Abstracts (Oral)

PL–1 Chemical Communication: Structural Principles, Evolution, and Perspectives

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Molecular recognition is prerequisite to the beginning of life, and thus, "chemical signalling" is the oldest means for the transmission of information.

During evolution, principles of chemical signalling may have been developed several times and for different reasons, typical elements being made up by a broad spectrum of secondary metabolites. These may originate from the ubiquituous streams of classic catabolism or metabolism.

Therefore, striking similarities are found between molecular structures of compounds which are used for chemical communication. This conservative scenario may not only indicate similar biogenetic pathways but also common roots including aspects of coevolution. It points to general concepts in the establishment of "chemical languages" and the principle development of appropriate receptor systems. A general question concerning an ethymology of chemical signals may be raised.

Most of the relevant compounds are represented by acetogenins, polyketides, and terpenoids, the biosynthesis of which is not restricted to animals, but is also valid in plants and microorganisms, in terrestrial as well as in aquatic ecosystems. The role of (endo)symbionts in the production and transformation of animal associated chemical signals is yet unknown.

Due to past processes of coevolution, the structures of odoriferous compounds from plants, insects, and microorgansms can be identical. The same compound may be used as a chemical messenger by quite different organisms and in entirely different ecological contexts. The information linked to a specific chemical structure is not necessarily constant and may change during evolution. This becomes particularly evident in compounds which cause a high status of alertness in the receiver and which may turn into signals causing attraction, aggression, defense or stampede; on the other hand compounds evolved in the context of defense, may change into attractants.

In this context, a more philosophical contemplation: Any chemical signal needs a receptor at the receiver-site - and one may ask whether semiochemicals may also be recognized by proteins that are not primarily involved in chemical communication. In other words, wouldn't it be wise to generally screen semiochemicals for biological activities other than their presently known function: Methyl *p*-hydroxybenzoate, a component of the honeybee queen pheromone, exhibits strong antibiotic properties—and there are quite some more examples that prove the physiological versatility of semiochemicals.

On another matter:

- There is much too little known about chemical ecology of marine organisms.
- There is much too little known about chemical ecology of microorganisms
  - — and almost nothing about chemical interactions between plants and microorganisms in the rhizosphere.

Finally:

There should be more research activities about hematophagous insects and other vectors of diseases.

Key words: semiochemicals, structural relationships, biogenesis, evolution

PL–2 Chemistry and Ecological Aspects of Marine Microalgal Toxins

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The significance of microalgal toxins is most obvious when a dense bloom of the organisms changes the color of the water, as referred to a red tide, and cause mass mortality of various marine lives. In addition to working on the red tide toxins, we have also disclosed that the majority of the toxins are produced by species unseen from the surface or occurring in too low abundance to be easily recognized. The key to the success in identifying such cryptic species was our effort to determine the chemical structures of the toxins and develop analytical method. As an example of such studies, ciguatera fish poisoning (CFP) will be explained. CFP is caused by ingestion of more than 300 species of fish normally safe to eat. We found an epiphytic dinoflagellate, *Gambierdiscus toxicus*, was the primary source of the toxins. The toxins enter the food chain via herbivorous fish and accumulate in a wide range of fish. During the transmission form fish to fish, the toxins are metabolically modified, producing analogs varying not only in structures but also in toxicological properties. Along with *G. toxicus*, a number of dinoflagellates were found to grow on algal surface or benthic substrata. Among them, *Prorocentrum lima* that produces the okadaic acids and *Ostreopsis siamensis* that produces palytoxin analogs are the two important species. When a coral reef ecosystem is damaged, calcareous alga replace corals, offering *G. toxicus* the surfaces to grow. The sudden increase of CFP incidents after destruction of coral reefs by construction works is thus explained by the underwater blooms of *G. toxicus*. The second example of cryptic species is a planktonic dinoflagellate *Dinophysis fortii* that produces okadaic acid and dinophysistoxin-1 responsible for diarrhetic shellfish poisoning. In the presence of very low number of this species in water, less than 200 cells per litter, bivalve shellfish would accumulate the toxins to the level enough to cause diarrhea upon consumption. In addition to the low cell density, *D. fortii* is an unculturable species. A sensitive analytical method played a critical role in confirming the toxins in *D. fortii*. In addition to the above two examples, the characteristic features of the toxin structures and their ecological significance will be also discussed.

PL–3 Balanced Olfactory Antagonism as a Concept for Understanding Evolutionary Shifts in Moth Sex Pheromone Blends

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In moth sex pheromone communication systems, both heterospecific sex pheromone blend components as well as individual conspecific pheromone components act as behavioral antagonists when they are emitted at excessive rates and ratios and thus the resulting blend composition does not comprise the sex pheromone of a given species. The only time any of these compounds do not act as behavioral antagonists is when they are emitted at optimal rates and ratios with other compounds. In such situations the array of possible blend compositions is centered around the characterized femaleproduced sex pheromone blend of a species. The resulting optimal attraction of males to a sex pheromone is thus a result of olfactory balance, compared to the would-be behavioral antagonism imparted by all such behaviorally active compounds when emitted individually or in other off-ratio blends.
I am proposing that in studies of moth sex pheromone olfaction we should no longer artificially compartmentalize heterospecific behavioral antagonists into a special category distinct from conspecific sex pheromone components. Indeed, continuing to impose such a delineation among these compounds may retard advances in understanding how moth olfactory systems can shift to be able to facilitate males' behavioral responses to novel sex pheromone-related compositions emitted by females.
Key words: insect sex pheromones, attractants, heterospecific antagonists, sex pheromone olfaction, insect behavioral response

PL–4 Organic Synthesis in Chemical Ecology — from Early Days to Future

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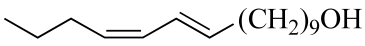
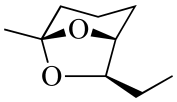
Synthesis and bioactivity of the following semiochemicals will be discussed to illustrate the the roles of organic synthesis in chemical ecology.

(1) Synthesis in early days.

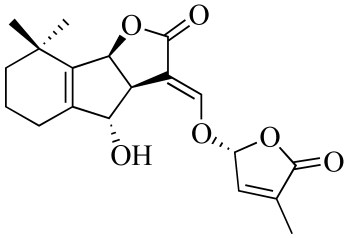


bombykol (1962)

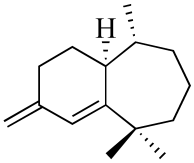


exo-brevicomin (1974)

(2) Synthesis to establish the structures.

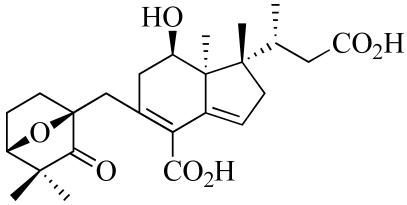


orobanchol (1999)

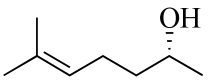


the flea beetle pheromone (2004)

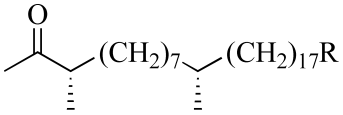
(3) Synthesis to supply samples.



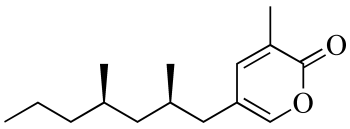
glycinoeclepin A (1989)



(*R*)-sulcatol (1975)

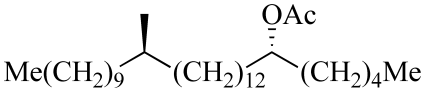


R = Me; R = CH_2OH; R = CHO
German cockroach pheromone (1978, 1990, 2007)

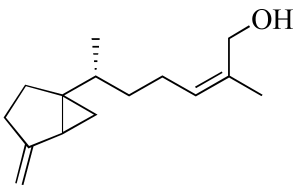


supellapyrone (2001)

(4) Recent synthesis.



screwworm fly pheromone (2004)



stinkbug pheromone (2007)

Key words: chirality, pheromones, semiochemicals, stereochemistry, synthesis

KS–1 Molecular Mechanisms Underlying Sex-Pheromone Reception in Insects and Mice

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The detection of chemicals in the external environment—so called, chemosensation—is essential for the survival in many animals [1]. Odorants—volatile 'smellable' chemicals—and pheromones—species and gender-specific chemicals—are two major olfactory cues by which information about food and suitable mating partners is transmitted.

The insect chemosensory system has been a subject of great interest because of its remarkable selectivity and because of its association with sexual behavior. We discovered two male-specific olfactory receptors in the silk moth, *Bombyx mori*, that are mutually exclusively expressed in a pair of adjacent pheromone-sensitive neurons in the male antennae: one that is specifically tuned to bombykol, the sex pheromone, and the other to bombykal, an oxidized form of bombykol [2]. This mutually exclusive expression pattern of two pheromone receptors in single sensillum may provide a paradigm to ensure detection of the specific ratios of a pheromone blend in antenna of various moths. We also investigated how the pheromone information is converted to an electrical signal via the pheromone receptors. Intriguingly, it turns out that the pheromone receptors form heteromeric complexes that comprise a novel class of ligand-activated nonselective cation channels [3]. The ionotropic mechanisms allow insects to more rapidly respond to odor or pheromone cues in the environment.

Social and reproductive behaviors in mammals are modulated by not only volatile pheromones but also non-volatile cues that are likely detected by vomeronasal sensory neurons. We identified a male-specific peptide that was encoded by a gene from a previously-unrecognized large family in mice [4]. This peptide, named ESP1, is secreted in tears of male mice and transferred to the female vomeronasal organ wherein it elicits an electrical response. We also found that ESP1 was recognized by the specific V2R-type pheromone receptor expressed in the vomeronasal sensory neurons. Mice appear to send socio-sexual information via an accessory olfactory pathway during direct contact. Each animal appears to have acquired a unique strategy to transmit volatile or non-volatile sex-specific cues by adopting their environment during the processes of evolution.

References: [1] Touhara: Microsco. Res. Tech. 58, 135, 2002. [2] Nakagawa et al.: Science 307, 1638, 2005. [3] Sato et al.: submitted 2007. [4] Kimoto et al.: Nature 437, 898–901, 2005.

Key words: pheromone, pheromone receptor, sex, insect, mouse

KS–2 Allelopathy of Tropical Plants in Thailand

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The research work on allelophathy from tropical plants in Thailand will be overviewed. Based on biological activity guide, several potent lead compounds could be separated. The insect antifeedant limonoids from the heartwoods of *Xylocarpus moluccensis* and *Xylocarpus granatum*, diterpenes from those of *Xylia xylocarpa* and pterocarpans and pterocarpol from those of *Pterocarpus macrocarpus* are among those examples exemplified to show the plantinsect interaction phenomenon. The plant-animal interaction phenomenon will be revealed from the work on agochemicals from *Datura metel.* Several tropical plants also displayed a good potential source for anti-phytopathogenic fungal activity. For plant-plant interaction, the isolation of allelochemicals from *Hydrocotyl umbellate* and *Hyptis suaveolens* will be presented. The fundamental knowledge of allelopathy on the plant-plant interaction could be used to develop more potent agrochemicals. The structure-activity relationship work searching for weed control agents disclosed two potent cinnamic acid derivatives as herbicides.

Key words: allelopathy, antifeedant, antifungal, herbicide, tropical plants

KS–3 Odor-Independent Effects of Human Chemosignals and Pheromones on Ovulation, Sexuality and Cognition

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Human pheromones, one type of social chemosignal, modulate neuroendocrine function by regulating the timing of the preovulatory surge of lutenizing hormone. Here, we demonstrate that natural compounds collected from lactating women and their breastfeeding infants not only modulated the timing of the menstrual cycle but also increased the sexual motivation of other women, measured as sexual desire and fantasies. Other social chemosignals modulate the adrenal axis, different types of psychological states and widely distributed yet specific areas in the brain. They also accelerate processing of social information, lightening both cognitive and emotional loads. In this newly emerging field, there appear to be directly conflicting results. Here I will argue that this apparent conflict arises, in many cases, from an exquisite sensitivity to social context that enhance or preclude a response. Thus, human social chemosignals may serve a broad range of neuroendocrine and social functions.
Key words: pheromone, chemosignal, lutenizing hormone

KS–4 Cuticular Pheromones and Sexual Isolation in Insects

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Since the discovery of the first cuticular pheromone, Muscalure, many others have been characterized in insects. All are long chain hydrocarbons with a small number of double bonds or methylbranchings on specific carbon numbers. They are usually abundant, mixed in the epicuticular layer and involved in the recognition of mates or colony partners in interaction with other sensory cues.

In the last twenty five years, Drosophila has become a popular model to study the role of these cuticular hydrocarbons in sexual isolation. Indeed more and more species have been discovered, adapted to different environments and their phylogenetic relations were easily established thanks to the molecular tools built for D. melanogaster. They are easily bred in the laboratory, in large numbers and at low cost. Among the various mechanisms of isolation, ethological ones are the most important. A sophisticated interplay of courtship behaviors performed by potential sex partners has been first described in D. melanogaster, which then appeared well conserved between species. Among others, chemical cues have been involved in homospecific stimulations and heterospecific discriminations, especially among species of two subgroups far apart, the melanogaster and virilis subgroups.

The presentation of two sets of studies will show that long chain hydrocarbons with double bonds localized on more or less internal double bonds play a major role in sexual isolation of sympatric species. Moreover there is often a sexual dimorphism in CHC which makes the chemical dialogue more informative, from each sex towards the other and between individuals of the same sex when all meet near food sources. The comparison of geographic variants of some of these species has then raised the hypothesis that these individual compounds were actually part of complex chemical signatures with genotype/environment specific compositions, including excitatory and inhibitory components. They lead to more or less courtships and mating between individuals of different homospecific populations. A further question was whether all components of the cuticular signature are only modulating positively or negatively the same Drosophila courtship step or acting on different behaviors?

More Drosophila species were studied even in the Hawaiian archipelago, and there species were found where branched hydrocarbons replaced unsaturated hydrocarbons as major CHC. In the planitibia subgroup of picture wings Hawaiian flies, very long dimethylalkanes with two methyls branched internally, for example 11,15-tritriacontadienes, are domineering cuticular compounds in both sexes. In these species are these CHC playing the same sex pheromonal role as alkenes in continental flies, or is their main function linked to male competitions in leks? Actually similar branched hydrocarbons are very abundant in the cuticle of ants. For example in a complex of three Pachycondila species, CHC are involved in species recognition and there is some suggestion that they might also affect colony recognition. There the perception of specific isomer mixtures by individuals of different ensembles triggers more or less antagonistic behaviors and thus modulates the ensemble closing. Is there a relation between some CHC structural traits and certain types of induced behaviors?

The biosynthesis of such species- and sex-specific signals has been studied in the melanogaster subgroup and a number of structural genes identified. Chertemps et al. have identified a number of genes controlling the biosynthetic steps of species recognition cuticular signals. The hypothesis that environmental factors might modulate the expression of these genes and control through adaptations to the evolution of the recognition signals will be discussed

Key words: hydrocarbons, cuticular signature, sexual isolation, biosynthetic genes and environment.

KS–5 Application of Pheromones in Area-Wide Integrated Pest Management Programs

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Identification of the sex pheromone structures revolutionised our understanding of insect olfactory orientation and made new methods of monitoring and pest control possible. Pheromone-based monitoring is the most effective survey method for detecting pest insects. It could be used for early detection and warning of pest invasion, taxonomic and biodiversity investigations, population density and dispersion trends estimation, forecasting and threshold determination, mapping of pest infested areas and risk assessment, recommendation of treatments and timing of application, measuring of treatment efficacy and impact on pest density. Production of pheromones in commercial quantities permitted the use of attract and kill, mass trapping and mating disruption methods for direct control of pest populations. Pheromone mediated mating disruption (MD) is now a major tool of sustainable and area-wide Integrated Pest Management (IPM) systems in Horticulture. For many insect species the release of large quantities of sex pheromone into a target crop can disrupt mate location and prevent or delay mating, thus reducing egg fertilisation and pest damage. In Australian orchards, hand-applied MD dispensers have been used successfully for long-term sustainable control of oriental fruit moth (OFM), codling and light brown apple moth for over 20 years. Initially the general approach was to treat individual orchard blocks and only known hosts with MD. An increased incidence of OFM damage on the borders of MD treated blocks stimulated an area-wide application of MD for better crop protection. An area-wide MD program, with more than 1,100 ha of 40 contiguous orchards covered with MD dispensers, applied to all fruit trees in the Cobram region of northern Victoria, Australia, substantially improved protection against OFM damage. Later, local growers took the initiative to continue this area-wide MD program as a self-sufficient community approach and re-established MD across the whole Cobram region. Although successful, the area-wide MD program was an expensive approach. To reduce the cost of area-wide MD program, trials were conducted, where only infested blocks and border areas were treated with MD. Such a selective approach successfully controlled localised pest outbreaks and areas of increased infestation. Other trials investigated use of a reduced application rate of MD dispensers, barrier MD treatments and new MD products, including sprayable microencapsulated and multispecies MD dispensers. Sprayable microencapsulated pheromone products for MD have the advantage of easy application with standard spray equipment and compatibility with most insecticides and fungicides. Results of our trials demonstrated that fortnightly spray intervals provided pest control equivalent to the performance of standard hand-applied MD dispensers and was more effective than monthly applications. New multispecies hand-applied MD dispensers were also evaluated in counter seasonal replicated trials in Victoria (Australia) and Michigan (USA) over three years. New host-plant attractants were tested to improve pest monitoring, particularly for mated females in orchards treated with MD. Also mass trapping of mated female moths was tested in MD treated orchards. Aggregation pheromone with co-attractant was successfully used on stone fruit to "attract and kill" a large number of dried fruit beetles to support pheromone-based IPM program in the region. Pheromone-based IPM and selective area-wide MD programs are now the key elements in development of cost-effective strategies for pest control, while protecting the environment by reducing pesticide pressure.

Key words: sex pheromones, monitoring, mating disruption, attract and kill, IPM

KS–6 Semiochemical Sociology of True Bugs (Hemiptera: Heteroptera)

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The first part of the talk will cover the history of pheromone chemistry for the group, including a description of the "defensive" metathoracic scent gland (MSG) morphology and chemistry, and selected examples demonstrating the complexity and chemical diversity of heteropteran pheromone chemistry. Examples of pheromones will include 1) predacious pentatomids (*Podisus* spp.) whose males produce aggregation pheromone blends of aliphatic, aromatic and monoterpenoid compounds from specialized glands opening underneath their wings; 2) phytophagous pentatomids (*Nezara*, *Acrosternum*, *Euschistus*, *Thyanta* and *Plautia* spp.) whose males produce aggregation pheromones (probably from cells in the abdominal epidermis) containing normal and sesquiterpene hydrocarbons, sesquiterpene epoxides, and/or methyl esters of 2,4- and 2,4,6-alkenoic acids; 3) milkweed bugs (Lygaeinae: *Oncopeltus*, *Lygaeus* and *Tropidothorax* spp.) whose males produce aggregation pheromones from the MSG (initially considered to serve only a defensive role in Heteroptera) that are mixtures of (*E*)-2-hexenyl and octenyl acetates, (*E*,*E*)-2,4-hexadienyl acetate, and the unusual terminally unsaturated (*E*)-2,7-octadienyl acetate; 4) the minute pirate bug, *Orius insidiosus* (Anthocoridae), whose females produce a sex pheromone from the MSG (only attractive to males) consisting of (*E*)-2-octenal (both sexes) and (*E*)-2,7-octenal (female-specific); 5) *Phytocoris difficilis* (Miridae) whose females produce a sex pheromone (hexyl, (*E*)-2-hexenyl and (*E*)-2-octenyl acetates), and whose males produce an anti-sex pheromone (hexyl and (*E*)-2-hexenyl butyrates) from the MSG and; 6) alydid bugs, which produce aggregation pheromones from their MSGs, in some species by the female (e.g. *Alydus eurinus*: (*S*)-(−)-2-methylbutyl butyrate and (*E*)-2-methyl-2-butenyl butyrate, mostly attractive to males), and in some species by the male (e.g. *Riptortus clavatus*: (*E*)-2-hexenyl (*E*)-2-hexenoate and (*E*)-2-hexenyl (*Z*)-3 -hexenoate from the MSG, plus the key component, myristyl isobutyrate, from the abdominal sternum). Almost all of the heteropteran pheromones discovered thus far are used as host-finding kairomones by various parasitoids, particularly tachinid flies.

The second part of the talk will cover recent, ongoing research concerning the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Pentatomidae), a newly invasive species in the eastern USA that is rapidly spreading from the original point of establishment in Allentown, PA. In its native east-Asian range, the BMSB is reportedly attracted to methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate, the maleproduced pheromone of another pentatomid common in the region, *Plautia stali.* Methyl 2,4,6-decatrienoates were field-tested in Maryland to monitor the spread of the BMSB from 2004 (none trapped) through 2007 (hundreds trapped). We also report the unexpected findings 1) that various isomers of methyl 2,4,6-decatrienoate attract the North American native green stink bug, *Acrosternum hilare*, although this bug apparently does not produce methyl decatrienoates and, 2) that the supposed attractant pheromone of *A. hilare* (*cis*- and *trans*-bisabolene epoxides, 95 : 5, respectively) did not attract green stink bugs in the field whereas the tachinid parasitoid of the green stink bug, *Trichopoda pennipes*, was attracted to the bisabolene epoxide blend. Other stink bugs and tachinids native to North America were also attracted to methyl 2,4,6-decatrienoates. These data indicate there are Heteroptera in North America, in addition to *Thyanta* spp., that probably use methyl 2,4,6-decatrienoates as pheromones. The evidence that some pentatomids exploit the pheromones of other true bugs as kairomones to find food or to congregate as a passive defense against tachinid parasitism is discussed.

Key words: Aggregation, cross-attraction, kairomone, pheromone, parasitoid

A–01 Bioactive Polypeptides of the Marine Venomous Animals

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Recent studies on the toxins from marine venomous animals, such as jellyfishes, crown-of-thorns starfish and sea anemones, have showed that marine noxious animals are rich sources of novel bioactive polypeptides. I will present here the recent results of our studies on bioactive polypeptides from marine venomous animals.

We have isolated and chemically characterized three major proteinaceous toxins from three species of venomous box jellyfishes. These were the first examples of the characterization of jellyfish toxins. Our continuing studies on the jellyfish toxins revealed that *Chiropsalmus quadrigatus*, which is the most venomous jellyfish in Japan, have the other protein toxins not only the main toxin CqTX-A. Besides CqTX-A, *C. quadrigatus* had a hemolysine and a cytotoxin. New hemolysine has a molecular weight of 44 kDa. These results suggest that the symptom occurred by this jellyfish stings is a complex phenomena caused by plural toxins.

Bioactive compounds from the deep-sea animals are hardly examined for the difficulty of collecting animals. Our research group has been making efforts to collect the deep-sea animals from the depth of 1,000 m using specially equipped nets in collaboration with Japan Agency for Marine-Earth Science and Technology. The bioactivity screening was done on several deep-sea jellyfishes. It was shown first time that the deep-sea jellyfishes actually have the proteinaceous toxins like shallow water common jellyfishes. Polypeptides from the deep-sea jellyfishes will be also presented.

Hydrozoa corals belonging to the genus *Millepora*, which are called fire corals, cause painful and damaging effects on skin by accidental contact. We isolated a hemolysin (ca. 100 kDa), a cytotoxin (ca. 20 kDa) and a fluorescent polypeptide (ca. 15 kDa) from *Millepora dichotoma.* The 100 kDa hemolysin showed potent hemolytic activity (EC_{50}, 36 ng/mL) toward 0.8% suspension of sheep erythrocytes. The 20 kDa cytotoxin (*Millepora cytotoxin*-1: MCTx-1) showed potent cytotoxicity (EC_{50}, 79 ng/mL) toward L1210 mouse leukemia cells. The complete amino acid sequence of MCTx-1 was determined. This is the first chemical characterization of a toxin from the fire corals. The amino acid sequence of MCTx-1 showed homology with that of a hemagglutinin formerly isolated from the horseshoe crab. MCTx-1 actually showed hemagglutinin activity on sheep erythrocytes. The isolated fluorescent polypeptide was revealed to be a new peridinin-chlorophyll a-protein. It was suggested that this fluorescent protein was produced by the symbiotic zooxanthellae in the fire coral.

Key words: jellyfish, deep-sea, fire coral, protein toxin, fluorescent protein

A–02 Differential Gene and Protein Expression During Larval Settlement and Metamorphosis in Response to Chemical Cues

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Although studies of the nature and origins of chemical cues that can trigger the larval settlement of marine invertebrates are decades old, the information remains limited, which negatively impact our understanding of mechanisms of larval metamorphosis and the development of environmentally friendly antifouling technology. Recent identification of effective inducers/inhibitors for larval settlement of the barnacle *Balanus amphitrite* and the polychaete *Hydorides elegans* allow us to investigate the differential gene and protein expression in larvae during settlement and metamorphosis in response to chemical cues in these species. Here we present our preliminary findings on the differential expression pattern of genes and proteins in larvae of *B. amphitrite* and *H. elegans* during settlement and metamorphosis in response to exposure to both negative (control) and positive chemical cues. In barnacles, we found that both number and intensity of protein spots dramatically decreased after cypris settlement triggered by positive chemical cues (the number was reduced by 20% to 30% depending on the type of chemical cues used). This implies the down-regulation of few key proteins during larval settlement and metamorphosis in barnacle. Meanwhile, expression of barnacle cypris-specific genes (bcs-genes) was suppressed when larvae exposed to positive cues and vice versa. For *H. elegans*, we also observed differential expression of genes (some up-regulated while other down-regulated) and proteins when larvae settled in response to chemical cues. This study represents the first application of a proteomics and gene expression approach to probe larval settlement mechanisms. Our on-going studies may identify marker and key proteins involved in larval settlement and provide insights into larval settlement mechanisms.

Key words: gene expression, protein expression, larval settlement, chemical cues, barnacles, polychaete

A–03 Tetrodotoxin Resistant Systems of the Toxin Possessing Animals

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Tetrodotoxin (TTX) and saxitoxin (STX) bind to a single site in the outer pore of the voltage gated sodium channels (Navs), formed by the amino-acid residues in the outer-pore loops (p-loops) located between the S5 and S6 segments of each of the homologous domain (I–IV) of the a-subunit. Since puffer fish and newts accumulate TTX at high concentration in their tissues, they are thought to have a special defense system against their own TTX. Indeed, we revealed that dissociation constants (K_d) for ^{3}H-STX to brain and skeletal muscle membranes of the puffer fish, *Fugu pardalis*, were 190- and 460-fold larger than those of rat, respectively. We obtained a cDNA encoding Nav from *F. pardalis* skeletal muscle (fNav1.4a). In fNav1.4a protein, the residues for ion-selective filter, voltage sensor, and the charged residues in p-loop regions in domain I–IV were conserved, but the aromatic amino acid Phe/Tyr-383 in domain I was replaced by Asn, and Gly-1569 in domain IV was replaced by Thr. The aromatic amino acid replacements by nonaromatic amino acids at this position have been reported in some TTX-resistant Navs. Recently, Venkatesh et al. reported that the mutant Y401N of rat Nav1.4 expressed in HEK 293 cells was 2000-fold less sensitive to TTX than WT, suggesting that this mutation was responsible for TTX-resistance of fNav1.4a. On the other hand, the effect of G1569T mutation on TTX affinity to the channels has never been examined. The similar mutation of Gly at this position by Gln was found in Nav1.4 of another TTX possessing puffer fish, *Tetraodon nigroviridis*. The Willow Creek (North America) population of garter snakes (*Thamnophis sirtalis*) were reported to be resistant to TTX to feed on TTX containing newts (*Taricha granulosa*) by Geffeney et al. In this garter snakes, the replacement of Gly at this position by Val (G1569V) was found together with other mutations in p-loop region of domain IV, I1556L, I1561V, and D1568N. We investigated the effect of G1718T mutation found in fNav1.4a of *F. pardalis* on TTX resistance by comparison of IC_{50}-TTX values determined electrophysiologically among the corresponding mutants F385N, G1718T, and F385N/G1718T of rat brain Nav (rNav1.2a) transiently expressed in HEK293 cells. In addition, we also determined IC_{50}-TTX values of F385A and F385Q of rNav1.2a. F385A type mutation was found by Kaneko et al. in TTX-resistant retinal neuron of the newt, Cynops pyrrohogaster, which possesses TTX. We also found garter snake-like mutations in domain IV of Nav1.4 of this newt. A dramatic increase of STX-resistance caused by mutation E945D of rNav1.2a (domain II) was also reported by Bricelj et al. This mutation was found in STX accumulated softshell clams, *Mya arenaria.* As the second TTX-resistant system of puffer fish, we reported a novel soluble STX and TTX binding protein from plasma of the puffer fish, *Fugu pardalis*, and named puffer fish STX and TTX binding protein (PSTBP). PSTBP possessed a K_d of 14.6 nM for ^{3}H-STX in equilibrium binding assays. ^{3}H-STX (10 nM) binding to PSTBP was half-inhibited by the presence of TTX and STX at 12 μmM and 8.5 nM, respectively. From the results of gel filtration chromatography (200 kDa) and SDS-PAGE (104 kDa), PSTBP was suggested to consist of noncovalently linked dimers of a single subunit. PSTBP was completely deglycosylated by glycopeptidase F, producing a single band at 42 kDa. Two highly homologous cDNAs to each other coding PSTBP (PSTBP1, PSTBP2, the predicted amino-acid identity 93%) were obtained from a cDNA library of *F. pardalis* liver. We prepared polyclonal antibody by immunization of a rabbit by injection of protein part of PSTBP expressed in *E. coli* (Re-PSTBP) and investigated distribution of PSTBP in puffer fish tissues, and other TTX possessing animals.

Acknowledgments: The electrophysiological experiments were performed by Prof. Kaoru Yamaoka, Hiroshima International University.
Key words: tetrodotoxin, saxitoxin, puffer fish, voltage gated sodium channel, binding protein

A–04 Surface Mediated Interactions in the Marine Environment – The Role of Natural Products

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Marine natural products play an important role in mediating ecological interactions between organisms. The majority of studies have focused on the role of natural products as feeding deterrents, while their role as mediators of interactions at biological surfaces is only beginning to be understood. One of the most obvious surface mediated interactions is biofouling, the settlement and growth of microbes, plants and animals on an immersed surface. Biofouling can be highly detrimental to the growth, reproductive output and survival of the organisms on which they settle. One of the proposed defences of marine plants and animals against fouling is the delivery of natural products that prevent the settlement and growth of fouling communities. However, to unequivocally demonstrate an antifouling role for natural products, their localization and presentation on the surface relevant to fouling needs to be quantified, as does their efficacy at ecologically relevant concentrations. This presentation uses red algae (*Delisea pulchra* and *Asparagopsis armata*) and starfish (*Linckia laevigata*, *Fromia indica*, *Cryptasterina pentagona* and *Archaster typicus*) as models to demonstrate unequivocally that natural products are a functional defence against the settlement and growth of fouling organisms. The localisation and delivery of natural products to the surface of model organisms are described using ultra-structure and chemical studies. The presentation of natural products at biological surfaces is subsequently confirmed using quantitative chemical analyses, in particular gas chromatography-mass spectrometry. Finally, isolated natural products, either individually or collectively, are demonstrated as effective defenses against ecologically relevant fouling organisms at natural concentrations confirming their role as defences against fouling.

Key words: marine chemical ecology, surface mediated interactions, biofouling, chemical defence, ecological relevance

A–05 Bioactive Small Molecules from Marine Organisms

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Marine organisms, such as sponges, tunicates, or soft corals, are well known to be the rich source of small molecules with unique chemical skeletons and biological activities. The roles of these small molecules in the animals are still to be disclosed for most of the cases. However, a variety of the biological activities of these molecules against human beings have been reported because of their potential as drug leads.

We have been searching for marine bioactive compounds to be developed as drug leads for the treatment of various diseases. To make the best of chemical diversity of marine organisms, we have been trying to apply as many types of bioassays as possible to the screening, such as cytotoxicity against several cell lines, inhibitory activities against various enzymes, along with traditional antimicrobial activities, and so on. Recently, we have focused on angiogenesis, which is recognized as one of the most promising target for the cancer chemotherapy.

Angiogenesis is a physiological process that is fundamental to the growth and development of normal tissue, wound healing, reproduction and embryonic development. In the normal tissue, angiogenesis is highly regulated by a delicate balance between pro- and antiangiogenic factors. In contrast, uncontrolled blood vessel formation occurs in many pathological conditions such as tumorigenesis, diabetic retinopathy, rheumatoid arthritis, or psoriasis. Antiangiogenic agents controlling new blood vessel formation are therefore potential drug leads for such diseases. Because angiogenesis is the complex biological process composed of multiple-steps, multiple targets-oriented strategy is effective for the search of anti-angiogenic agents. In our starategy, two types of bioassays, phenotype- and target-oriented, are employed. The former is to evaluate effects at the cell level, while the latter is to evaluate inhibitory activities against target molecules, which play key roles in the process of angiogenesis. Combination of these bioassays makes it possible to search wide variety of active compounds and to study angiogenesis in the versatile ways using these agents. The research by such strategy is the new area of chemistry called chemical genetics.

In this presentation, examples of anti-angiogenic agents obtained from Japanese marine invertebrates will be presented.

B–01 Chemical Communication in Humans

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Recent studies on human body odor or pheromone indicate the relevance of chemical communication in various social situations. Stern and McClintock showed evidence indicating that the menstrual synchrony is mediated by pheromones, axillary compounds, which have two opposing effects. Axillary compounds from donor women in the follicular phase (FP) shortened both the time to ovulation and the length of the menstrual cycle in recipients and those in the ovulatory phase (OP) delayed ovulation and lengthened the total cycle. From animal studies, it is thought that sexual pheromones activate the hypothalamus-pituitary-gonadal axis (HPG) and this leads to an increase in the frequency of pulsatile luteinizing hormone (LH) secretion and induces preovulatory events culminating in the LH surge, which will elicit ovulation. We therefore examined the effects of axillary compound on LH pulse. As a result, we found that the frequency of pulsatile secretion of LH in humans is increased by exposing recipients to axillary compounds in the FP and decreased by axillary compounds in the OP. These findings indicate that FP compounds facilitate the growth and maturation of ovarian follicles, whereas OP compounds retard them. We further studied the effect of a candidate human pheromone, 3α-androstenol, on the frequency of pulsatile secretion of LH. 3α-androstenol decreased the frequency of pulsatile secretion of LH. Furthermore, the women who showed menstrual synchrony had a high sensitivity to 3α-androstenol when we examined the relationship between menstrual synchrony and the ability to smell the 3α-androstenol. It is therefore possible that 3α-androstenol may be an axillary compound, which influence menstrual cycles. Therefore, it is likely that in humans, pheromones may modulate the timing of ovulation by changing the frequency of pulsatile LH secretion, which controls the rate of follicular growth and maturation.

Recently, we study about early human chemical communications between mother and child. Mother's breast milk odors have attractive effect on their infants, which is one of the fundamental behavioral responses that human infants share with other mammals. In addition to this effect, we showed easing effects of the mother's breast milk odors on their infant's pain stress response. We examined whether the mother's breast milk odors ease the stress responses to a capillary puncture on the heel (heelsticks) during routine blood draws to screen for congenital diseases in 5 days-old human infants. We found that stress responses (crying, grimacing, body movement) to the heelsticks were significantly attenuated by their own mother's breast milk odor. However, breast milk odor of mother of different infant or formula milk odor did not affect stress responses. Therefore, our findings suggest that maternal breast milk odor has not only an attracting effect but also a calming effect.

On the other hand, we also investigated whether the body odor of newborn infants influence on moods in postpartum mothers, because it is known that mothers are attracted to the body odor of newborn infants. Postpartum mothers were exposed to odors of a part of the undershirt their infant wear and recorded changes in their mood. We found that the infant body odor increased positive mood such as pleasantness, willingness, euphoria, friendliness and motherhood and attenuated negative mood scores such as anxiety and fatigue. Our findings suggest that body odors of newborn infants attract their mothers because they have a calming effect on postpartum mothers.

B–02 Behavioral Adaptations Using Chemical Cues in Rodents

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In animal kingdom, chemical cues from other conspecifics have great influences on behavioral responses. Chemical cues can contain various aspects of animal information, such as sex, age, kinship, individuality, and social status. Therefore, animals react to encountering co specific based on social information emitted from the other dyad, which means that social cognition based on chemicals has an essential role in behavioral responses in social contexts. In this talk, the following three topics are picked up and I will discuss how the chemical communication is important in these three social contexts. First example is the "aggression" in mice. A male mouse can distinguish between male and female mice based on chemical information, and he then shows different behavioral responses, namely aggression toward males and sexual approach to females. Besides sexual discrimination, individual recognition also plays a critical role in behavioral responses. A male mouse can distinguish between familiar and unfamiliar males, and does not show vigorous aggression toward familiar individuals. Second example is the "partner preference" in mice. Growing neonates memorize social odors while receiving parental cares, and in the future they express partner preference based on thus acquired olfactory memory, i.e., a potential sexual partner which emits similar odor tends to be avoided. Third one is the alarming pheromone, which is released from stressed animal and increased behavioral and physiological vigilance to danger in other conspecifs. This alarm pheromone could be divided into two categories, that is, the one released from whisker pad evokes behavioral responses testosterone dependently, and the one released from perianal region aggravates autonomic response in a testosterone-independent manner. In addition, the vomeronasal organ-excised (VNX) rats failed to show pheromonal effects, suggesting that this pheromone was perceived by the vomeronasal system. These behavioral phenomena indicate that social cognition is important, probably more than we have expected, for animal reproduction and survival.

B–03 Alternative Forms of Reproductive Regulation in Honeybees: Semiochemical Contests vs Physical Contests

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Exploring reproductive regulation in honeybee colonies will be the subject of this presentation. As a result of work that has been conducted over the last decade, it has become clear that this regulation is complex and involves interactions between queens to produce monogyny, between queens and workers to ensure worker 'sterility', and between workers themselves when they get the opportunity to reproduce.

Reproductive regulation in honeybee colonies depends on interactions between queens, between the queen and her workers, and between workers as a function of the colony social organization. In a normal queenright colony, the queen regulates the reproductive activity of her workers through the pheromones that she produces. Should a worker start laying eggs in such a queenright colony then her sister workers remove the eggs, resulting in worker policing. In this case, semiochemcials from the queen act as the primary control mechanism. Worker policing acts as a physical secondary form of regulation to maintain the reproductive monopoly of the queen. When colonies are preparing to reproduce by swarming, a number of virgin queens are produced. Once the primary swarm has left the colony with the old queen, the virgin queens remaining in the colony engage in physical contests that eliminate all except one queen. This individual becomes the single reproductive within the colony.

In queenless colonies, workers compete for the opportunity to reproduce. In these colonies, the workers engage in semiochemical contests that result in a limited number of individuals becoming reproductive.

In the Cape honeybee, *Apis mellifera capensis*, workers are capable of mimicking the semiochemcials produced by the queens and hence the semiochemical contests between workers are particularly intense. Reproductively dominant individuals are capable of regulating their fellow workers. Furthermore, if these dominant workers are able to invade queenright colonies, they are able to compete with the queen in the colony for reproductive dominance. Here the contest has both a semiochemical component that allows these individuals to be identified and a physical component in that both queens and workers attack these individuals.

Under certain experimental conditions, it is possible to have multiple queens in colonies that appear to coexist without conflict and all contribute to the reproductive output of the colony. The conditions in which physical conflict is avoided appear to be contingent on the possession competitive strategies that are context dependent.

The experimental studies that provide the evidence for the descriptions that have been given above will be discussed. They will demonstrate that reproductive regulation in honeybees is dynamic, context dependent and subject to a degree of behavioural plasticity that was not recognized until recently. Finally, exploration of the capacities for reproduction in Cape honeybee workers has led to the recognition that they are able to act as social parasites. Such social parasitism has been observed in a population of honeybees for the first time.

Key words: reproductive regulation, honeybees, pheromones, contests, social parasitism

B–04 Semiochemicals Inducing Social Behaviors in Honey Bees

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Social behaviors in honey bees can serve as models for investigating recognition and communications among intra- and interspecies as well as those towards environmental conditions. In response to the stimuli, individuals recognize the environmental conditions surrounding the colony. Semiochemicals play crucial roles in recognition and pheromones do in communication. Individual recognition and communications among colony members would lead to wide variety of social behaviors. Therefore, understanding the basic mechanisms of social behaviors attracts attentions of researcheres in the field of micro machines and brain sciences. How the flexibility of the honey bee colony arises, is one of the most attractive issues in our research. In this paper, aquisition and development in behavioral repertory between these two honey bees were investigated in connection with differences in recognition and responses to semiochemicals. In Japan, there are two *Apis* species, domestic honey bee (*Apis cerana japonica* Rad.; Acj) and imported honey bee, *Apis mellifera* L. (Am). There are behavioural differences between the two species. For example, Am collect propolis but Acj does not. Acj performs hygienic allo-grooming behavior against *Varroa* mites but Am seldom does it. When Acj workers with different ages (0, 7, 14, 17 days after eclosion) were exposed to *Varroa* mite extract, day 0 workers did not respond (0%), however 7 days and older workers responded (100%). It was observed that the older workers responded more quickly and persistantly upon exposure to the extract. Therefore, allo-grooming behaviour in Acj develops in accordance with the age after eclosion. Development of allo-grooming behaviour in Acj may be under hormonal control as is age-polyethism.

B–05 Cuticular Hydrocarbons, the Most Important Semiochemicals in Maintaining Complex Ant Societies

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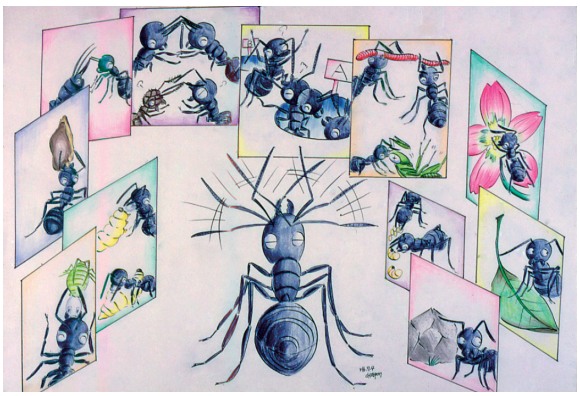
Ants are the most abundant and prosperous animal on earth, estimated to number ten to the sixteenth power. They are organized into complex societies and communicate with each other using several kinds of pheromones, such as alarm pheromone, trail orientation pheromone, sex pheromone, nestmate recognition pheromone, and others. Nestmate recognition pheromones are one of the most important pheromones system because they are basic to the maintenance of colony special structure. Individuals ants of the same species but different colonies recognize that they are different and fight each other.

The nestmate recognition chemical cues have been shown to be blends of colony specific cuticular hydrocarbons (CHC).(1) A special sensory sensillum on the antennae functions in nestmate discrimination. The sensillum is multiporous and responds to the non-nestmate CHC blends(quantitative differences) and also to different CHC compositions of the other ants species(qualitative differences). The cuticular surface of insects is composed mainly of CHC blends and the compositions are different and species-specific, like a bar code. On the other hand, ants must must be able to sense other objects they encounter inside or outside of the nest. During foraging they use their antennae to recognize volatile compounds in the air, as well as by touch the nonvolatile chemicals on the surface of objects.

When they encounter plants they never attack them because plants cuticular wax contains only nalkanes hydrocarbons that are not detected by th antennae of ant. Plant surface wax primarily consist of polar components. Some plants though have evolved ways to make ants serve as assistants, for example, in the case of seed dispersal of plants by ants, special olefin hydrocarbons present on the seed surface induce many species of ants to carry back the seeds to their nests. On the other hand, some plants evolved a strategy to prevent ants from stealing nectar from flowers. The CHC compositions on the surface of the flowers is different on the inside and outside. Ants are inhibited to go inside the flower, because of the presence of repellent olefin hydrocarbons on the inside surface of the flower.



Another example, generally when ants encounter insects they are perceived as pray and are usually, attacked, killed and carried back to their nest. But the situation is different if they encounter symbiont aphids, they never attack them but instead tap them with their antennae and the aphid responds by releasing nectar that the ant ingests. To the non-symbiont species of aphids, ants walk around over them looks like ants walk over plants. There are large differences between CHC compositions of the two types of aphids. Non-symbiont aphids are mainly covered by n-alkanes like the cuticular surface of plants. On the contrary, symbiont aphids possess n-alkanes, branched alkanes and olefins.

Another interesting example of differential and recognition focuses on the relationship between ants and lepidopteron larvae and pupae, ants never attack naked pupae of swallowtail butterfly but attack silkworm pupae without their cocoon. The former's surface is covered only by n-alkanes like the plant surface, but the latter are covered by ant sensitive hydrocarbons, e.g. branched alkanes and olefins.

Key cuticular hydrocarbons of animal kingdom and plant kingdom is basically different. This is the main reason why plant and animal can coexist friendly on the earth.

(1) Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. M. Ozaki, A. Wada, K., R. Yamaoka, et al. Science, 309, 311–314 (2005).

C–01 Green Leaf Volatiles: Inter- and Intra-Plant Signal Molecules

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Green plants form and emit C6-aldehydes, C6-alcohols, and their acetates. These green leaf volatiles (GLVs) are biosynthesized via the lipoxygenase/hydroperoxide lyase (HPL) pathway from polyunsaturated fatty acids such as linoleic and linolenic acids. It has been reported that GLVs negatively affect herbivore performance and play an important role in the recruitment of the carnivorous natural enemies of herbivores. Further, GLVs are known to have antifungal activity. In intact, healthy plants the amounts of GLVs are low, but they are rapidly formed after mechanical wounding on plant tissues. These cumulating evidences indicate that GLVs are involved in plant responses against various stresses that would cause disruption of plant tissues. In order to estimate significance of GLVs in plant resistance against pathogens, we have been analyzing *Arabidopsis-Botrytis cinerea* as a model system. The amounts of GLVs in *Arabidopsis* were rapidly increased after inoculation of a necrotrophic fungal pathogen, *B. cinerea.* When intact *Arabidopsis* plants were exposed to GLVs, the plants showed higher resistance to *B. cinerea*, than the unexposed plants. This higher resistance could be at least partly accountable to upregulation of a subset of defense genes, lignification of cell walls, and accumulation of antifungal compounds[1]. When a transgenic *Arabidopsis* whose *HP* gene expression was suppressed was infected with *B. cinerea*, higher susceptibility could be observed. In the *HPL*-suppressed plant, expression of *PR3* that is involved in resistance response against the pathogen was significantly retarded. Taken together, it was suggested that GLVs were one of important signal molecules to elicit resistance responses in *Arabidopsis* as well as fungicidal compounds that inhibited growth of *B. cinerea* on the plants.

In this experiment, plants were exposed to vapor of GLV, from which it was expected that GLVs formed from infected plants could induce defense response in the neighboring plants. Analysis of volatiles emitted from *B. cinerea*-infected plants showed that GLVs were major components formed upon infection among other compounds such as 1-octen-3-ol and benzylaldehyde. When a healthy plant was placed next to the infected plant for 12 h, it showed higher resistance to *B. cinerea* that was infected on the treated plants subsequently. Because higher resistance could also be found when the plants were exposed to vapor of artificial volatile blend made according to the volatiles emitted from infected plants, it could be concluded that volatiles including GLVs were cues to elicit defense responses in the neighboring plants.

These observations indicated that GLVs could be signal molecules within and between plants at least under a specified condition. Further study to estimate their relevance in natural habitats must be performed.

[1] Kishimoto, K., Matsui, K., Ozawa, R., Takabayashi, J., *Plant Cell Physiol.* **2005***, 46*, 1093.

Key words: green leaf volatiles, *Arabidopsis*, *Botrytis cinerea*, induced defence

C–02 Natural Flavonoids in Plants as Ultraviolet Shields

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Ultraviolet-absorbing substances in two plant species, *Rheum nobile* (Polygonaceae), which grows in the alpine zone of the eastern Himalayas, and *Davidia involucrata* (Davidiaceae), which is endemic to China, were isolated from the translucent bracts. Five kinds of the UV-absorbing substances were found by HPLC and PC surveys from their species, respectively. All of the five major compounds were flavonoids. The flavonoids in *R. nobile* were identified as quercetin 3-glucoside, quercetin 3-galactoside, quercetin 3-arabinoside, quercetin 3-rutinoside and quercetin 3-[6″-(3-hydroxy-3-methylglutaroyl)-glucoside] by UV, ^{1}H and ^{13}C NMR, MS, and direct TLC and HPLC comparisons with authentic specimens. Of their flavonoids, the latter one was reported in nature for the first time. On the other hand, the flavonoids in *D. involucrata* were identified as quercetin 3-glucoside, quercetin 3-galactoside, quercetin 3-arabinoside, quercetin 3-sambubioside and quercetin 3-acetylglucoside. The translucent bracts of *R. nobile* accumulate a substantial quality of flavonoids (3.3–5 mg per g dry material for the major compounds).

References: Iwashina, T. et al., J. Plant Res. **117**: 101–107 (2004). Takemura, S. et al., Polyphenols Communications p. 255–256 (2006).

Key words: quercetin glycosides, flavonoids, UV-absorbing substances, *Rheum nobile*, *Davidia involucrata*

C–03 Allelopathy of Semi-Arid Region Plants

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Secondary metabolites are released from plants that might be beneficial from plants or detrimental to the growth of receptor plants. These compounds are involved in the environmental complex of managed or natural ecosystem. Allelopathic compounds have been shown to play important role in determination of plant diversity, dominance and succession and climax of natural vegetation and in the plant productivity of agro ecosystems. The indiscriminate use of harmful synthetic herbicides often causes environmental hazards, imbalance of soil microorganisms, nutrient deficiency and change of soil physico-chemical properties resulting in decrease of crop productivity. The incorporation of allelopathic substances into agriculture management may reduce the synthetic herbicides, fungicides, and insecticides and thus lessens the environmental deterioration. It is well known that most of the volatile compounds such as terpenoids are released from plants grown in draught or arid region.
Some common trees such as *Prosofis juliflora*, *Prosofis cineraria*, Parthenium hysterophorus, *Lantana camara*, *Broussonetia papyrifera* are some common exotic species in semi-arid zones, Rawalpindi and Pothohar region of Pakistan. These plants are exotic species and grow luxuriantly on sandy soil in arid zones due to their fast growth in soil binding capacity.
We have studied the allelopathic behavior of *Prosofis juliflora*, *P. Cineraria. Lantana camara*, *Broussonetia papyrifera* and *Parthenium hysterophorus* on germination and seedling growth of lettuce by sandwich method. Among these *Prosofis juliflora* has been found allelopathically most active. Chromatographic identification of the phytotoxins present in these plants will also be carried out along with the soil analysis collected under these trees for their seed germination and seed growth inhibition potential.
Key words: exotic species, arid zone, allelopathy and sandwich method

C–04 Invasive Plants and Allelopathy

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Exotic plants threaten the integrity of agricultural and natural systems throughout the world. Many invasive species are not usually dominant in their native regions, but once they invaded into new regions, they competitively eradicate their new neighbors. Callaway and his colleague published many papers demonstrating the importance of allelopathy of dominating invasive alien plants in North America (Science, 2000).

In Japan, the "Invasive Alien Species Act" (Law No. 78) has just started 2006, with the purpose of preventing adverse effects of IAS to Japanese ecosystems, human safety, or agriculture. Allelopathy and toxic chemicals involved in invasive alien plants are serious problems. We started a new national project named "Risk assessment of alien plants and their control". To know the allelopathic or toxic nature of invasive alien plants, we started several examples of allelochemicals from alien plants in Japan will be demonstrated.

1) *Leucaena leucocephala*: An invader to tropical and subtropical island has an unusual amino acid, mimosine, isolated as the major allelochemical in this leguminous tree.

2) *Bishofia javanica*: Native to south east Asia, but now becoming invader in Ogasawara Island, has tartaric acid as allelochemicals.

3) *Prosopis juliflora*: Leaching of the allelopathic substance, L-tryptophan from the foliage of mesquite was demonstrated. Juliflorine, a unique alkaloid, was also detected as major allelochemical.

4) *Sphenoclea zeylanica*: A serious paddy weed in the Asian tropics. We have isolated a new type of allelochemicals and named as "zeylanoxide". This compound is new type of dithiolane compound.

Allelopathic activities of alien plants were evaluated using sandwich method (for root exudates), plant box method (for leaf leachates), dish-pack method (for volatile chemicals). As a result of these three assay, potentially dangerous alien plants with high allelopathic activity found to be: *Coccinia grandis*, *Rottboellia cochinchinensis*, *Fumaria capreolata*, *Phalaris brachystachys*, *Physalis angulata*, *Gypsophila paniculata*, *Oenothera hookeri*, *Trifolium incarnatum*, *Ipomopsis rubra*, *Silene armeria*, *Avena strigosa*, *Anisantha madritensis.* Estimation of their invasion and toxicity to the environment, and the potential allelochemicals will be discussed.

Key words: Invasive Alien Plants, Allelochemicals, Sandwich Method, Plant Box Method, Dish-pack Method

C–05 Induction of Rice Signaling Pathway and Defense Related Genes by Infection of Pathogen of Rice Blast Disease (*Magnaporthe grisea*)

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Allelopathy includes the chemical relations of plant to other plants, microorganisms, insects and pathogens. The pathogen of rice blast disease, *Magnaporthe grisea*, could invade rice plant through leaves or root. Can the invasion through root systems trigger the signaling pathway and defense related genes both in root and in leaves? Near isogenic lines of rice with or without resistant gene were used along with spores of specific isolates of *Magnaporthe grisea* for inoculation through leaves or roots of rice seedling. Salicylic acid (SA) and jasmonic acid (JA) as well as their inhibitors were used to induce or stop to induce the relative genes for defense. By means of RT-PCR, Real-time PCR and the tests of key enzyme activity, the differences of genes expression, the quantity of expression and the location of expression were found. The inoculation of *M. grisea* in root system could increase the enzyme activity levels of phenylalanine ammonia-lyase (PAL) and Lipoxygenase (LOX) both in root and in leave. The infection of *M. grisea* to rice root could directly induce the expression of the defense response genes and some key enzyme genes which involved in the signal transduction pathway, such as OsLOX, *OsAOS*, *OsPAL*, *OsPR*, *OsMyb*, and *OsBBPI* in root, and also could indirectly induced those genes in leaves. The infection of *M. grisea* to rice leaves could have the same systematic induction effect. The expressions of the major related genes in leaves were much higher than the same genes in roots no matter directly or indirectly induced. The cross talk between SA and JA pathway within rice plant were found.

Key words: rice blast disease, jasmonic acid, salicylic acid, signal transduction

D–01 Chemical Input and Neuronal System for Aggressiveness in a Japanese Carpenter Ant

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Ants have developed a sophisticated chemical communication system that enables them to reject non-nestmate conspecifics and to accept nestmates. Many behavioral experiments have suggested that their aggressive behavior against non-nestmates is evoked by contact chemosensory detection of differences between colony-specific chemical signals of cuticlar hydrocarbons.

Previously, we describe a novel contact chemosensillum on the antennae of the Japanese carpenter ant *Camponotus japonicus* that functions in nestmate discrimination. This sensillum is multiporous and houses >100 receptor neurons. Within the sensillum, we found a chemosensory protein, which can dissolve the CHCs in the hydrophilic surroundings of the receptor membranes. Using the recombinant protein, we dissolved the CHCs derived from different colonies in stimulus solutions and gave them to the sensillum. The results indicated that the sensillum responded only to the non-nestmate CHCs with vigorous impulse discharge but not to the nestmate CHCs. Thus, we proposed that in addition to the traditional dynamic "neural template hypothesis", a peripheral mechanism may act as a "filter" for nestmate recognition.

Through this discriminative peripheral system, chemical information of non-nestmate is changed to electrophysiological impulses and sent to the brain. By following the axonal fibers of the receptor neurons in these particular CHC sensilla, we histologically identified the neuronal center for aggressiveness in the antennal lobe, a primary center of olfaction. This region consisted of 136 glomeruli. Histological staining for detecting NOS activity in the ant brain suggested that almost all regions in the antennal lobe produce NO as a neurotransmitter, but this region would not be stained with this method. Comparing with other casts, we observed that males and queens had no such region in the antennal lobe. They also have colony specific CHCs on the body surfaces but would not be so aggressive as workers, when meet each other.

Additionally, we comparatively investigated nestmate and non-nestmate discrimination in a representative unicolonial ant species, Formica Yassensis, whose workers can associate with each other beyond nests. This species also has the CHC sensilla, but those sensilla could respond not only to non-nestmate CHCs but also nestmate CHCs at some magnitude. Thus, this species should determine a behavioral threshold at the brain level.

Key words: ant, pheromones, nestmate recognition, chemosensory, antennal lobe

D–02 The Complexity of Fire Ant Nestmate Recognition

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Fire ants, *Solenopsis invicta* and *Solenopsis richteri*, were inadvertently introduced into the United States in the early 1900s and currently inhabit over 129 million hectares in Puerto Rico and twelve southern states from Texas to Virginia. Imported fire ants have also become established in isolated sites in California, New Mexico, and Maryland. They are expected to move upward along the Pacific coast, southward into Mexico and the Caribbean, and northward in Oklahoma, Arkansas, Tennessee, and along the eastern seaboard into Maryland and possibly Delaware. Within the past 10 years populations of *S. invicta* have been found in Australia, Taiwan, and mainland China. Fire ant population densities in the United States are very high. Workers are aggressive and have a potent sting and they have a high reproductive potential. The medical, agricultural, and other costs of fire ant control and damage in the United States are estimated at nearly six billion dollars per year.

Mature monogyne (single queen) fire ant colonies contain 100,000 to 250,000 workers and reach infestation rates of over 130 mounds per hectare. In the last several decades, polygyne fire ant colonies (multi-queen colonies) appear to be proliferating in the southern United States, with mound densities up to 500 per hectare. Monogyne *S. invicta* colony workers are territorial and aggressive toward members of other fire ant colonies. In contrast, polygyne colony workers are not aggressive toward non-nestmates, presumably due to broader exposure to heritable and environmentally derived nestmate recognition cues (broad template). The nestmate recognition system for the two social forms is very complex, and may by correlated with a single gene, *Gp-9*, especially for acceptance or rejection of newly mated queens. Workers from both monogyne and polygyne fire ant colonies execute newly mated queens after mating flights. We discovered that after removal of their colony queen, monogyne worker aggression toward non-nestmate conspecifics quickly drops to investigative levels; however, heterospecific recognition/aggression remains high. Queenless monogyne or polygyne worker groups were also not aggressive toward newly mated queens. Queenless worker groups of both forms that adopted a monogyne-derived newly mated queen became aggressive toward non-nestmate workers and newly mated queens. We suggested that this powerful effect of queens on conspecific nestmate recognition is caused by a queen produced recognition primer pheromone that increases the sensitivity of workers to subtle quantitative differences in nestmate recognition cues. Biogenic amines have been reported to modulate the sensitivity of insects to stimuli. We used this information to probe the primer pheromone/endocrine basis of nestmate recognition in fire ants. Queenright colonies were divided into three components: queenright, queenless, and queenless fed the biogenic amine, octopamine (OA). Queenright colonies maintained high aggression levels. In contrast, queenless workers fed only crickets and aqueous sucrose had low aggression levels. Workers that were fed octopamine had aggression levels that were not significantly different from queenright workers. Feeding OA to fire ant workers was adequate to simulate the presence of the queen, in terms of nestmate recognition. Thus, we have strong evidence that the queen recognition primer pheromone acts on workers to maintain high levels of OA that up—modulates worker sensitivity to the subtle changes in intraspecific nestmate recognition cues. This work was supported in part by BSF Grant No. 2003367.

Key words: fire ant, nestmate recognition, biogenic amine, aggression

D–03 Odor and Taste Receptors in *Drosophila melanogaster*: A Functional Analysis

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An animal's sensory systems extract a representation of the vast information content in the environment, a representation that we assume is relevant to its survival and reproduction. We are investigating the receptors for smell and taste in the fruit fly *Drosophila melanogaster* to understand how such a representation of the chemical world is constructed. The olfactory receptors we study belong to a highly diverse family of 60 genes. A detailed investigation of one olfactory receptor gene, *Or22a*, has shown that it is expressed by a subset of olfactory receptor neurons (ORNs) in the antenna which respond preferentially to a number of esters, including ethyl butyrate. These ORNs lack odor sensitivity entirely in a deletion mutant missing *Or22a*; rescue experiments whereby the receptor is supplied on a transgene fully restore the odor response. We have developed this mutant neuron into an *in vivo* expression system that allows us to decode odor specificities of many of the receptors. Ectopic expression of different receptors in this system has shown that they can be narrowly or broadly tuned, and that in addition to ligand specificity they may determine other response properties including the temporal dynamics of odor response. By comparing the ligand specificities of receptors ectopically expressed in the *in vivo* system with the known ligand specificities of the ORNs in the antennae, we have started to construct the odor-to-receptor-to-neuron map of the *Drosophila* olfactory system. Though many of the olfactory receptors mediate responses to chemicals released by plants and their fruits, a subset of four receptors appear to mediate responses to chemicals in hexane extracts of the flies, including to the candidate anti-aphrodisiac pheromone *cis*-vaccenyl acetate. A second family of receptor genes identified from the genome database was proposed to encode gustatory receptors. This *Gr* gene family is similar in size to the *Or* gene family suggesting that the "gustatory world" of flies may rival the breadth of olfactory perception. We have shown that one member of this family, *Gr5a*, is necessary for the reception of the sugar trehalose and we are investigating the specificity of the receptor protein. Identification of the receptors and their ligands, both food- and fly-related, will allow us to develop tools to dissect what role the representation of the chemical world plays in the life of the fly.

Key words: Drosophila, olfaction, receptor genes, odor coding

D–04 Insights into the Mechanism of Ligand Binding and Release of the Pheromone-Binding Proteins from the Gypsy Moth, *Lymantria Dispar*

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The great selectivity of moths to pheromone sources is a very interesting topic in the field of olfaction. At the molecular level, the abundant pheromone-binding protein (PBP) in the moth antennae, has been proven to be essential for insect olfaction. We have investigated the binding process of the two known PBPs from the gypsy moth, *Lymantria dispar*, with different ligands. Specifically, kinetic studies have revealed that the binding process is biphasic, and the slow phase leads to equilibrium ligand binding. Other studies have revealed that equilibrium binding occurrs at the known internal binding site of the protein. The slow process follows first-order kinetics in both protein and ligand concentrations when ligand is insufficient and is independent of ligand concentration when it is in excess. This result implies that ligand binding occurs in at least two stages: first, the protein and the ligand must collide and second, the ligand moves into the internal binding pocket. The selectivity of the moth olfaction might come from the different speeds of the ligand migration into the binding pocket.

Key words: pheromone-binding proteins, ligand association and dissociation kinetics, mechanism, ligand selectivity

D–05 Insect-Machine Hybrid Systems — Novel Neuroethological Approaches for Analyzing Adaptive Behaviors —

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Insects can adapt to various environmental changes and perform sophisticated behaviors by the processing of the simple nervous system as so called microbrain systems. Since the adaptive behaviors are elicited by interactions between insects and environments, it is preferable to analyze them under closed-loop conditions. In order to understand the neural mechanisms underlying these behaviors, here we propose two novel approaches: (1) an insect-controlled robot and (2) a behavioral simulator driven by neural activities. In these approaches, we extrapolated machines or computers into the closed-loop to modify the interactions artificially between insects and environments. In the first approach, we built an insect-controlled mobile robot, which was a two-wheeled mobile robot. The robot moved based on locomotion of a male silkmoth (*Bombyx mori*) on board. In order to produce unintentional movements to the moth on the robot, we artificially altered motor gains of the robot in relation to actual locomotion of the moth. Under these conditions we evaluated how the insect could adapt to the new circumstances. In the second approach, we built up a behavioral simulator composed of two parts: a recording system of neural activities (i.e., controller) and a computer-based behavior simulation (i.e., virtual agent). We reproduced a pheromone-searching behavior on the computer by controlling the model silkmoth using real neural activity of the neck motor neurons which contain steering information. Thus, we built a closed-loop conditions and modified various parameters around the loop. These new methods extrapolating controllable systems into the closed-loop will contribute to evaluating and understanding the adaptive behaviors in insects.

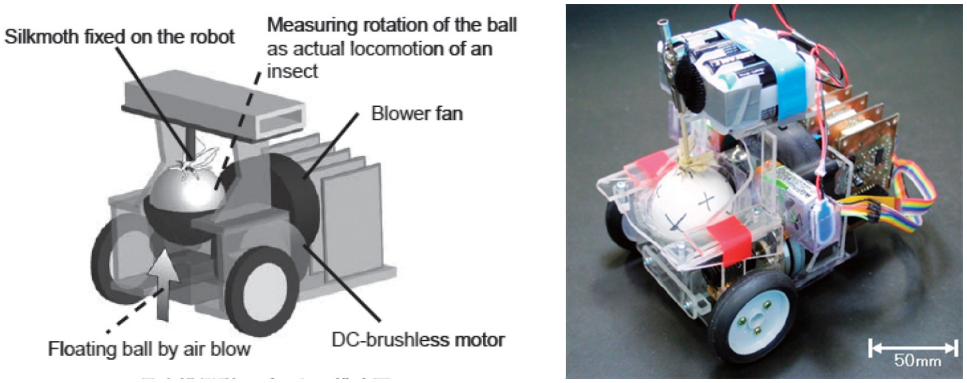

Fig.1. An insect-controlled robot.

This study was supported by MEXT (Scientific Research on Priority Areas 454 (mobiligence)) and JSPS (Exploratory Research 18656075).
Key words: insect, silkmoth, adaptive behavior, robot, pheromone, olfaction, hybrid system, extracellular recording, microbrain, motor neuron, BMI

E–01 Diversity of Anti-Herbivore Defenses in *Macaranga myrmecophytes*

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Plants develop various modes of anti-herbivore defenses to reduce herbivory damage. In general, plant species are armed with chemical or physical defenses against herbivores, and sometimes further adjust life histories to accommodate seasonal changes in potential herbivory. In the tropics, many plants in diverse taxa have been evolutionarily specialized to have mutualistic relations with ants that function as effective anti-herbivore agents. Above all, "myrmecophytes", which are defined as plants that provide nest sites and sometimes nutrients for the ants, are highly specialized to symbioses with ants. They derive benefit from symbiosis because ants protect their nest plants from various invaders.

Macaranga (family Euphorbiaceae) is a tree genus of approximately 280 species, distributed from West Africa to South Pacific islands. The genus includes at least 20 myrmecophytic species. Most of the myrmecophytic species have symbioses with their specific *Crematogaster* ant species. Some of these *Macaranga* myrmecophytes often co-occur and widely spread into various microenvironments in tropical rain forests.

As well as most other myrmecophytes, *Macaranga* myrmecophytes intensively depend on their symbiont ants for anti-herbivore defenses. However, symbioses with ants are not necessarily beneficial for them, because ant defenses require some metabolic costs from the plants. To provide ants with nutrients and energy sufficient for defensive activities against herbivores, plants turn resources from growth, reproduction, and non-ant defenses (chemical and physical anti-herbivore defenses) toward symbiotic ants. Therefore, under constraint of limited resources, myrmecophytes are expected to face trade-off between ant defenses (anti-herbivore defenses by symbiotic ants) versus non-ant defenses. However, the questions remain to be solved how do myrmecophytic plants deal with the trade-off between ant defenses versus non-ant defenses, or between ant defenses versus other components in the life history, and what environmental or biological factors affect the plant traits involved in the tradeoff? For understanding the trade-off, we attempted to determine interspecific variation in balance of two-modes of anti-herbivore defenses, *i.e.* ant defenses versus non-ant defenses. Targeting several sympatric *Macaranga* myrmecophytes and non-myrmecophytes, the balance was quantified by assessment of the intensity of the two modes of defenses. For assessment of ant defenses, we conducted field-experiments with exclusion of symbiont ants, assayed ant aggressiveness and measured the ratio of ant colony biomass to host plant biomass. For assessment of non-ant defenses, we conducted a bioassay with larvae of a polyphagous moth, *Spodoptera litura.* In the bioassay, we determined the growth performance and survival of larvae when they were fed with artificial diet (suitable food), fresh leaves of the several target *Macaranga* species, and artificial diet mixed with different dosage of dried-powder of the leaves.

The results demonstrated the clear trend that non-ant defenses were more effective in the *Macaranga* that was less defended by ants. This trend was true in comparison among myrmecophytic species and also in comparison between myrmecophytic versus non-myrmecophytic species. These trends in interspecific variation in the balance between the two modes of defenses are likely consistent with the constraints inferred from the trade-off that *Macaranga* might face.

We will discuss the factors affecting plants' diversification in relation to the trade-off and the plausible consequences of the diversification in the *Macarang*-centred community.

Key words: mutualism, ant-plant interactions, Crematogaster ants, symbiosis, Southeast Asia

E–02 Diverse Ingredients in Plant Latex and their Defensive Roles against Herbivorous Insects: Case Studies in Papaya, Fig and Mulberry Trees

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Plant latex is widely found in plant species (ca. 12,000–35,000 species). The hypothesis that the role of plant latex is defense against insect herbivory is prevalent, but supported only by a few well-known examples (such as milkweed latex with noxious cardenolides and their defensive roles against herbivorous insects), and the ecological roles of latex and their material bases remain unstudied in most latex-exuding plants. We here show novel examples from our studies where diverse latex ingredients, proteins and chemicals, play crucial roles in plant defense against insect herbivory.

The first example is about cysteine proteases in papaya and fig latex and their defensive roles against caterpillars. We found that leaves of the Papaya tree (*Carica papaya*) and a wild Fig tree (*Ficus virgarta*) are fatally toxic against lepidopteran larvae such as oligophagous *Samia ricini* (Saturniidae) and two notorious polyphagous pests, *Mamestra brassicae* (Noctuidae) and *Spodoptera litura* (Noctuidae), though no apparent toxic factors from these species had been reported. A serious damage was observed in the midgut epithelium layer of the larvae fed papaya leaves. When the latex was washed off, the leaves of papaya lost toxicity. Latexes of both papaya and the wild fig were rich in cysteine-protease activity. E-64, a cysteine protease-specific inhibitor, completely deprived the leaves of toxicity when painted on the surface of papaya and fig leaves. Cysteine proteases, such as papain, ficin, and bromelain all showed toxicity. The results suggest that plant latex and the proteins in it, cysteine proteases in particular, provide plants with a general defense against herbivorous insects.

The second example is about sugar-mimic alkaloids and a novel defense protein in mulberry latex. Since leaves of the Mulberry trees (*Morus* spp.) have been used to rear the silkworm, *Bombyx mori*, and the silkworm grows well on them, their toxicities and defensive activities against herbivorous insects have been neglected. However we found that mulberry leaves are highly toxic to caterpillars other than the silkworm, *B. mori*, (such as *S. ricini* and *M. brassicae*), due to the ingredients of the latex. The toxicity of mulberry leaves was lost when latex was washed off, and latex-added artificial diets showed toxicity. Mulberry (*M. australis*) latex contained very high concentrations of alkaloidal sugar-mimic glycosidase inhibitors, such as 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1), 1-deoxy nojirimycin (DNJ), and 1,4-dideoxy-1,4-imino-D-ribitol. Their concentrations, altogether, in latex reached 1.5–2.5% (8–18% to dry weight) in several mulberry varieties, which were 100 times the concentrations previously reported from whole mulberry leaves. The sugar-mimic alkaloids account for approximately half of the defense activity of mulberry latex against lepidopteran larvae. The remaining half comes from novel defense protein that we found recently in mulberry latex. The defense protein showed growth-inhibitory effects to lepidopteran insects in low concentrations, and the sequence data showed that it is a novel defense protein deferent from all other known defense proteins. Interestingly, the sugar-mimicking alkaloids toxic to *S. ricini* (generalist) larvae were not at all toxic to the silkworm, *B. mori* (mulberry-specialist). While sucrase in digestive juice and tissue trehalase activities of *S. ricini* was inhibited efficiently by sugar-mimic alkaloids, those of *B. mori* was not at all inhibited even by very high concentrations of sugar-mimic alkaloids, suggesting that *B. mori*, has adapted themselves to latex-borne defense (sugar-mimic alkaloids) by enzyme-level adaptation.

The above findings suggest that plant latexes are treasuries of bioactive substances (chemicals and proteins) that play key roles in plant defense and plant-insect interactions.

Literature

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Key words: Plant latex, Plant Defense, Plant-Herbivore Interaction, *Carica papaya*, *Morus australis*

E–03 Behavioral Sabotage of Plant Defense: Chemical Releasers of Trenching by Insect Herbivores

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Over 20,000 species of plants release latex from elongate canals when damaged. Other plants emit resin, mucilage or other fluids. Insect herbivores on these plants commonly sever veins or cut trenches in leaves before feeding beyond the cuts. The insects thereby depressurize the canals and reduce their exposure to exudates during feeding. Whether the cuts function to reduce insect exposure to noxious chemicals in exudates, to adhesives, or both has been debated. Using a trenching bioassay with cabbage loopers, *Trichoplusia ni*, I have documented that adhesives are relatively unimportant in eliciting trenching. Three compounds that trigger trenching have been identified: lactucin from lettuce latex, myristicin from parsley, and lobeline from cardinal flower. All three compounds affect the nervous system of mammals. Other neuroactive chemicals, including some neurotransmitters, insecticides and drugs, also elicit trenching. The known toxicity of many of these compounds supports the hypothesis that cabbage loopers trench specifically to reduce their ingestion of noxious exudate constituents. Trenching assays thus have potential for directing the isolation of novel plant compounds with insecticidal properties and potential applications in pest management or pharmacy. Most insects that employ vein cutting or trenching are dietary specialists. Their ability to disable canals by severing veins has not resulted in host range expansion. I will explore the hypothesis that the diverse chemical constituents within exudates and unavoidable contact with these chemicals during canal-cutting and feeding favor specificity despite the vulnerability of canals to behavioral sabotage.

Cabbage looper cutting trench in prickly lettuce, *Lactuca serriola*, (Asteraceae).

Key words: insect-plant interactions, latex, trenching, neurotoxins, *Trichoplusia ni*

E–04 Addiction to Pyrrolizidine Alkaloids in Male Danaid Butterflies: Delving into the Evolutionary Origin of Pharmacophagy

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Close associations of some lepidopteran taxa with pyrrolizidine alkaloids (PAs) have long drawn attention and have been well documented in regard to pharmacophagy. In butterflies, adults, particularly males, of the Danaidae and Ithomiidae are often observed to congregate on decaying or dead plants containing PAs in the families Boraginaceae, Asteraceae, and Fabaceae, from which they imbibe the alkaloids. In Lepidoptera, ingested PAs have been shown to be utilized as precursors for pheromone biosynthesis and also for chemical protection against predators. Male adults of most danaids have two types of androconial organs, the hairpencil and the sex brand, from which they are believed to disseminate aphrodisiac pheromones. Another PA-insect relationship is found in the egg-laying by a primitive danaid butterfly, *Idea leuconoe*, which exploits macrocyclic PAs present in its host plant as oviposition stimulants. We have been studying danaid-PA association for several years from the evolutionary viewpoint of their pheromone system. Major findings we obtained through the investigation on two danaids, *Euploea mulciber* and *Parantica sita*, are summarized below:

〈***Euploea mulciber***〉

1. Male adults secrete 9,10-epoxytetrahydroedulan (ET) and (2*S*,3*S*)-viridiflorine β-lactone (VL) from the hairpencils. ET is biosynthesized *de novo* only after eclosion from nutrients ingested during the larval development. **2.** Males are attracted exclusively to particular PAs such as intermedine/lycopsamine (I/L), and have a strong feeding preference for them. VL is produced only when the butterfly ingests I/L. Males are endowed with gustatory organs specifically responsive to certain PAs on mid- and/or hind-legs as well as on the proboscis. **3.** Both ET and VL act as sex pheromones in the sequence of courtship, but even males lacking VL can mate at a success rate of ca. 30%. However, males seem to devise a strategy for enhancing the rate of mating success by making use of PA-derived VL in addition to 'self-made' ET. **4.** Uptake of I/L by males markedly increases the fertility of eggs deposited by females that mated with those males. Male ingestion of PAs thus elevates female fitness. Consequently, males that acquire PAs can transfer more genes of their own to their progeny than those that do not. This would certainly operate to maintain the male trait of PA feeding.

〈***Parantica sita***〉

1. Male adults secrete two well-known dihydropyrrolizines, i.e., danaidone (DO; major) and (*R*)-hydroxydanaidal (HD) from both the hairpencil and the sex brand. **2.** DO and HD are efficiently biosynthesized when males ingest I/L, which they prefer over other PAs, while intake of heliotrine or retronecine results in the production of a smaller amount of DO. **3.** The two components are first produced in the sex brand, and then in the hairpencil. Their formation in the hairpencil, however, requires physical contact between the two androconial organs. **4.** Both components serve as the sex pheromones; males without the pheromone can rarely mate.

The retention of PA-feeding habit by males may be well explained by their use of PA-derived sex pheromones. However, taking account of the limited availability of PAs as pheromone precursors, influence of PA ingestion on the fertility of eggs, and other findings, we propose that pharmacophagy found in danaids and related taxa originates from their ancient use of particular PAs as physiologically important agents crucial for successful reproduction rather than defensive or aphrodisiac use.

Key words: danaid butterfly, pyrrolizidine alkaloid, pharmacophagy, androconial organs, sex pheromone

E–05 An Appreciation of the Elegant Utilisation of a Potent Plant-derived Attractant, Methyl Eugenol by the Male Oriental Fruit Fly: from Ecology to Physiology

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Sexually mature male Oriental fruit fly *Bactrocera dorsalis* is strongly attracted to feed voraciously on methyl eugenol, a naturally-occurring phenylpropanoid found both as a plant secondary metabolite, and a component of essential oils found in over 200 species of plants from 46 families. It is now widely acknowledged that the central role this important attractant plays in the interrelationships between methyl eugenol-sensitive fruit fly species with their host plants and predators. Consumption of this phenylpropanoid is known to confer male mating advantage and protection against vertebrate predators. Further, methyl eugenol and its derivatives have also been shown to be involved in the pollination of certain *Bulbophyllum* orchids by *Bactrocera* fruit flies. These findings highlight the fascinating interactions between insects and their non-host plants in the natural ecosystems. Our investigations into the physiological transport of the attractant-derived sex pheromonal components in *B. dorsalis* began when we reported ME-fed male producing chemical cues that were more attractive to conspecific females compared with ME-deprived male. Upon consumption, the male fly biotransforms methyl eugenol into sex pheromonal components, 2-allyl-4,5-dimethoxyphenol and (*E*)-coniferyl alcohol in the crop organ. The rectal gland then sequesters these phenylpropanoids, prior to their release during dusk. We wanted to ascertain if these pheromonal components were transported from the crop organ to the rectal gland via the hemolymph or the midgut. As these pheromonal components are also male attractants, we then developed a biodetection, a simple technique using conspecific and sexually mature male flies to detect for the presence of these components in the hemolymph and midgut. The pheromonal components were detected in the hemolymph and not the midgut. These results were later substantiated by GC-MS analyses. Further, using physiological experiments—parabiosis, transplantation and transfusion, we demonstrated that the hemolymph was responsible in transporting the pheromonal components from the crop to the rectal gland. Subsequently, using column chromatography, bioactive fractions containing the methyl eugenolderived sex pheromonal components from the hemolymph have been separated and identified using biodetection and GC-MS analyses. We have also been able to detect the appearance of peptide bands in the fractions using the SDS-PAGE technique. These findings suggest the role of the bioactive peptides as pheromone-binding peptides in the transport of the attractant-derived sex pheromonal components.

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Key words: *Bactrocera dorsalis*, biodetection, hemolymph, methyl eugenol, sex pheromone

F–01 Mating Disruption for Control of the Sugarcane Click Beetle, *Melanotus okinawensis* Ohira (Coleoptera: Elateridae), with Synthetic Sex Pheromone

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The sugarcane click beetle, *Melanotus okinawensis* Ohira (Coleoptera: Elatelidae), is the most destructive insect pest of sugarcane in Okinawa and Kagoshima Prefectures. Larvae of this species injure underground buds, which results in germination failure, dead-hearts, and rationing failure. To reduce crop damage, a large amount of insecticide is applied to the sugarcane fields before planting or during the early growth period to control the larvae. However, water pollution is a great concern in this area because people rely on groundwater as the source of their drinking water. Therefore, alternative tools for controlling this pest are desired. Mass trapping has been conducted to control the click beetle species in various regions. However, after ten years of control by mass trapping on several islands, ratooning failure of the sugarcane due to wireworm attacks is still a major problem. To control the adults of *M. okinawensis*, mating disruption using sex pheromone was attempted on cultivated land and in Japanese pampas grass fields on Minami-Daito Is. (3,057 ha), from early March to early June to cover the adult emergence period from 2001 to 2007. Polyethylene tubing dispensers were obtained from Shin Etsu Chem. Co., Ltd. (Tokyo). One roll of dispensers was 80 m long and contained 75g of dodecyl acetate. The polyethylene tube was sealed with a 2 mm-wide strap every 20 cm to prevent liquid leaking. One roll dispensers per hectare was set in ratooning and harvested sugarcane fields; three roll dispensers per hectare were set in newly planted sugarcane fields. Eighty-meter-long dispensers were set evenly in the field and were supported by bamboo sticks. A total of 1,850 roll dispensers was set up in the sugarcane fields. Dispensers were also set up at the edges of tree belts and attached directly to the trees at 1 to 1.5 m above the ground at the rate of 80 m of dispensers per 100 m of tree belts. A total of 900 roll dispensers were set up in the tree belts. A total of 1,000 roll dispensers were cut in 120 cm long and were scattered in the Japanese pampas glass field on the outer fringe of the island using a helicopter. Therefore, a total of 3,750 roll dispensers were used for mating disruption. The mean trap catch of 24 monitoring traps in 2001 (7.08/trap) indicated a 96.1% reduction from that of 2000 (181.51/trap: untreated). The mean trap catch in the treated area yearly decreased to 26.6% (1.88/trap) in 2007. Furthermore, a total of 72 wild adults was captured by hand in the treated area in 2001, and the number of wild adults decreased yearly to 12.5% (nine adults) in 2007. However, the number of wild adults captured in the untreated area on Miyagi Island throughout the seven years did not exhibit a similar reduction. Annual mating ratios of wild females on the treated cultivating land (14.3 to 71.4%) were significantly lower than those on untreated Miyagi Island (96.9 to 100%). Poor trap shutdown (39.6%) was observed in the Japanese Pampas grass field in 2002. Nevertheless, the mean trap catch by 18 monitoring traps in 2007 showed a 96.2% reduction from that of 2002. These results indicated that mating disruption with sex pheromone effectively reduced an isolated population of *M. okinawensis*.

Key words: wireworm, sex pheromone, communication disruption, mating rate, pheromone dispenser

F–02 Utilization of Mating Disruption Products in Japan

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More than 40 years have passed since the possibility of mating disruption (MD), as a control method, by using synthetic sex pheromone was published. Treated area of MD has remarkably expanded in the last decade, and the total area in 2006 was about 590,000 ha throughout the world. The use of MD for the gypsy moth and codling moth was especially spread. It was 200,000 and 150,000 ha, respectively. On the other hand, treated area of MD in Japan was about 20,000 ha. It was only 5% of the world.

MD products in Japan are roughly divided into two types. One is single-purpose type, and the target pest is one or closely related species (Table 1). Most of MD products marketed in the world are classified in this type. The other type is multi-purpose type that loads a mixture of synthetic sex pheromones for important pest insects of a target crop. The active ingredients are decided depending on pest insect species on the target crop. Then, the target insect species belong to a different family, (Table 2). In the tables below, product names, target crops and pests, treated area in 2006, and year registered in Japan of single- and multi-purpose dispensers are shown.

The first MD product registered in Japan is single-purpose type, Hamaki-con (1983). Shinkui-con (1984), Sukashiba-con (1988), and Konaga-con (1989) followed. However, these products did not become popular because there are two or more important pests that are needed to be controlled in the target crop. It was requested to develop multi-purpose type for integrated pest management with MD. Therefore, we introduced multi-purpose dispenser in 1990, and the first product, Confuser-A, was registered in 1996. Confuser-A was widely used in apple orchards in Fukusima and Nagano prefectures. After 1990, multi-purpose types were mainly developed.

Table 1. Target crops and pest insects, and treated area of single-purpose products in Japan (2006)

Product name	Crop(s)	Pest insects	Area (ha)	Registration in Japan
Hamaki-con (for tea)	tea	*Adoxophyes honmai*, *Homona magnanima*	3,070	*1983*
Hamaki-con (for fruit)	apple, peach, pear	*A. honmai*, *A. orana*, *Homona magnanima*	1,360	*1983*
Shinkui-con	apple	*Carposina niponensis*	240	*1984*
Sukashiba-con	Japanese plum, peach, cherry, persimmon	*Synanthedon hector*, *S. tenuis*	3,860	*1988*
Konaga-con	vegetables	*Plutella xylostella*	560	*1989*
Yotoh-con	*ditto*	*Spodoptera exigua*	120	*1990*
Yotoh-con	*ditto*	*S. litura*	380	*1996*

Table 2. Target crops and pest insects, and treated area of multi-purpose products in Japan (2006)

Product name	Crop(s)	Pest insects	Area (ha)	Registration in Japan
Confuser-A	Apple	*Carposina niponensis* *Grapholita molesta* *Phyllonorycter orientalis* leaf rollers	580	*1996*
Confuser-P (-MM)	Peach, Pear	*C. niponensis* *G. molesta* *Lyonetia clerkella* leaf rollers	2,540	*1998*
Confuser-R	Apple	*C. niponensis* *G. moleata* leaf rollers	3,390	*2002*
Confuser-N	Pear	*ditto*	3,660	*2003*
Confuser-V	Vegetables	*Spodoptera exigua* *S. litura* *Plutella xylostella* *Autographa nigrisigna*	760	*2004*

F–03 Semiochemicals Communication and their Application in China

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We summarized the semiochemicals communication and their application in China in recent years. Many agricultural, forestry pests seriously affect the yields of crop and forest products. In order to solve these problems, semiochemicals have been employed to understand the species signal communication and practical application. We give a few examples to indicate the recent research progresses in China. First, biological activity of female sex pheromone extracted from gland of *Holcocerus hippoophaecolus* Hua (Lepidoptera: Cossidae) was reported; Second, the improvement of the trap efficiency of stem borer, *Chilo suppressalis* using different blends of sex pheromones. Also, the effectiveness of the synthetic sex pheromone (10,14-dimethylpentadecyl isobutyrate) of tea tussock moth, *Euproctis pseudoconspersa* as a trapping lure was tested in Duyun, Guizhou Province. Third, Meng et al. developed a method for synthesis of the intermediates of sex pheromone of pine sawfly. In addition, the component of sex pheromone of female *Leposcelis entomophia* was identified. Regarding to beetles, our group improved the attractants for pine sawer, *Monochamus alternatus*. The trapping efficiency has been tested for several years and its application with fungi was confirmed to be effective in controlling the pine wilt disease (pine nematode) vector.

F–04 Mass Trapping as a Control Strategy for Tropical Coleoptra

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Insecticide-based management insect pests of tropical crops requires multiple applications often on a year round schedule. Attendant problems of environmental contamination, worker health and resistance management are principal forces behind the search for alternative methods of pest control in these high pressure environments. In the early 1990's we and others began a search for pheromones of several of the major coleopteran pests of palms, sugarcane and banana. A few years of intensive identification effort yielded sex and aggregation pheromones as well as some kairomones for the primary target insects. A few additional years of equally intensive effort on the part of numerous private sector and government personnel yielded robust insect pest management techniques utilizing the discovered semiochemicals. This presentation will describe the control of several important Coleopteran pests of tropical crops by mass trapping.

Male palm weevils of the genus *Rhynchophorus* produce aggregation pheromones that strongly attract both sexes only when presented with food. In tropical America *R. palmarum* causes direct larval damage and vectors red-ring nematode. The author's commercial group and collaborators developed a management system of trapping and removal of red-ring nematode infested palms that is the only economically viable technique in the Americas to combat this weevil and its associated nematode. Use of 1 trap/5 ha reliably lowers weevil associated damage by 80% over a year.

In the Middle East and Southern Europe *R. ferrugineus* (red palm weevil) is the most important Coleopteran pest of date palm. Collaboration of the authors commercial group with governments in the Middle East yielded a pheromone/food based trapping system that significantly lowers damage due to this pest at 1 trap/ha.

Oryctes rhinoceros is a pest of oil palm in Southeast Asia. The aggregation pheromone for this pest was also identified in the author's former laboratory in Canada. A robust managment system has been developed by the commercial group of the author and Southeast Asian collaborators. At 1 trap/2 ha pheromone-baited traps are as effective in lowering damage to young palms as the alternative insecticide treatment.

Cosmopolites sordidus (banana corm weevil) is a world-wide pest of banana and plantain. The author's commercial group and collaborators from the Costa Rican Ministry of Agriculture developed a pheromone-based trapping system that is effective at 4 traps/ha in lowering corm damage to less than 10% and increasing yields 10–20% over a crop cycle. Each of the above semiochemical-based trapping strategies is cost effective vs insecticide treatment. It is estimated that approximately 500,000–600,000 Ha are currently under management by mating disruption while 120,000–150,000 Ha are under management by mass trapping.

Key words: *Rhynchophorus*, *Cosmopolites*, *Oryctes*, mass trapping, aggregation pheromone

F–05 Monitoring Stored-Product Insects by Pheromone Traps in Food-Processing Facilities in Japan

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Stored-product insects infest grains and cereals and cause post-harvest losses in dry-food production and storage facilities. The sex pheromone (Serricornin) of the tobacco beetle, which is one of the most serious insect pests in those facilities, was identified earliest by Chuman et al. (1979). Thereafter, over 60 insect pheromones have been identified. Almost all of the pheromones have been used to monitor insects as pheromone traps not to control them. This is closely related to the fact that pheromones used to control insect pests are regarded as insecticides, so registering the pheromones is very expensive.

For this reason, pheromone traps for monitoring have been developed and used in many dry-food processing and tobacco facilities. The traps consist of an attractant lure and an apparatus to capture the individuals attracted. Pheromones and/or food volatile substances are used as attractants. Sticky traps or non-sticky traps (plastic cases or probes) with specially designed shapes are used as the capturing apparatus.

In Japan, pheromone traps for monitoring stored-product insects have been used since the 1980s and have been related with Integrated Pest Management (IPM). Based on the monitoring data, trap users evaluate results of pest control conducted in each production or storage facility and decide the control methods and timing of the control. They also attempt to predict future population dynamics or risk of the insects contaminating commodities.

Though pheromone traps are widely used for monitoring in food production and storage facilities, there are no standards for evaluating capture efficiency of each pheromone trap, trap placement and monitoring interval, or analyzing monitoring data for predicting future population dynamics and the contamination risk.

In this presentation, therefore, we summarize the current monitoring by pheromone traps in Japan and propose some ideas to establish standard methods.

Key words: pheromone trap, monitoring, stored-product insect, contamination risk

G–01 Olfactory and Visual Responses to Herbivore-Infested Corn Plants by the Parasitoid Fly *Exorista japonica*

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In contrast to hymenopteran parasitoids, the cues important for host habitat location by dipteran parasitoids remain largely unknown. Since parasitoid flies possess well-developed visual and olfactory senses, we presume they use both chemical and visual cues for orientation to the food plants of host insects. Flight responses of the parasitoid fly, *Exorista japonica*, to corn plants infested with final instar larvae of the noctuid moth, *Mythimna separata*, were examined in a wind tunnel. Mated female flies were conspicuously attracted to the infested plants but responded less to healthy plants. When the healthy plants were accompanied with headspace volatiles collected from the host-infested plants, female flies showed a high rate of landing on the healthy plants. They also responded to the healthy plants with a blend of 9 chemicals released from the host-infested plants. These results indicate that *E. japonica* is attracted to the volatiles emitted from corn plants infested with *M. separata* larvae. In order to test the effect of plant colour, 4 colours (blue, green, yellow and red) of paper models were separately placed in front of the host-infested plants hidden behind a mesh screen in the wind tunnel. The landing rate of the flies was the highest on green and the lowest on red paper models. Few female flies responded to the green model without the host-infested plants. When the 4 colour models were placed together in the cage with odours of the host-infested plants, they chose the green model. These results show that *E. japonica* recognizes plant colour and prefers green when odours of the hostinfested plants exist. *E. japonica* appears to use both olfactory (odours of host-infested plant) and visual (plant colour) cues to locate the host habitat.

Key words: *Exorista japonica*, host habitat location, insect-plant interaction, plant volatiles, Tachinidae

G–02 Why dose Sagebrush Emit Volatile Cues that Cause Neighbor to Become more Resistant to Herbivory?

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Recent work has demonstrated that communication between plants can increase resistance against herbivores when a neighbor plant has been damaged. Volatiles are known as one of the most common the plant communication tools. The plant which received the volatiles from a damaged neighbor gets some benefits. The ecological interest of this phenomenon has been in question why they emit volatile cues that cause neighbor to become more resistant to herbivory.

Here, we report the study of volatile induced plant resistance in Sagebrush. We found that pairs of sagebrush plants that were up to 60 cm apart were influenced by experimental clipping to a neighbor. We observed that most individuals had conspecific neighbors that were much closer than 60 cm. According allozyme analysis, there is close kinship within 2 m. These results might be one of the benefits for an emitter sagebrush since it makes its relative resistant. Furthermore we found that even same plant uses volatiles for systemic induced resistance. Reports from the literature indicated that sagebrush is highly sectorial, as are many desert shrubs. Branches within a plant do not freely exchange material via vascular connections and apparently cannot rely on an internal signaling pathway for coordinating induction of resistance to herbivores. Instead, they may use external, volatile cues. This result suggests that communication among individuals may be a by-product of volatile communication among branches of a single individual.

Key words: plant communication, volatile, systemic, induced resistance, sagebrush

G–03 Induced Direct and Indirect Defenses and Transcriptional Responses in Rice after Infestation by *Chilo suppressalis*

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After attack by herbivores, plants produce volatile and nonvolatile defense chemicals to resist herbivores directly or indirectly by attracting natural enemies of the herbivores. These herbivore-induced plant defense responses are involved in a large number of signal pathways and defense-related genes. In this study, the volatile and nonvolatile defense chemicals, defense-related transcriptome and the signaling pathways of rice plants induced by the rice stem borer *Chilo suppressalis* were investigated. *C. suppressalis* infestation resulted in dramatic increases in levels of volatiles, which plays an important role in the host searching behavior of the parasitoid *Cotesia ruficrus*, non-volatiles, diterpenoids, flavonoids and trypsin inhibitors, and signal molecules, jasmonic acid, salicylic acid, ethylene and hydrogen peroxide. Macroarray analysis showed that a lot of defense-related genes were up-regulated by the herbivore attack, which are involved in signaling pathways, transcriptional factors, secondary metabolism, abiotic stress responses, and so on. By using Agrobacterium-mediated transformation system, homozygous rice lines with antisense inhibition of a *lox* gene were screened. Bioassay showed that the *lox* gene plays an important role in interactions between rice plants and herbivores.

Key words: rice, *Chilo suppressalis*, tritrophic interactions, induced plant defense response

G–04 Early Herbivore Alert: Insect Eggs Induce Plant Defence

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Plants are able to mobilize defensive responses to egg deposition by herbivorous insects. Thus, they react to herbivores in a very early phase of attack, i.e., prior to feeding damage by herbivorous larvae. On the one hand, oviposition-induced defence can act directly against the egg-laying female or the eggs, and on the other hand, such defence triggered by oviposition can act indirectly by arresting or attracting parasitoids killing the eggs. Tritrophic interactions between egg-laden plants, herbivorous insects, and egg parasitoids will be addressed by focusing on the following questions: (1) Which molecular, physiological, and chemical plant responses are elicited by insect egg deposition?, (2) what do we know about egg-associated elicitors of plant defensive responses?, and (3) which are the plant cues the egg parasitoids are responding to? Both parallels and differences of plant responses to egg deposition and feeding damage will be highlighted.

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Key words: insect egg deposition, plant defence, elicitor, tritrophic interactions, egg parasitoid

G–05 Induced Disease Resistance in Rice Plants Infested with Whitebacked Planthopper, *Sogatella furcifera*

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The white-backed planthopper (WBPH), *Sogatella furcifera*, and the rice blast caused by *Magnaporthe grisea*, are economically important sucking insect pest and disease respectively, of rice (*Oryza sativa* L.). We found that pre-infestation of WBPH conferred resistance to *M. grisea* in rice plants. Development of blast lesions on rice plants was distinct from WBPH-infested regions, indicating that this was not a local form of resistance. Infestation with either male or female WBPH-induced resistance, thus their feeding behavior was responsible for the resistance. Simple mechanical wounding of rice plants did not induce the resistance. Synthesis of two phytoalexins (momilactone A and sakuranetin) which indicate strong antimicrobial activity to fungus was also detected in rice leaves which were infested with WBPH, but did not detected in rice leaves which were mechanically wounded. Moreover, this induced resistance is effective also against bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae.* We also confirmed that these diseases were suppressed by WBPH infestation under field conditions. Interestingly, this induced resistance was not observed after pre-infestation with blown planthopper, *Nilaparvata lugens*. We analyzed gene expression in WBPH-infested rice plants, using rice cDNA microarray system. It revealed that WBPH feeding increased the expression of many defense related genes.

Key words: induced resistance, planthopper, *Sogatella furcifera*, Magnaporthe grisea, *Xanthomonas oryzae* pv. *oryzae*

G–06 Do Host Plant Volatiles Always Attract the Parasitoids of Herbivores?

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In the last twenty-five years there have been major advances in our understanding about the upregulation of induced plant defenses in response to attack by herbivores and pathogens. The use of host plant volatiles, produced in response to herbivory, by natural enemies of insect herbivores has received considerable attention, and the generally accepted paradigm is that these are important
foraging cues.

However, a great deal of the information has been generated under laboratory conditions with a limited number of model systems. Furthermore, the relative importance of these volatile cues in tritrophic interactions under natural, and thus variable, ecological conditions has received little attention.

In this presentation I will present results of experiments examining the response of the aphid parasitoid *Aphidius nigripes*, in a wind tunnel to potato plants that were clean or infested with the potato aphid, *Macrosiphum euphorbiae*. Host plant volatiles had very little effect on the upwind flight of either virgin or mated females, whether naive or experienced with respect to oviposition. Our findings suggest that such cues were of minor importance for this aphid parasitoid and possible reasons for such a difference between this and other study systems will be discussed.

In order to determine if our finding on potato hold with other aphid parasitoids we are currently carrying out similar experiments on *Aphidius ervi*, a parasitoid of the pea aphid. The results obtained will be compared with those obtained for *A. nigripes* in an effort to determine whether our original findings represent a specific case or are of more general application.

Key words: multitrophic interactions, aphid parasitoids, female foraging, host plant volatiles

H–01 Chemical Diversity of Arthropod Semiochemicals

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The structure of compounds used by insects or other arthropods for chemical communication are derived from the biosynthetic machinery available to them. Such compounds can be formed *de novo* by synthesis from readily available starting material within the body, can be taken up from the outside and used with no or minor modification, or can be provided by symbiontic microorganisms. From these considerations one might think that a relatively common set of structurally related compounds is used in chemical communication. In contrast, chemical defense might be more chemically diverse because common compounds or similar modes of action would favor adjustement to common defense mechanisms, leading to a more diverse group of defensive compounds.
General similarity can be found for example in the sex pheromones of female moth, which are derived from the fatty acid biosynthetic pathway, either by introduction of double bonds by desaturases, modification of end groups, or decarboxylation and epoxidation processes. In other orders, e.g. Coleoptera, this uniformity cannot be found, and similarity is restricted to smaller groups of related beetles. In contrast, pheromone systems of male Lepidoptera are often behaviorally less well defined, but are chemically much more diverse than those used by the females. Compounds derived from the terpene pathway, fatty acid metabolites, aromatic compounds, and even alkaloids can be found, the origin of which is often unknown. We will present here some of the results obtained from day flying butterflies like *Heliconius* or *Pieris*, focusing on structural motifs like lactones and compounds of uncertain biosynthetic origin. The chemical communication systems of spiders, often linked to silk production, have been investigated only rarely, making a comparison to insect pheromones difficult. Nevertheless, some pheromones show a remarkable structural similarity with compounds of the primary metabolism, e.g. the citric acid circle components, not found in insects so far. Some examples will be described, as well as some thoughts on the involvement of microorganisms in pheromone production.
Key words: Pheromones, biosynthesis, Lepidoptera, Arachnida

H–02 Functional Diversity of Boring Insect Pheromones

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Some herbivorous insects bore into trunks or twigs of their host trees. There are the variety of approaches to their mates. I will show some examples of boring insect's pheromones and examinations of their messages.

Cossus insularis (Lepidoptera: Cossidae), sex pheromone: (*E*)-3-Tetradecenyl acetate (95–98%), (*Z*)-3-Tetradecenyl acetate (5–2%)

Female releases male attractants. Aggregate larvae feed on woody material in living tree.

Anaglyptus subfasciatus (Coleoptera: Cerambycidae), sex pheromone: (*R*)-3-Hydroxy-2-hexanone, (*R*)-3-Hydroxy-2-octanone

Male releases female attractants, and female produce chemically unidentified contact conspecific and/or sex distinctive pheromone. Adult beetles feed on flower pollen and nectar, whereas larvae feed on woody materials in dying branch and then on living inner bark.

Platypus quercivorus (Coleoptera: Platypodidae), aggregation pheromone (and sex pheromone): (1*S*,4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol

Male attracts males and females. Mass attack of the beetles kill host oak tree, then parents and larvae feed on ambrosia fungus in their gallery.

Endoclita excrescens (Lepidoptera: Hepialidae), sex pheromone: (1*R*,3*S*,5*S*)-1,3,8-trimethyl-2,9-dioxabicyclo [3.3.1]non-7-ene

Flying male releases sex distinctive pheromone in the lek. Larvae feed on callus of inner bark, not on woody material.

Key words: pheromone, boring insect

H–03 Sex Pheromone Communication Systems in the German Cockroach: A Tribute to Ritz

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Volatile sex pheromones, responsible for bringing of the sexes together, have been studied extensively. On the other hand, contact sex pheromones which are vital for reproductive success in many species, have received much less attention and little is known about their chemistry and biochemical and hormonal regulation. An exception is the German cockroach, *Blattella germanica*, whose contact sex pheromone has been elucidated in the late 1970s by the Nishida group. Upon contacting the cuticular surface of a sexually mature female, the male German cockroach exhibits a characteristic courtship behavior: He turns away from the female and raises his wings, thereby exposing tergal glands. The nutritious glandular secretion stimulates the female to mount the male and feed, thus positioning her appropriately for copulation. A multi-component female contact sex pheromone is responsible for eliciting courtship behavior. The most abundant pheromone components are 3,11-dimethylnonacosan-2-one and 3,11-dimethylheptacosan-2-one, both oxidation products of the abundant hydrocarbon analogs 3,11-dimethylnonacosane and 3,11-dimethylheptacosane, respectively. The C_{29}-dimethyl ketone is thought to be further metabolized to two less abundant pheromone components, 29-hydroxy-3,11-dimethylnonacosan-2-one and 29-oxo-3,11-dimethylnonacosan-2-one. Based on this proposed biosynthetic pathway of pheromone production, we hypothesized that 3,11-dimethylheptacosan-2-one also would be oxidized to give two candidate pheromone components, 27-hydroxy-3,11-dimethylheptacosan-2-one, and 27-oxo-3,11-dimethylheptacosan-2-one. Using behaviorally-guided fractionation and chemical analyses of organic extracts of virgin females, and synthesis of each of the two predicted pheromone components, we now show that the epicuticle of the German cockroach does indeed contain these two compounds. The contact sex pheromone the female German cockroach thus consists of six biosynthetically related components.

In addition, we describe two intriguing phenomena where courtship is directed toward non-receptive individuals: The German cockroach male courts immatures of its own species, as well as members of 5 of 20 other cockroach species that we screened. We used a similar behavior-guided chemical fractionation approach to purify and identify from nymphs compounds responsible for eliciting courtship. Last instar female nymphs share common pheromonal components with the adult female contact sex pheromone. Nymphs may elicit courtship as a potentially adaptive strategy of sexual mimicry, whereby they avoid aggression from males or gain nutritional benefits from the courting male.

We identified two active compounds from the cuticular extract of the Oriental cockroach, *Blatta orientalis*—11-methylheptacosan-2-one and 27-oxo-11-methylheptacosan-2-one; the former was confirmed by synthesis. Each of these compounds can effectively and independently stimulate courtship in German cockroach males. These compounds share common features with, but are distinct from any of the known contact sex pheromone components. This suggests that the sex pheromone receptor(s) of male German cockroach is unusually promiscuous, accepting a wide range of compounds that share features with its native pheromone, thus resulting in a broad spectrum of behavioral response to heterospecifics. We propose that several features of their mating system—chiefly, absence of closely related species in the anthropogenic environment resulting in relaxation of selection on sexual communication and a highly male-biased operational sex ratio—have driven males to respond with extremely low thresholds to a wide spectrum of related compounds.

Key words: 27-oxo-3,11-dimethylheptacosan-2-one, 27-hydroxy-3,11-dimethylheptacosan-2-one, sex pheromone, chemical mimicry, cockroach

H–04 Diversity of Sensory System in Pheromonal Communication

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Insects show diverse sensory system for pheromone communication both in morphology and in physiology, as well as some common features across different species. From peripheral receptors to central processing system, species-specific architectures for pheromone reception can be observed. Numerous studies have showed that highly specific receptors and responses for conspecific pheromones are common. Chemical compositions of cuticular hydrocarbon of pheromone sensilla appeared to be different among different species. Nano-scale observation of pheromone sensilla surface using AFM (atomic forced microscopy) and CFM (chemical forced microscopy) also suggests that species-specific sensory system may develop at the sensilla surface, too.
Key words: communication, cuticular hydrocarbon, pheromone, sensilla, sensory system

H–05 Sex Pheromones and Reproductive Isolation in Three Species of *Adoxophyes* in South Korea

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Adoxophyes spp. (Lepodoptera: Tortricidae) are pests of pear, apple, and peach throughout South Korea. The larvae feed on leaves and fruits but cause serious economic damage, especially on fruits, resulting in priceless fruits. In order to develop a method for monitoring as well as mating disruption, pheromone compositions of *A.* spp. were surveyed in populations collected from three locations (Cheonan, Sangju, and Naju) in South Korea. (*Z*)-9-tetradecenyl acetate (*Z*9–14: Ac), (*Z*)-11-tetradecenyl acetate (*Z*11–14: Ac), (*E*)-11-tetradecenyl acetate (*E*11–14: Ac) and 10-methyldodecyl acetate (10me–12: Ac) have been reported as the sex pheromone components of the genus *Adoxophyes.* Gas chromatography (GC) analysis of gland extracts of females from Cheonan (midwest of South Korea) and Sangju (middle of South Korea) revealed the presence of two components, *Z*9–14: Ac and *Z*11–14: Ac. Females from Cheonan produced primarily *Z*9–14: Ac with mean ratios of *Z*9–14: Ac and *Z*11–14: Ac of 80 : 20, while females from Sangju produced primarily *Z*11–14: Ac with a mean *Z*9–14: Ac and *Z*11–14: Ac ratio of 3 : 97. On the other hand, females from Naju (southwest of South Korea) produced sex pheromone blend consisting of four components, *Z*9–14: Ac, *Z*11–14: Ac, *E*11–14: Ac and 10me–12:–Ac, at a ratio of 31 : 62 : 6 : 1. Field trapping tests in pear orchards with two major components, *Z*9–14: Ac and *Z*11–14: Ac indicated that maximum captures of the males at Cheonan, Sangju, and Naju were obtained with traps baited by 80 : 20, 10 : 90, and 30 : 70 blends, respectively. Addition of *E*11–14: Ac to the two-component blend of *Z*9–14: Ac and *Z*11–14: Ac strongly inhibited captures at Cheonan and Sangju, whereas capture at Naju was not affected. Conversely, addition of 10me–12: Ac to the two-component blend enhanced trap captures in all three locations. Thus, the relative amount of major components (*Z*9–14: Ac and *Z*11–14: Ac) and the presence of *E*11–14: Ac were important factors in the reproductive isolation of three populations. These results suggest that at least three pheromone dispensers should be developed for monitoring and mating disruption of *A.* spp. in South Korea. In addition, our observation emphasizes the need to clarify the taxonomic status of these species.

Key words: *Adoxophyes*, sex pheromone, (*Z*)-9-tetradecenyl acetate, (*Z*)-11-tetradecenyl acetate, (*E*)-11-tetradecenyl acetate, 10-methyldodecyl acetate

H–06 Pheromonal Communication in Selected Species of Lymantriid Moths

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The talk will review the diversity of sex pheromones in the *Lymantriidae*, particularly in the genera *Lymantria* and *Leucoma.*

Using selected species (gypsy moth, *Lymantria dispar*; *L. dispar albescens*; nun moth, *L. monacha*; Indian gypsy moth, *L. obfuscata*), the talk will provide evidence in support of the concept that community composition is a biological factor that shapes the communication system of its constituent member species. For sexual communication, *L. monacha* uses a multicomponent blend [(7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane＝(＋)-disparlure; (7*S*,8*R*)-*cis*-7,8-epoxy-2-methyloctadecane＝(－)-disparlure; (7*R*,8*S*)-*cis*-7,8-epoxyoctadecane＝(＋)-monachalure; (7*S*,8*R*)-*cis*-7,8-epoxyoctadecane＝(－)-monachalure; (*Z*)-2-methyloctadec-7-ene＝2me-*Z*7-18Hy] of which four components [(－)-disparlure, (＋)- and (－)-monachalure, 2me-*Z*7-18Hy] are antagonistic to male *L. dispar*, if both moth congeners inhabit the same community. In communities in which *L. monacha* is absent, such as that of *L. dispar albescens* on Okinawa or that of Indian gypsy moth, *L. obfuscata*, in northern India, (－)-disparlure has no antagonistic effect. Indeed, *L. obfuscata* uses 2me-*Z*7-18Hy as a pheromone component. Even though (－)-disparlure is not a behavioral antagonist for males of *L. dispar albescens*, single-cell recordings with (－)-disparlure, and analyses of odor-binding proteins, show that males have the ability to perceive this compound through their olfactory system.

The talk will conclude with evidence that sexual communication in *L. monacha* (and others) involves acoustic signals during close-range mate location or courtship behavior. We have acquired and characterized these bioacoustic signals, and in bioassays have shown that they mediate orientation of males to females.

Key words: lymantriid moths, pheromonal communication, community composition, acoustic signals

I–01 Quormone: A Bacterial Cell–Cell Communication Signal Has the Function as the Cross-Kingdom Signal

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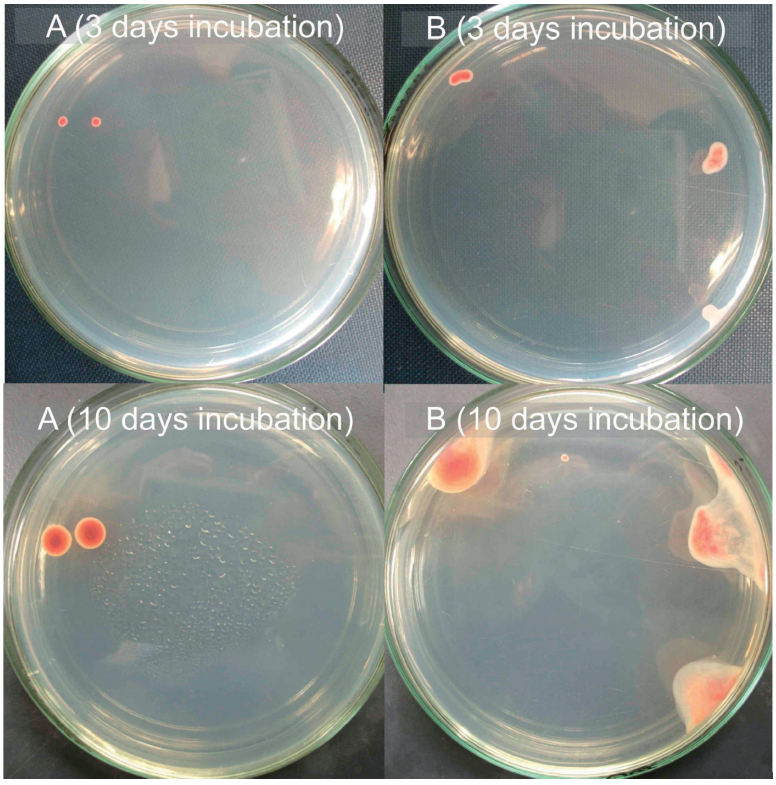
Quorum sensing is a mechanism of cell density-dependent phenotypic expression adopted by many prokaryotes. It is regulated by easily diffusible, low-molecular-weight substances, quormones. If a quormone accumulates to a concentration above a certain threshold, it activates expression of various genes. Many pathogenic bacteria regulate the virulence by quorum-sensing mechanisms. If the quormone is inactivated, or its signal is blocked, the pathogenic bacteria can not express the virulence genes. A method of disrupting the quorum-sensing mechanism of bacteria is called "quorum-quenching". For an example, a quormone-degrading enzyme is able to prevent expression of virulence genes of *Ralstonia solanacearum.*



A: *Ralstonia solanacearum* MAFF 301487 was grown on a plate containing quormone-degrading enzyme. Colonies of MAFF 301487 exhibited low fluidity and low EPS production. B: Control, a plate containing no quormone-degrading enzyme. Colonies exhibited high fluidity and high EPS production.

Quorum sensing is also exploited as cross-kingdom signaling. Zoospores of green seaweed Ulva are attracted to quormones produced by bacteria. A human hormone, epinephrine, activates expression of virulence genes of enterohemorrhagic *Escherichia coli* serotype O157 : H7. Paraoxonase, a human enzyme in the liver and plasma, degrades quormones of Pseudomonas aeruginosa, an important agent of nosocomial infections. Plants secrete substances mimicing quormone and affecting the behaviors of bacteria.
This presentation outlines mechanisms of quorum-sensing and describes functions of quormones as bacterial cell-to-cell communication signals and a cross-kingdom signals.
Key words: quorum sensing, quormone, quorum-quenching

I–02 Molecular Mechanisms Underlying Bombykol Biosynthesis in *Bombyx mori*

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Pheromonogenesis, which requires the concerted actions of multiple gene products, can be defined as a dynamic cellular process that produces sex pheromone components in the pheromone gland (PG) cells of female moths. Recently a number of PG-specific genes have been cloned and *in vitro* characterizations have indicated functions consistent with roles in pheromone production. The molecular mechanisms underlying female moth pheromonogensis, however, still remain far from fully understood.

Bombykol biosynthesis in the silkmoth, *Bombyx mori*, is under the control of the neurohormone PBAN (pheromone biosynthesis activating neuropeptide) and is triggered upon PBAN binding to its cognate PBAN receptor (PBANR) on the PG cells. The pheromonogenesis of *B. mori* is divided into two major processes depending on the developmental stage; the first one proceeds in the late pupal stage when the newly formed PG cells become functionally competent and are primed for bombykol biosynthesis, while the second one proceeds after eclosion with PBAN binding and the subsequent signal transduction cascade culminating in bombykol production and release.

Taking advantage of the genome information in *B. mori*, we have recently characterized PG-specific genes [i.e. PG fatty acyl reductase (*pgFAR*), *B. mori* PG Z11/Δ10,12 desaturase (*Bmpgdesat1*), PG acyl-CoA-binding protein (*pgACBP*), midgut ACBP (*mgACBP*), and pheromone biosynthesis activating neuropeptide receptor (*PBANR*)] and successfully demonstrated their specific roles during pheromonogenesis by using an RNA interference-mediated loss-of-function approach. With all efforts to understand the molecular mechanisms underlying bombykol production, however, little is known regarding how the external PBAN signal is converted to the calcium signal that drives bombykol production and release. To address this issue, I will present in my talk our recent approach and results regarding the intracellular PBAN signal transduction cascade in *B. mori*.

Key words: moths, sex pheromone, pheromone biosynthesis, PBAN receptor, signal transduction

I–03 "Talking Trees": How Do Apple Trees Signal to Lightbrown Apple Moth Parasitoids?

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Lightbrown apple moth *Epiphyas postvittana*) (Lepidoptera: Tortricidae), a leafroller native to Australia, feeds on a wide range of plants, including apples (*Malus domestica*) as a preferred host, although they did not co-evolve together. Despite this, an Australian braconid endoparasitoid wasp (*Dolichogenidia tasmanica*) responds to infested apple trees in wind tunnel choice tests. The comparison of headspace around infested and uninfested seedling apple trees indicates that a number of compounds showed higher release rates from foliage after larval feeding. In addition, at least eight compounds were only detected after larval feeding, in particular terpenoids, and benzene derivatives known to be associated with plant defence. Electroantennogram recordings with females of *D. tasmanica* indicated that several of these key compounds could be detected by the wasp. Preliminary assays, where larval regurgitate was applied to apple leaves, suggested the presence of an inducer compound(s) from the caterpillar spit. Two candidate compounds have been identified and work is underway to ascertain their role in the volatile response of apple trees to herbivory.

Key words: Tortricidae, parasitoids, signaling, apple, lightbrown apple moth

I–04 Identification, Synthesis, and Applications of Pheromones of Mealybugs

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Mealybugs (Homoptera: Pseudococcidae) comprise a group of small sucking insects that are characterized by the "mealy" wax covering that gives them their name. In many species, females are sessile and holometabolous, whereas males undergo a complete metamorphosis to winged, nonfeeding adults. Because males do not feed, they live only a few days, and hence are under intense pressure to locate a mate before they run out of energy and die. Thus, females produce a powerful sex pheromone for attraction of males.

For the past several years, we have been working with a group of mealybugs that are important pests of grapes and many other crops in California and many other areas of the world. These include the vine mealybug *Planococcus ficus*, the obscure mealybug *Pseudococcus viburni*, the longtailed mealybug *Pseudococcus longispinus*, and the grape mealybug*Pseudococcus maritimus.* Pheromones for the first three species have been identified, synthesized, and field tested, and the identification of the pheromone for grape mealybug is in progress. The syntheses of the pheromones will be described. Field trials have shown that the pheromones are extraordinarily powerful, with lures loaded with 25 micrograms or less of pheromone remaining active for periods of longer than 8 weeks under field conditions. Field trials have also been conducted by collaborators worldwide in a wide variety of crops, and the pheromone has proven to be consistently active when used in monitoring lures in all cases. Mating disruption trials have also been carried out with vine mealybug pheromone, using two types of dispensers. Overall, these pheromones are already being commercialized and incorporated into IPM programs.

The chemistry of these and many other mealybug pheromones is also extraordinary. All of the pheromones of the above species, as well as most of the pheromones that have been identified from related scale and mealybug species, are esters of irregular, non-head-to-tail terpenoids. In the case of the obscure mealybug, the highly substituted cyclopentanoid core of the molecule represents the first example of an entirely new family of monoterpenes, with a skeleton apparently formed from 2-2′-4′-3 connections between two isoprene units. The chemistry of this and several related mealybug pheromones will be discussed.

Key words: mealybug, terpenoid, mating disruption, monitoring, pheromone

M–01 Clearwing Moths Use Visual Cues with Olfactory Cues on their Mating Behavior

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Many lepidopterans recognize mating partners with visual and/or chemical cues. Mainly nocturnal moths use volatile sex pheromone, and contrary diurnal butterflies use visual cues to recognize their mating partners. On the other hand, information about the mating behavior of diurnal moths is very limited. It is expected that no little diurnal moths possibly use visual cue on their mating in a diurnal condition, but both usage of the visual cue and female pheromone have been reported only in a few Zygaenidae species (Zagatti & Renou, 1984; Koshio & Hidaka, 1995).
Since the clearwing moth (Sesiidae), which flies only daytime and has a specific colorful wasp-mimicry body, seems to be one of the best insects to study the relationships of visual cues and sex pheromones on the mating behavior, we started identification of the sex pheromone of two sesiid species, *Nokona pernix* and *Synanthedon hector*. GC-MS and GC-EAD analyses identified (*E*,*Z*)-3,13-octadecadien-1-ol and the (*Z*,*Z*)-isomer in a pheromone extract of the former species and two acetate derivatives in that of the latter species. Each component has been identified from other Sesiidae species, but the mixtures attracted only the corresponding males in a field, indicating that each species secreted the pheromone blend with high species-specificity. However, while the synthetic pheromones baited to rubber septa showed strong attraction activities, a copulatory behavior to the lures was not observed. Interestingly, visual female models (black or wasp-mimic pattern) were necessary for the male moths to recognize their partners and try copulation.

M–02 Aggregation Pheromone of the Brazilian Stalk Weevil, *Sternechus subsignatus* (Coleoptera: Curculionidae)

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Volatiles from both sexes of *Sternechus subsignatus* Boheman (Coleoptera: Curculionidae) were collected by aeration and the behavioral response of males and females was evaluated using Y olfactometer. The results obtained demonstrate that the communication among *S. subsignatus* conspecifics is mediated by aggregation pheromone, since there was significant attraction of both sexes to male insect volatiles when added to the host plant. Seven male-specific compounds were detected in the chromatographic analysis, in ratios of 9.7: 2.7: 7.1: 41.4: 0.2: 1.6: 37.3, providing a chemical support to the behavioral data. (*E*)-2-(3,3,-Dimethylcyclohexylidene) ethanol (*E* isomer of grandlure II) is the major component and the chemical structures of the minor constituents were revealed by MS analyses and derivatizations as; *cis*-methyl-2-(1-methylethenyl)cyclobutane ethanol, grandisol (grandlure I, ratio-9.7), *gamma-iso*geraniol, (ratio 2.7), (*Z*)-2-(3,3,-dimethylcyclohexylidene) ethanol (grandlure II, ratio-7.1), (*Z*)- and (*E*)-2-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure III, ratio-0.2, and IV, ratio-1.6), and the (*E*)-2-(3,3-dimethylcyclohexylidene) acetic acid, (ratio 37.3), which is describe to the first time as a natural product. Enantioselective gas chromatography proved the natural grandisol to be the (1*R*,*S*)-stereoisomer. The major component, (*E*)-2-(3,3,-dimethylcyclohexylidene) ethanol, was attractive alone in the olfactometer, and work is now in progress to evaluate the biological activity of the full blend under laboratory and field conditions. Release of these male-specific volatiles is dependent on the presence of the host plant, since the amount of compounds differs significantly when volatiles are collected from weevils with or without access to food. The production takes place mainly during photophase, showing a peak between 4 to 6 hours after its beginning, which is also a peak of the insect activity in the field.
Key words: soybean weevil, semiochemicals, pheromone production, olfactometer

M–03 Does Male Odor Influence Female Mate Choice in the Mediterranean Fruit Fly?

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In the Mediterranean fruit fly, *Ceratitis capitata*, the basis of female choice is not understood, but recent studies indicate that male exposure to the aroma of certain plant structures or essential oils may increase their mating success. In particular, exposure to the aroma of ginger root oil (GRO) has been shown repeatedly to enhance male mating frequency. The purpose of the present study was to investigate the mechanism underlying female preference for GRO-exposed males. Two sets of experiments were conducted. First, we monitored female attraction to i) freshly killed flies or ii) hexane extract-containing paper discs from varying treatments. In these tests, females were sighted more frequently near i) GRO-exposed males than non-exposed males and ii) paper discs containing hexane wash from GRO-exposed males than discs with hexane wash from non-exposed males. These findings suggest a 'perfume effect', whereby female mate choice was mediated by olfactory differences between GRO-exposed and non-exposed males. Second, we compared mate choice between intact females and females from which both antennae had been surgically removed. Intact females preferred GRO-exposed males, while females lacking both antennae mated very rarely and showed no preference between GRO-exposed and non-exposed males. In the opposite treatment (intact females but surgically altered males), GRO-exposed males lacking both antennae mated as frequently as GRO-exposed, intact males. These data suggest that female choice was dependent on olfactory perception of male odor but that male mating success did not depend on olfactory perception of GRO aroma.

Key words: Mediterranean fruit fly, female choice, ginger root oil

M–04 Ménage-à-trois for Insects: Mobile Mating Disruption and Other Flights of Fancy?

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We propose a new cross-species approach that might be capable of interrupting mating or other behaviors of one species, using another insect species as the mercenary agent. We argue that insects treated with a sufficiently powerful attractant for a second species might interfere with mating of one or both species, for example by leading males astray in pursuit of the false trails created by suitably dosing individuals of the first species. Our reciprocal test systems used 1) methyl eugenol, an attractant for male Oriental fruit flies (*Bactrocera dorsalis*), applied to melon flies (*B. cucurbitae*) and 2) cuelure (a lure for male melon flies (*B. cucurbitae*)), applied to *B. dorsalis.* Toxicology tests indicated no mortality after a week from either attractant applied to individual flies at doses up to 100 ng, which was effective in attracting insects in a field cage and in the field. In wind tunnel choice tests, 100 ng of either lure topically applied to tethered flies attracted fruit fly males of the second species, which exhibited prolonged bouts of physically disruptive behaviors including chasing and bumping. In small cages, the presence of lures on males did not translate into a reduction in mating of either species, with one group of three (ménage) per cage. However, in large field cages with multiple pairs of both species present, there was a significant reduction in the mating rate of Oriental fruit flies resulting from cuelure applied to males, compared to untreated controls. These results do not yet provide the practical proof of this new concept for pest management, but we suggest other strategies which are proposed for further research.

Key words: mating disruption, attractants, fruit flies, ménage-à-trois, chemical ecology

M–05 PBAN Signaling in *Bombyx mori* Involves Gq-Linked Activation of Store-Operated Calcium Channels

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Similar to most species of female moths, de novo sex pheromone production in the silkworm, *Bombyx mori*, is regulated by pheromone biosynthesis activating neuropeptide (PBAN), a 33 amino acid neuropeptide that exerts its effects via its cognate G protein-coupled receptor, the PBAN receptor. Generally, activated G proteins are utilized to relay extracellular signals to downstream effector molecules such as calcium channels and adenylyl cyclase. While the role of calcium in PBAN signaling has been clearly demonstrated, the involvement of cAMP is not as straight-forward. Indeed, despite recent successes in elucidating many of the molecular components comprising the sex pheromone biosynthetic pathways, the molecular identity of the processes that transduce the PBAN signal remain unknown. To address this issue, we have taken a multifaceted approach to identify and characterize the steps leading up to, and including, calcium influx. Using molecular cloning methods, we have identified 4 G-protein alpha subunits, as well as homologs of the calcium signaling molecules, STIM1 and Orai1, from *B. mori* pheromone-producing cells. The pharmacological profile of PBAN signaling is consistent with the involvement of store-operated calcium channel activation by Gq alpha subunits. RNAi-mediated knockdown of individual signal transduction components further demonstrated that the pathway is Gq-linked. In contrast, targeted disruption of Gs subunits had no effect on bombykol production, an indication that, in *B. mori*, the second messenger cAMP is likely not utilized. Taken together, these results suggest that the PBAN signal transduction pathway in *B. mori* proceeds via Gq-mediated stimulation of store-operated calcium channels.
Key words: PBAN, G protein, signal transduction, calcium signaling, sex pheromone

M–06 Olfactory Response of the Decapitating Phorid Fly, *Pseudacteon tricuspis* to the Red Imported Fire Ant, *Solenopsis invicta*

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In the past decade, a few species of *Pseudacteon*phorid flies (Diptera: Phoridae), including *P. tricuspis* and *P. curvatus* have been released in many parts of southern United States for biological control of invasive imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae). However, little is known about the cues used by these phorid flies to locate host imported fire ant workers. To test the hypothesis that host location is mediated by fire ant semiochemicals, we investigated the electroantennogram (EAG) and behavioral responses of *Pseudacteon tricuspis* of different sex and mating status to red imported fire ant, *Solenopsis invicta* host-related odor stimuli, including live fire ant workers, extracts of worker whole body, head, thorax, and abdomen, and (*E*,*E*)-α-farnesene, a trail pheromone component of fire ants. In EAG experiments, female and male *P. tricuspis* showed significant EAG response to extracts of worker whole body, head, and abdomen, and to a less extent, thorax extract, but not to (*E*,*E*)-α-farnesene. Females showed slightly greater EAG response than males, but EAG response was not affected by mating status. Results from Y-tube olfactometer bioassays demonstrated the attraction of mated female *P. tricuspis* to live *S. invicta* workers. In addition, extracts of *S. invicta* worker whole body and thorax elicited strong olfactometer response in mated and unmated female flies and mated males, but not in unmated males. No significant attraction of *P. tricuspis* of different physiological states was elicited by extracts of *S. invicta* worker head and abdomen, or by (*E*,*E*)-α-farnesene. These results suggest that ant defensive compounds (including alarm pheromones), which are produced in the abdomen and the head and trail pheromones are not likely to be used as attractants by phorid flies. The data further suggest that fire ant thorax is likely the source of kairomones used by *P. tricuspis* to locate host fire ant workers.
Key words: *Pseudacteon tricuspis*, *Solenopsis invicta*, olfactometer, electroantennogram, semiochemicals

M–07 Floral Synomone Cocktail and Trap Architecture of Two "Fruit Fly Orchid" Species to Attract *Bactrocera* Fruit Flies

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Many orchid flowers attract insect pollinators by using floral fragrance combined with a chemical reward (not nectar). Two species of "fruit fly orchids" *Bulbophyllum cheiri* and *B. vinaceum* offer a cocktail of phenylpropanoids as an attractant and chemical reward to methyl eugenol (ME)-sensitive male fruit flies (*Bactrocera* spp.). Although there are differences in the chemical compositions of these two attractants, ME still features as the largest component for both orchids. ME is ingested by male fruit flies and biotransformed into male sex pheromonal component(s) in order to attract conspecific females. The sex pheromone is temporarily stored in the male rectal gland and later released during courtship. Both *B. cheiri* and *B. vinaceum* have highly movable floral lips that can temporarily trap an attracted male fruit fly, causing it to either remove or deposit the pollinarium. The architecture of the floral lip is different between the two species—in the *B. cheiri* flower the hinged see-saw lip is located above the floral column while in the *B. vinaceum* the hinged lip is located below the column and is furthermore spring loaded. This paper discusses the similarities and differences between the two species, in light of floral adaptations to enhance cross pollination; and the true mutualism, in which both parties gain direct reproductive benefits, between the orchid and fruit fly.
Key words: *Bulbopohyllum* orchids, *Bactrocera* fruit fly, pollinators, floral phenylpropanoids, lip architecture

M–08 Chemical Communication and Division of Labor in Social Aphids

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Together with bees, wasps, ants, and termites, aphids represent a major insect group that includes many social species with individuals, called soldiers, that altruistically sacrifice their own fitness for the benefit of their colony mates. In highly social aphids, soldiers are morphologically differentiated from normal nymphs and unable to grow, constituting a sterile caste. To understand the full picture of aphid social system, we investigated the mechanisms of division of labor and chemical communication in a gall-forming social aphid, *Tuberaphis styraci*, which is a model species representing social aphids. In *T. styraci*, division of labor was based on soldier age polyethism, where younger soldiers preferentially performed gall cleaning inside the gall while aged soldiers exclusively performed colony defense outside the gall. Both gall structure and soldiers' physiological conditions were important for this age-dependent division of labor. Chemical analyses and behavioral observations revealed two aphid pheromones, alarm pheromone and corpse-recognition pheromone, and two plant-gall signals mediating aphids' alarm communication. Aphid colonies regulated task allocation by utilizing various pheromones and plant-gall signals and exhibited a flexible division of labor. Based on these results, we discuss the ecological and physiological mechanisms that coodinate the aphid social system.

N–01 Efficacy of Some Multipurpose Tree Spices (MPTS) Extracts on Wheat and Some of its Weeds

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Studies were undertaken in Weed Research Laboratory, Department of Weed Science, NWFP Agricultural University, Peshawar, Pakistan to investigate the allelopathic potential of aqueous extracts of the leaves of *Prosopis julifera* and *Eucalyptus camaldulensis* and the bark of *Acacia nilotica.* The concentrations studied included 0, 50, 100 and 150 g L^{-1} (w/v). A check, tap water (0 g L^{-1}) was also included for comparison. The dry plant materials were ground and soaked for 24 hours in water. Ten seeds each of *Triticum aestivum*, *Avena fatua*, and *Carthamus oxycantha* were used in each Petri dish. Each treatment was replicated twice and there were two identical runs of the experiment. The data were recorded on germination percentage, seedling length (mm) and biomass (mg) $plant^{-1}$. The data for each parameter were converted to the percent of check and subsequently analyzed statistically. A varying inhibition was recorded in all the species tested for all the parameters studied, but it was most pronounced in the germination percentage. *C. oxycantha* was the most inhibited species. Wheat was the most tolerant. Only 20% seeds as compared to the respective check germinated in this species when exposed to *E. camaldulensis* @150 g L^{-1}. All other concentrations of *P. julifera* and *E. camaldulensis* severely inhibited germination of *C. oxycantha.* For other parameters the inhibition was observed at the higher concentrations of *P. julifera* and *E. camaldulensis. A. nilotica* emerged as the weakest species in inhibiting the growth parameters of the tested species. The findings reveal that the allopathic potential of the effective species could be exploited for weed management in wheat due to the tolerance of what to the extracts.
Key words: allelopathy, *Prosopis*, *Eucalyptus*, *Acacia*, inhibition, phytotoxicity

N–02 Analysis on the Characteristics of Microbial Flora in the Rhizospheric Soils of Different Allelopathic Rice Accessions (*Oryza sativa* L) at Seedling Stage by BIOLOG Method

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In order to elucidate the relationship between allelopathic potential of rice and the microbial flora in rhizospere, agar plate counting, fumigation-extraction methods, accompanied BIOLOG analysis, were employed to investigate the characteristics of microbial flora and its functional diversity in the rhizospheric soils of rice PI312777, IAC47, Iguape Cateto and Lemont. The results showed that the microbial flora in the rhizospheric soils of different rice cultivars was dominated by bacteria, which bacteria occupied by 58.4–65.6%, actinomycete by 32.2–39.4%, and fungi by 2.2–2.8%. BIOLOG analysis indicated that the value of Average Well Color Development (AWCD) showed significantly higher among allelopathic rice accessions, PI312777, IAC47, Iguape Cateto than non-allelopathic rice Lemont. There were 3 principal component factors (PCF), screened out of 31 factors by Principal Component Analysis (PCA), which explained for 70.08%, 11.33% and 7.02% of variation respectively, and 19 kinds of carbon sources were significantly positive related to the 3 principal components, they were phenolic acids, carbohydrates, amino acids and amides, which were significantly related to principal component 1, and phenolic acids, carbohydrates and fatty acid significantly related to principal component 2, while carbohydrates and hydroxylic acids to principal component 3. Amino acids and amides lead to the separation of the 3 principal component factors.
Key words: allelopathy, rhizospheric soil, microbe, function diversity, BIOLOG

N–03 The Study on Effect of Natural Compound and Temperature on Storage Behavior of Sweet Cherry (*Prunus avium*) C. V. "Siah-e-Mashhad"

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The research was conducted to identify the effect of natural compound [grape seed extract, Clove (*Eugenia caryophyllum*) and Black zira (*Bunium persicum*) essential oils] in two temperature regimes (10℃ and 25℃) on storage behavior of sweet cherry. The treatments were 500, 1000 ppm each oil or grape seed extract as emulsion and essential oil fumigation as 1 ml/package. Before treatment, physical and biochemical properties of the samples such as (fruit weight, TSS, TA and pH) were determined. After treatment, each package were studied for the measurement of weight loss, fruit quality, stem browning, fungal spoilage and fruit surface shriveling, 48 h intervals. At the end of the experiment, TSS, TA and pH also were measured. In ambient temperature (25℃) after 2 weeks and in refrigerator temperature (10℃) after 4 weeks all the samples had good conditions. Our results also indicated that low temperature (10° C) especially in covered boxes in comparison with open boxes, had positive effects. All treatments had significant effect on the measured factors during experiment. Clove essential oil (1000 ppm) had the best results in all measured factors. Fumigation method, because of the high concentration decrease fruit quality.
Key words: fruit quality, natural compound, storage, sweet cherry, temperature

N–04 Relationships between *Cuscuta japonica* Growth, Movements and its Hosts Volatile

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Relationships between general stem parasite plant *Cuscuta japonica* growth, movements and its 4 kind of hosts of different parasitized degree, of which the order was from preferred to less-preferred according to long time fields observation were investigated in laboratory. Solid-phase micro-extraction (SPME), minus-pressure cryotrap and hydro-distillation methods were used to extract the volatiles from the tested hosts' leaves, compounds and their relative content were detected by GC-MS. The results demonstrated that SPME method's result could reflect volatile releasing from hosts in the nature. *C. japonica*'s movement and growth were influenced by the volatiles of hosts. The volatiles released from fresh leaves, extracted by hydro-distillations and pure compounds have significant different effects on movements and growth of *C. japonica.* The pure compounds had the highest effect on the movements and growth of *C. japonica*, and the volatiles released from fresh leaves had the lowest. The impact degree of preferred host volatiles on the movements and growth of *C. japonica* were less than the less preferred ones. *B. papyrifera*, *I. cairica and L. camara* fresh leaves of healthy, parasitized and the neighboring leaves of parasitized hosts were respectively collected. The Volatiles of these leaves were tested by SPME-GC/MS. The results showed that before and after *C. japonica*'s parasitizing, leaves of hosts released different components and their relative content also varied, furthermore, these changes depended on the hosts, too. It revealed that the *C. japonica* could affect its hosts releasing volatiles, but this kind of effect ecological implications is undiscovered.
This project supported by the National Natural Science Foundation of China (30470192)
Key words: *Cuscuta japonica*, volatiles of host, growth and movement

N–05 Influence of Host Plant Odors on Invasion of Rice Leaf Bug into Paddy Fields

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The rice leaf bug, *Trigonotylus caelestialium* is one of the most serious rice pests, causing pecky rice, in the northern areas of Japan. Invasion of the bug to paddy fields from gramineous weeds rapidly increases with the heading of rice. The olfactory responses of the bugs to their host plants, rice, *Olyza sativa*, and four species of gramineous weeds, *Poa anua*, *Alopecurus aequalis*, *Digitaria ciliaris* and *Eleusine indica*, were investigated with an olfactometer to clarify the role of host-plant odours on their invasion into paddy fields. The bugs were attracted to four gramineous weeds tested. Attractancy of rice to the bugs differed with the growth stage and part of the plant. Although adult females were significantly attracted to stems and leaves in the panicle-formation stage, and panicles in the flowering stage, they were not attracted to the other rice structures tested. Adult males were significantly attracted to only stems and leaves in the panicle formation stage. The preference of the bugs for rice and gramineous weeds differed with the growth stage of rice. Although the bugs preferred weeds to rice before flowering of rice, they had a little preference for rice than grass weeds after flowering. Headspace components of rice at each growth stage were analyzed by GC-MS. Females were attracted to β-caryophyllene, which increased in the panicle formation stage and flowering stage. The findings suggest that the invasion of rice leaf bug into paddy fields is caused by seasonal changes of rice odor.
Key words: rice leaf bug, invasion, pecky rice, host plant odor, olfactometer, attractant

N–06 Effect and Durative of Indoor Plants on Removing Formaldehyde in 72 hrs

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The capability of plants removing indoor air pollution was reported a lot recently, but the ability published is inconsistent. In this study a fumigation lasting for 72 hrs was run to investigate the continuous purification ability of indoor plant. Four plants of different adsorbability were chose according to our previous study: *Syngonium podophillum*, *Aglaonema crispum* '*Silver Queen*' and *Dracena deremensis* '*Virens Compacta*', The plants were divided into wholeplant fumigation and leaf fumigation in self-designed glass box in which a lasting-released formaldehyde source was put, and the contents of formaldehyde were measured in every 12 hrs from the beginning. The results showed that, compared with the content of blank box which was increased from 0.78 mg/m^3 to 2.20 mg/m^3 in 72 hrs, the whole plant could decreased formaldehyde content to the following level at the first 12 hrs and then maintain it until 72 hrs: *Syngonium podophillum* could maintain the content from 0.27 to 0.33 mg/m^3, *Aglaonema crispum* '*Silver Queen*' was at 0.36–0.49 mg/m^3, *Dracena deremensis* '*Virens Compacta*' at 0.46–0.54 mg/m^3, and *Aloe vera* var. chinensis at 0.37–0.53 mg/m^3. In addition, the ratio of leaf purification to whole plant was increased during 72 hrs, *Syngonium podophillum* increased from 68.5% to 73.5%, *Aglaonema crispum* '*Silver Queen*' from 76.4% to 96.0%, *Dracena deremensis* '*Virens Compacta*' from 39.7% to 82.6%, and *Aloe vera* var. *chinensis* from 12.7% to 58.2%. The data above supported the point that indoor plants had lasting ability of removing formaldehyde, at least in 72 hrs.
Key words: indoor plants, formaldehyde, whole plant purification, leaf purification, lasting adsorbability

N–07 Biological Activity of Troponoid Compounds to Insect Pests

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Hinokitiol (β-thujaplicin) is a volatile compound that is contained in *Thujopsis dolabrata* var. *hondai* Sieb. et Zucc., and has a seven-membered ring. Troponoid compounds such as hinokitiol show antimicrobial activity, phytogrowth-inhibitory effects, etc. However, little is known about their effects on insects, which include insecticidal activity against termites and repellency against cigarette beetles. We investigated the repellency of troponoid compounds to adzuki bean beetle. Tropiliden, which is only a seven-membered ring with no functional groups, showed the lowest activity. Tropone, which has a keto group, showed higher activity than tropiliden, and tropolone, which has a keto and a hydroxyl group, showed higher activity than tropone. The activity of Thujaplicin, which is tropolone with an isopropyl group, varies with the position of the group. The activity of hinokitiol was higher than tropone but slightly weaker than that of tropolone. The insecticidal activity was similar to the repellency. The activity of tropiliden was the lowest. Tropone with a keto group showed the highest activity but tropolone with a hydroxyl group showed lower toxicity than tropone, indicating that a hydroxyl group inhibited the toxicity of the keto group. With an isopropyl group added to tropolone, hinokitiol showed lower toxicity than tropolone and tropone. Antifeeding activity of thujaplicins to cabbage armyworm was also tested. It was found that the position of the isopropyl group influenced the antifeeding activities. We will show that the biological activity of troponoid compound to insects depends on the existence and position of functional groups on the seven-membered ring.

Key words: troponoid compound, repellent, toxicity, *Callosobruchus chinensis*, *Mamestra brassicae*

N–08 Larvae-induced Volatiles from Maize Affect Behaviors of Conspecific Adults and Neonates in the Asian Corn Borer *Ostrinia furnacalis*

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Maize (*Zea mays* L.) volatile variations induced by the 3rd instar larvae of the Asian corn borer (ACB) *Ostrinia furnacalis* (Guenée) were determined and their effects on the behaviors of conspecific adults and the neonates of attacking larvae were investigated. After 48 h feeding by 3rd ACB larvae, maize plants emitted 19 chemicals characteristized by high contents of terpenes. There were some differences between adult females and males in responses to larvae-induced maize volatiles in gas chromatography and electroantennographic detection (GC-EAD). ACB neonates were tested for orientation to induced volatile collection and some individual chemicals. The neonates were volatile attracted to the whole volatile collections and farnesene, but repelled by (*Z*)-3-hexan-1-ol. Gravid females laid eggs on the larvae-damaged plants than on the mechanical-damaged plants or undamaged plants. In single chemical tests, ACB adult deposited fewer eggs on the wax paper with (*E*)-2-hexanal or (*Z*)-3-hexan-1-ol than on the wax paper with hexane (control). The results suggested that the changes of constitutions and contents of volatile compounds in maize induced by ACB larvae affected the orientation and oviposition of conspecific neonate larvae and adults, respectively. The sensitivity differences between ACB adults and larvae to induced maize volatile components were discussed.

Key words: behaviors, conspecific adults and larvae, induced volatiles, *ostrinia furnacalis* (Guenée), *Zea mays* L.

N–09 Ovipositional Deterrents in Sweet Pepper (*Capsicum annuum*), against *Liriomyza trifolii*

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Liriomyza trifolii (Burgess) is a serious leafminer pest to a variety of vegetable and ornamental plants which belong to families of Solaneceae, Cucurbitaceae, Compositae and so on. Female leafminer punctures the leaves of plants for feeding and oviposition. The larval stage of the flies feed within the leaves of host plants, and at high fly densities the feeding severely reduces yields or kills the plants. One of the difficulties in controlling this species is its ability to develop resistance to insecticide. In a greenhouse, we observed that leaves of sweet pepper (*Capsicum annuum*, Solaneceae) at a mature stage were rarely attacked by this insect but at a young stage the plant was fiercely attacked by the flies. Based on bioassay guided fractionation, five compounds namely luteolin 7-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside, phytol, 4-aminobutanoic acid, (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid and 4-amino-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone were isolated from the leaves of sweet pepper at the mature stage. These compounds, had significant oviposition deterrence effect towards adult flies of *L. trifolii* from laying their eggs on host plant leaf treated at 4.90, 35.2, 3.70, 16.60 and 6.45 $\mu g/cm^2$, respectively. These results might contribute to the development of an integrated pest control method against *L. trifolii* that is safe and environmentally compatible, which will decrease our reliance on use of insecticides.
Key words: *Liriomyza trifolii*, leafminer, ovipositional deterrent, *Capsicum annuum*

N–10 Production of Transgenic Tobacco Expressing Yam Tuber Lectin (DB1) in Order to Confer Insect-Resistance

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Lectins are carbohydrate-binding proteins that are wide-spread in the biosphere and found in almost every living organism. In plants, lectins are accumulated in storage organs such as seeds, tubers, bulbs and rhizomes. Lectins have been proposed to serve as defense proteins against insects, fungus and microorganism. Monocot mannose binding lectin (e.g. snowdrop lectin, GNA) is known to bind to the mid-gut in insects, and interfere with the growth and development of insects. Several lectins are expressed in transgenic plants and provide increased protection against insects. DB1 is a storage protein isolated and characterized from the yam tuber, *Dioscorea batatas*, and it has 65% homology to GNA. In artificial diet, DB1 has previously been shown to be toxic against moth larvae. In this study, we produced transgenic tobacco and rice expressing DB1 protein. Accumulation of DB 1 protein was estimated to be up to 2.4% of total soluble protein in the leaves of transgenic tobacco. We are currently investigating the toxic effect of DB1, which was introduced via artificial diet or transgenic plants, on the growth inhibition of *Myzus persicae.*
Key words: transgenic plants, plant lectin, insect resistance, yam tuber lectin

LS–01 Chemical Ecological Interface between Parasitic Plants and Fungi

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Seed germination of root parasitic plants, *Striga*, *Orobanche*, and *Alectra* in the family Orobanchacea, is induced by germination stimulants produced by and released from roots of host and some non-host plants. At least three different classes of plant secondary metabolites, dihydroquinones, sesquiterpene lactones, and strigolactones, have been shown to induce seed germination of root parasites. Among these germination stimulants, strigolactones appear to be widely distributed in the plant kingdom and thus play pivotal roles in the interactions between root parasites and their hosts, since these compounds are also important host recognition signals for arbuscular mycorrhizal (AM) fungi with which $>80\%$ of land plants form symbiotic relationships.

To date, 6 strigolactones have been isolated from root exudates of different plant species; strigol and strigyl acetate from cotton, sorgolactone from sorghum, alectrol from cowpea, orobanchol from red clover, and 5-deoxystrigol from *Lotus japonicus* and gramineous plants. In addition to these known strigolactones, at least 10 additional strigolactones have been detected in root exudates of tomato, pea, carrot, tobacco, and other plant species.

Characterization of strigolactones in the root exudates from various plant species grown hydroponically has been conducted by comparing retention times of germination stimulants on ODSHPLC with those of synthetic (or natural) standards and by using LC/MS/MS. All the plants examined so far have been found to produce at least two different strigolactones, indicating that bouquets of strigolactones are involved in the host recognition of both AM fungi and root parasites. Through these studies, 6 novel strigolactones have been purified from root exudates of tobacco, flax, sorghum, and pea. Isolation and structural determination of these novel strigolactones, their distribution in the plant kingdom, and in addition, effects of environmental conditions including nutrient availabilities on the production and exudation of strigolactones will be presented and discussed.

Key words: arbuscular mycorrhizal fungi, host recognition signal, parasitic plants, strigolactones

LS–02 Scope of Chemical Communication

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Technology fiction (TF) is a dream, which can come true in a technological sense, but due to present limitations it has yet to be realized. What is actually TF in chemical communication is to introduce chemical communication often used in the world of animals, plants and microbes into the human world. The dream will be realized by stepping up one by one from chemical ecology study. 1) By using semiochemicals or their mimics, a new dimension of agricultural technology can be brought about. Examples from bean weevils, rice weevil, cheese mite, stink bugs, and pine wilt disease are given. 2) By detecting and analyzing unintentional signals emitted from plants and animals control measures are provided. 3) By developing chemical sensors and the mimics, wide use can be opened beyond physical detection. 4) By utilizing chemicals, which act on human sense organs or inner receptors, various messages beyond taste or olfaction can be transmitted among man to effect physiological and/or psychological impacts. 5) By constructing a system consisting of message-chemical-sensor-transducer-computer, a novel communication technology using chemicals as media can be produced. For the realization of the above, there is need to gather experts and to create a clear image of the above proposals. Next, there is need to define technological limitations in accomplishing the above and to identify technical goal for overcoming such limitations. 1) to 4) is in the order of increasing technological difficulty. 1) is dependent on human and financial resources; 2) is drawing from much industrial expectation, but needs more study in physiology of sensory system; 3) has been carried out on a limited concept on olfaction and taste, requiring more imagination and basic study for expansion; and 4) requires thorough investigation into the concept of transmission of message by chemical molecules and overview on the potential uses which are different from or superior to physical communication.

Key words: chemical communication, technology fiction, semiochemicals, novel communication technology