The chemical structures of those active compounds were further identified by comparing their mass spectra and retention indices with those of synthetic standards. Finally, the capture efficacy of synthetic baits containing the identified compounds was evaluated against that of fermenting fruits, as well as other available fly attractants.

## POSTER

K5

### ATTRACT AND KILL TECHNOLOGY TO CONTROL TICKS.

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Three species of medically important ticks, *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum*, are arrested by chemicals extracted from the tarsal gland secretions of white-tail deer. The activity of these compounds is retained when gland extracts are incorporated into Last Call, a proprietary matrix used commercially in attract and kill formulations for several insect species. The chemical identity of the deer-secreted arrestant remains to be determined. Arrestant activity varied from deer to deer. This finding is important because it ensures that ticks will not only touch, but will remain on the surface of a Last Call acaricidal formulation, thus receiving a lethal dose of any toxicant. Also, the research has established that ticks that contact the Last Call matrix containing 6% permethrin are intoxicated and die in 24 h or less.

LAST CALL ATTRACT AND KILL: Bioassays were conducted in IPM Technologies' laboratory using a modification of the method of Carroll (1998), which simulates the selection of a resting or ambush site by a questing tick in the wild. Two flat wooden toothpicks were affixed at opposite ends of a rectangular island (1.5 cm wide by 3 cm length by 1 cm high) of modeling clay centered in a glass petri dish containing water to form a moat. One toothpick was dipped in aqueous methanol and the other in dissolved tarsal gland materials or the upper half of the toothpick was smeared with a small quantity of Last Call containing a candidate tarsal gland extract. Five ticks were placed on each clay island. After 4-5 h each bioassay unit was opened and ticks that were in the water were repositioned on the island. All three species of tick exhibited significant arrestant response to the treatment in the choice bioassays ( 2 analysis,  $P < 0.05$ ). Host odor baited Last Call attract and kill technology shows promise in management of these species of ticks.

EXPERIMENTAL CARBON DIOXIDE TRAP: CO<sub>2</sub> was generated ( 1.5 liters per hour) by adding water to a proprietary mixture of chemicals within the trap. All three species of tick were attracted from at least 4 feet away to these traps in field trials.

**MOLECULAR ECOLOGY OF RIBOSOMAL ITS1 POLYMORPHISMS IN  
*HELICOVERPA ARMIGERA* POPULATIONS IN THAILAND**

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We are investigating the molecular ecology of *Helicoverpa armigera* populations in Thailand. The bollworm *Helicoverpa armigera* is a serious agricultural pest in several different areas in Thailand. In cultivated areas, it infests a variety of different host plants including cotton, tomatoes and potatoes. We are using molecular markers to try and determine if there are genetic differences among populations defined geographically or on the basis of host plant preferences. These markers may also be useful for studies of pest movements through migration and/or invasions of new habitats.

In the search for appropriate molecular markers, we have determined the DNA sequence of the internal transcribed spacer region 1 (ITS1) of the ribosomal genes in a number of individuals collected on different host plants and from various geographic areas in Thailand. Using the variability we have uncovered in the DNA sequence data from these individuals, we have devised a method to identify specific marker types based on restriction fragment length polymorphisms. We are currently making additional collections and screening for additional markers to aid in this analysis.

**MOLECULAR ECOLOGY OF THE *BACTROCERA TAU* SPECIES COMPLEX**

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Evolutionary relationships of *Bactrocera tau* species and populations have been examined using nucleotide sequences of a heat shock cognate 70 like gene (hsc70-like gene). *B. tau* belongs to a group of tephritid fruit flies which are destructive pests in Southeast Asia. The name *B. tau* initially referred to a widely distributed complex of species. By identification of some biological differences, distinguishable groups of *B. tau* have been discerned and designated as eight forms labelled A, B, C, D, E, F, G, and I. However, the evolutionary and genetic relationships among these forms has not been clarified. Establishment of a molecular phylogeny may promote better understanding of the diversity and evolution of fruit flies within this complex.

For this purpose, part of an hsc70-like gene was amplified by PCR and cloned from genomic DNA of *B. tau* specimens. DNA sequences and genetic distances measures were obtained for the hsc70-like sequences. These were used to postulate evolutionary histories based on maximum likelihood, neighbor-joining, and maximum parsimony approaches to reconstruct phylogenetic relationships.

The best resulting phylogenetic tree topologies derived from all of the approaches were congruent. These results suggested that the *B. tau* forms could be divided into three subgroups. The first subgroup consisted of only *B. tau* form A. The second closely related subgroup consisted of forms B, E, F, and G, and the third consisted of forms C, D, and I. However, this same approach did not resolve phylogenetic relationships of *B. tau* (form A) geographical populations. In conclusion, the hsc70-like sequence appears to be suitable for establishing molecular phylogenies at the level of species but not populations in the *B. tau* species complex.

L3

### **POLYMORPHISM OF *DROSOPHILA* PHEROMONES: MOLECULAR STUDIES**

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The cuticle of *Drosophila melanogaster* is covered with a layer of lipids including long-chain hydrocarbons. Two of them are mainly produced by mature males (7-tricosene, 7T and 7-pentacosene, 7P) and can act on conspecifics either as attractants for females or repellents for other males. The main mature female hydrocarbons are longer and act as contact pheromones after males tap female cuticle (7,11heptacosadiene, 7,11 HD and 7,11nonacosadiene, 7,11 ND). Contrary to those of the rest of the world, females from sub-saharan African strains show low levels of 7,11HD and high levels of its position isomer, 5,9 HD. Other species, as the sympatric sibling species, *D. simulans* are sexually monomorphic, with a large production of 7T and 7P together with 2methyl-branched HCs as in *D. melanogaster* flies of either sex; in 5 such species of the *melanogaster* subgroup, 7T acts as a sex pheromone. The biosynthesis of these compounds shares a number of steps (synthesis of unsaturated fatty acids, desaturation, elongation and decarboxylation) while some steps are sex-specific, especially for female diene production (female-specific desaturation of monoenic fatty acids and their elongation and decarboxylation.) The common steps involve among others D9 desaturase and one or more elongase(s). We have characterized molecularly and functionally a D9 desaturase in *D. melanogaster* and in *D. simulans*, *desat1*, which transforms palmitic acid into palmitoleic acid and seem to lead to w7-HCs. Indeed in position 7 *desat1* mutants show low levels of such unsaturated hydrocarbons. Another desaturase gene has been characterized in *D. melanogaster*, which is translated only in females of the 5,9 HD rich morph. *desat2*, located near *desat1*, encodes another D9 desaturase which uses myristic acid as substrate leading to the expected w5-fatty acids necessary for the diene biosynthesis. We are searching for the enzymes involved in the female-specific steps and a number of preliminary data will be presented and discussed.



## POSTER

L4

### PHYTOCHEMICAL VERSUS MOLECULAR EVALUATION OF *PHYLLANTHUS NIRURI* L.

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The significance of *Phyllanthus niruri* in the treatment of various diseases especially liver complication has emphasized the necessity for adequate standardization. The ultimate goal of our research was to establish a phytochemical and a molecular standardization protocol to ensure the quality, safety and efficacy of this herb, as well as to avoid taxonomic confusion and adulteration in commerce. Eight species of *Phyllanthus* were screened in this survey. The thin layer and gas chromatographic profile of the hexane extract was recognized as the best method for phytochemical evaluation. Lignan constituent, i.e. lintetralin, niranthin, hypophyllanthin, phyllanthin and nirtetralin were only available in *P. niruri* and were reliable chemotaxonomic markers for this particular taxon. Meanwhile, Random Amplified Polymorphic DNA (RAPD) technique was used to distinguish the molecular variation. Polymorphisms in all the *Phyllanthus* species were detected by twenty random decamer primers in the polymerase chain reaction (PCR). The comparisons between the molecular and phytochemical evaluation were made. The great resolution contributed by the results obtained in the identification and standardization of *P. niruri* was briefly discussed.