

Abstracts (Poster)

P-001 Different Chemical Cues Inducing Predator-Avoidance Behavior in Two Anuran Tadpoles

Teruhiko Takahara^a, Yukihiro Kohmatsu^b and Ryohei Yamaoka^c

^a Venture Laboratory, Graduate School of Science and Technology, Kyoto Institute of Technology, Matsugasaki-gosyokaido, Sakyo, Kyoto 606-8585, Japan

^b Research Institute for Humanity and Nature, Kita, Kyoto 603-8047, Japan

^c Chemical Ecology Laboratory, Graduate School of Science and Technology, Kyoto Institute of Technology, Matsugasaki-gosyokaido, Sakyo, Kyoto 606-8585, Japan

taka02@kit.ac.jp

In freshwater systems, little is known about substances which induce defensive responses in prey species, such as predator chemical cues. To assess the characteristics of chemical cues exuded from the dragonfly nymph *Anax parthenope julius* which induced activity reduction as predator-avoidance behavior of tadpoles in two anuran species, *Rana (Rugosa) rugosa* and *Hyla japonica*, we conducted preliminary chemical analysis and a bioassay. The tadpoles of *H. japonica* responded to dragonfly nymph's incubation water that had volatile substances removed, as well as non-treated incubation water, suggesting that chemical cues inducing the activity reduction have non-volatile characteristics. The activity reduction of *R. rugosa* got gradually weaker, as water, with dissolved dragonfly nymph's chemical cues, became more diluted. To compare the characteristics of chemical cues inducing an activity reduction in two anuran species, dragonfly nymph's incubation water was fractionated by a solid-phase extraction with non-polar C18 cartridges. As a result, *R. rugosa* responded to the fraction that contained dissolved substances with non-volatile and low lipophilic characteristics. *H. japonica* responded to the fraction that contained dissolved substances with a non-volatile and non-lipophilic characteristics. Our study shows that predator-avoidance behavior in two prey species is induced by chemical cues having different lipophilicity. Prey species may perceive several substances to act as cues to efficiently avoid encounters with and/or attacks by predators in their habitats.

Key words: bioassay, chemical cues, cue characteristics, freshwater system, predator-avoidance behavior

P-002 Effects of Chemical Cues from Damaged Conspecifics and Heterospecifics on the Clumping Behaviour and Byssus Production in the Green-lipped Mussel, *Perna viridis*

Yang, F. Y., Shin, P. K. S. and Cheung, S. G.

Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon Tong, Hong Kong

50250330@student.cityu.edu.hk

Forming clumps is one of the behavioural features of mussels which can effectively reduce the predation risk, especially for small mussels. Early studies have shown that mussels at the centre of a clump suffer lower predation than those at the edge. However, their growth and reproduction are also reduced as compared with mussels located at the edge of a clump or being solitary. In view of the above trade-off, we hypothesized that the location of the mussels determines the extent the anti-predatory responses are induced upon exposure to predation-related chemical cues. The hypothesis was tested in a laboratory experiment with clumping behaviour and byssal attachment strength of juveniles of the green-lipped mussel *Perna viridis* (Bivalvia: Mytilidae) being investigated in response to waterborne cues from damaged conspecifics and damaged heterospecifics, the black mussel *Brachidontes variabilis*. The formation of clumps was monitored for 48 hours, whereas the attachment strength of mussels locating at different positions of the clumps was assessed by measuring byssal thread length and thickness. The number of byssus produced was also counted at the end of 48 hours. Isolated mussels and those found at the edge of the clump in the damaged conspecifics treatment produced significantly more byssal threads which were also longer than those within the clump. The number and the diameter of the threads from the conspecifics treatment were significantly higher than those from the damaged heterospecifics and the control groups. No significant differences, however, were observed in the formation of clumps among different treatment groups. Results have indicated that mussels occupying different positions in a clump would alter their byssus production according to the predation risk level.

Key words: chemical cues, clumping behaviour, byssus production, mussel

P-003 Antifouling Compounds against Barnacle Larvae from the Red Algae *Laurencia* spp.
Tatsufumi Okino^a, Minoru Suzuki^a, Takahiro Ishii^a, Takashi Kamada^a, Yuko Oguri^a, Erina Yoshimura^b and Yasuyuki Nogata^c

^a Graduate School of Environmental Science, Hokkaido University, Kita 10-Nishi 5, Sapporo 060-0810, Japan

^b Ceres, Inc., 1-6-1, Ogawacho, Kanda, Chiyoda, 101-0052, Japan

^c Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry, 1646 Abiko, Abiko 270-1194, Japan

okino@ees.hokudai.ac.jp

Natural products are possible sources and potential leads of environmentally safe antifoulants. As a part of our screening program for antifouling compounds from marine organisms, algal metabolites were investigated for antifouling activity against larvae of the barnacle *Amphibalanus amphitrite*. Especially we focused on the red algae *Laurencia* spp. which were known to be rich sources of halogenated compounds. For example, elatol from *L. majuscula* was reported to show potent antifouling activity. As a result of our screening for over 100 algal metabolites, several halogenated compounds showed antifouling activities against barnacle larvae at concentrations of 0.1–1.0 $\mu\text{g}/\text{mL}$. Especially a brominated acetogenin inhibited larval settlement at 0.2 $\mu\text{g}/\text{mL}$, but did not show toxicity even at 100 $\mu\text{g}/\text{mL}$.

Key words: antifoulant, acetogenin, settlement, rhodophyte

P-004 A Possible Symbiotic Relationship through Norzoanthamine

Takahisa Genji, Seketsu Fukuzawa and Kazuo Tachibana

Department of Chemistry, School of Science, The University of Tokyo, Hongo 7-3-1, Tokyo 113-0033, Japan
ktachi@chem.s.u-tokyo.ac.jp

There are two generally accepted presumptions for ecological roles of marine natural products. One is that most of them are involved in defensive systems of their host organisms. The other is that those in multicellular animals are produced not by hosts but by microorganisms. Therefore, it is probable that symbiotic relationships are established between the host organisms and the microorganisms where such marine natural products play important roles. In this study, we show an example among the colonial zoanthid *Zoanthus* sp., its symbiotic fungus *Aspergillus fumigatus*, and a marine alkaloid norzoanthamine.¹ This *Zoanthus* sp. contains the alkaloid at more than 0.19% of wet weight in its epidermal tissue as shown by quantitative determination with high-performance liquid chromatography and its ionic signal mapping on slices of the animal with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). At this level of concentration, norzoanthamine is shown to suppress degradation of proteins, indicating that its biological function in *Zoanthus* sp. could be to protect the epidermal tissue from external stress. As another finding, we isolated a fungus *A. fumigatus* whose population is more than 29,000 cells per 1 g wet weight of *Zoanthus* sp., indicating this fungus is a symbiotic fungus of it. Furthermore, production of norzoanthamine by the fungus was indicated by MS/MS analysis on the extract from the cultured fungus. These results imply a symbiotic relationship between *Zoanthus* sp. and the fungus through norzoanthamine. *Zoanthus* sp. supplies nutrition and residence to the fungus, and the fungus pays norzoanthamine as the rent.

1. Fukuzawa, S. *et al. Heterocycl. Commun.* **1995**, *1*, 207–214.

Key words: marine natural products, symbiotic relationship, biological function, norzoanthamine

P-005 Induction of Sea Cucumber (*Stichopus japonicus*) Larval Metamorphosis by Neurotransmitters

Hiroshi Matsuura^a, Ikuko Yazaki^b and Tatsufumi Okino^c

^a Graduate School of Environmental Science, Hokkaido University, N10W5, Kita-ku, Sapporo, 060-0810, Japan

^b Faculty of Science, Tokyo Metropolitan University, Minamiosawa, Hachioji, Tokyo, 192-0357, Japan

^c Faculty of Environmental Earth Science, Hokkaido University, N10W5, Kita-ku, Sapporo, 060-0810, Japan
matsuura@ees.hokudai.ac.jp

In Japan, the demand of aquaculture of sea cucumber (*Stichopus japonicus*) is increasing recently. To improve the aquaculture technique, it is important to understand the mechanism of larval metamorphosis. Sea cucumber doliolaria larvae, which were competent to metamorphose, were placed in 12-well tissue culture dishes at densities of approximately 10 per well. To search for inducers of sea cucumber larval metamorphosis, 30 compounds including amino acids and neurotransmitters were tested. As a result, dopamine, L-DOPA, L-adrenaline and L-noradrenaline induced metamorphosis of sea cucumber larvae at concentrations of 5–10 μM . The metamorphosis by these compounds completed in 60 hours.

Key words: doliolaria larva, dopamine, L-DOPA

P-006 How Induce Morphological Changes of Zooplankton by Diel Vertical Migration of *Chaoborus* larva?

Mariko Nagano and Akihiko Yagi

Aichi Institute of Technology, 1247 Yatigusa Yakusa-cho Toyota 470-0392, Japan

naganoma@hotmail.co.jp

Chaoborus (Diptera: Chaoboridae) kairomone induce in morphological changes of zooplankton and undergo important for interaction of lake ecosystem. Seasonal diel vertical migrations (DVMs) of *Chaoborus* larvae were studied from 2003 to 2006 in Lake Fukami-ike (maximum depth 7.75 m, Central Japan). The number of larva in summer and autumn were high density 379 and 494 ind. m^{-2} respectively. In April and December, the larvae were appeared at the sun set to sunrise, although the larva was not observed in water column at daytime. During the summer stratification period, the number of larvae stayed in anoxic layer (4 m to 7 m depths) at daytime and migrated into whole water column at night, respectively. In case of the night distributions, the larvae were observed through the all layers in October, while the larvae accumulated in layers from 2 m to 3 m depths in summer stratification period. Typically these migration cycles were made complex aquatic ecosystem. It is clear that changing *Chaoborus* predation pressure, density and structure of instar induce in morphological changes.

Key words: *Chaoborus* larva, seasonal DVMs, zooplankton

P-007 Natural Inducers for Coral Larval Metamorphosis

Makoto Kitamura^a, Peter Schupp^b, Tomoyuki Koyama^c, Yoshikatsu Nakano^d, Daisuke Uemura^a

^a Department of Chemistry, Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan

^b Marine laboratory, University of Guam, Mangilao GU 96923, USA

^c Laboratory of Nutraceuticals and Functional Foods Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology; 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

^d Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Motobu, Okinawa 905-0227, Japan

m.kitamura@attglobal.net

External chemical signals used by scleractinian corals to recognize suitable substrata for larval settlement and metamorphosis were identified from crustose coralline red algae (CCA). A fragment of coral rubble with CCA induced larval metamorphosis of the scleractinian coral *Pseudosiderastrea tayamai*. A natural inducer and compounds that enhanced its effect in larval metamorphosis were isolated from the methanol extracts of coral rubble with CCA. A bromotyrosine derivative, 11-deoxyfistularin-3 (10^{-7} M) isolated from the CCA, induced the metamorphosis of *P. tayamai* larvae ($27.5 \pm 24.0\%$). In the presence of fucoxanthinol (10^{-9} M) and fucoxanthin (10^{-9} M), the percentage of metamorphosis induced by the bromotyrosine derivative was further enhanced to 87.8 ± 13.0 and $88.4 \pm 17.8\%$, respectively. Both carotenoids are also found in the coral rubble with CCA. These results suggest that bromotyrosine derivative and carotenoids have a synergistic effect in the metamorphosis of *P. tayamai* larvae.

Key words: larval metamorphosis, *Pseudosiderastrea tayamai*, crustose coralline red algae (CCA), 11-deoxyfistularin-3, carotenoids

P-008 Okadaic Acid Binding Proteins from the Sponge *Halichondria okadai*

Naoyuki Sugiyama^a, Keiichi Konoki^b and Kazuo Tachibana^c

^a Human Metabolome Technologies, Inc., Mizukami Kakuganji 246-2, Tsuruoka, Yamagata 997-0052

^b Department of Chemistry, Graduate School of Science, Osaka University, Machikaneyama 1-16, Toyonaka, Osaka 560-0043, Japan

^c Department of Chemistry, Graduate School of Science, The University of Tokyo, Hongo 7-3-1, Bunkyo, Tokyo 113-0033, Japan

konoki@ch.wani.osaka-u.ac.jp

Okadaic acid, first isolated from a marine sponge *Halichondria okadai*, is a potent inhibitor of protein phosphatases 1 and 2A (PP1, PP2A). OABP1 and OABP2 were purified from *H. okadai* as guided by binding affinity of [$27\text{-}^3\text{H}$] okadaic acid. Molecular cloning and sequencing identified OABP1 as 88% identical to the rabbit PP2A β catalytic subunit. HPLC analysis revealed that OABP2 consists of three 22 kDa proteins OABP2.1, OABP2.2 and OABP2.3. The complete amino acid sequences for the three components were also deduced from Edman degradation and molecular cloning. Homology of OABP2.1 to OABP2.2 is 96%, while 66% to OABP2.3. All of them shows little homology to any protein phosphatases known to date. Photoaffinity labeling experiments performed with biotinylated photoreactive okadaic acid revealed that OABP2 only exists in *H. okadai* but not in the sponge *Halichondria japonica* or the dinoflagellate *Prorocentrum lima*. OABP2 binds to okadaic acid with K_d of 0.97 nM, though it does not exhibit phosphatase activity. We thus speculate that OABP2 may be involved in detoxifying okadaic acid or mediating translocation of okadaic acid.

Key words: okadaic acid, symbiosis, defense mechanism, protein phosphatase

P-009 The Effects of Artificial Reefs on Nutrient Dynamics in Seabed Sediments

Wai, H. Y., Kwok, S. T., Tsui, S. Y., Cheung, S. G. and Shin, P. K. S.

Department of Biology and Chemistry, City University of Hong Kong, 83, Tat Chee Avenue, Kowloon Tong, Hong Kong

50404309@student.cityu.edu.hk

Artificial reefs have been widely employed as sanctuaries for fish and they can also act as a platform to increase assemblages of marine organisms for enhancing marine biodiversity. Nonetheless, the ecological function of artificial reefs in marine ecosystems has rarely been studied in detail. In this study, the ecological value of artificial reefs system was investigated. Sediments were collected in traps which were deployed above and below a reef that was located in Long Harbour, east of Hong Kong. Sediment cores along a transect at 1 m, 5 m, 20 m and 50 m upstream and downstream of the reef were also collected for analysis. Sediment physico-chemical parameters including silt-clay fraction, water content, total organic carbon content (TOC), total Kjeldahl nitrogen (TKN), total phosphorus (TP) and fatty acid profiles were analyzed. Sedimentation rate was also calculated in the sediment collected from the sediment trap data. Results showed that the fatty acid profiles of upstream and downstream sediments were similar to that of the sediments collected from bottom sediment traps. Furthermore, sedimentation rate in the bottom traps was higher than that in the upper traps. This implied that biofiltration caused by organisms on the reef surface might have modified the food quality for infauna in the adjacent and/or nearby sediments and lead to an increase in food availability to other benthic organisms. TOC and TP levels and silt-clay fraction were found lower in sediments which were closer to ARs, suggesting that biofiltration was carried out by the epifauna on the ARs and that the egestion of pseudofaeces and faeces by these organisms might affect sediment particle size distribution. Further investigations will be planned to examine which sediment parameters are best to be used for indicating the relationship between hard reef systems and seabed sediment characteristics.

Key words: artificial reefs, nutrient dynamics, chemical ecology, fatty acid profiles

P-010 Synthesis and Identification of an Endogenous Sperm Activating and Attracting Factor from Ascidian *Ciona intestinalis*

Hiroshi Tsuchikawa^a, Kouichirou Ootou^b, Tohru Oishi^b, Michio Murata^b, Manabu Yoshida^c and Masaaki Morisawa^d

^a Department of Chemistry, School of Science and Technology, Kwansei Gakuin University, Sanda, Hyogo 669-1337, Japan

^b Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

^c Misaki Marine Biological Station, Graduate School of Science, University of Tokyo, Miura, Kanagawa 238-0225, Japan

^d Department of Biology, Faculty of Science, Yamagata University, 1-4-12, Kojirakawa, Yamagata, 990-8560, Japan

murata@ch.wani.osaka-u.ac.jp

Chemotaxis of sperm toward eggs during fertilization is a crucial event for species conservation, particularly for animals living in aquatic environments. Relevant chemical attractants have been found from a few marine organisms such as sea urchins and corals. We previously showed that sperm of the ascidian *Ciona intestinalis* is activated and then attracted toward the egg by a factor released from the egg, which led to the purification of sperm-activating and attracting factor (SAAF) from the eggconditioning medium of *Ciona intestinalis*. The structure of SAAF was proposed to be a novel polyhydroxysterol sulfate on the basis of 2D-¹H NMR and FAB-MS/MS analysis using no more than approximately 4 μg of a specimen. However, there still remained small ambiguity that the biological activity might be attributed to a minor constituent. Synthesis is, therefore, essential for the unequivocal identification and for the complete structure elucidation including the stereochemistry at C25. The first synthesis of the two diastereomers was accomplished from chenodeoxycholic acid in 19 steps and we could determine the complete structure of SAAF. Synthetic SAAF activated the sperms of ascidian *Ciona intestinalis* at 3.7 nM and concurrently exhibited the attracting activity at < 10 nM, which led to the verification of its dual activity. It is noteworthy that 25-*epi*-SAAF turned out to possess comparative activities as those with SAAF. The second generation synthesis of SAAF was achieved by using highly diastereoselective reactions to construct a series of C3-C5 stereochemistry, which enabled us to synthesize its derivatives including desulfated, biotinized, and photoaffinity-labeled derivatives.

Key words: chemotaxis, SAAF, *Ciona intestinalis*, synthesis, structure determination

P-011 The Fatty Acid Composition of the Pacific Copepods Compared with Those of the Atlantic Ones

Hiroaki Saito^a, Gerhard Kattner^b and Martin Graeve^b

^a National Research Institute of Fisheries Science, Yokohama 236-8648, Japan

^b Alfred Wegener Institute for Polar and Marine Research, Bremerhaven 27570, Germany
hiroakis@affrc.go.jp

The lipid and fatty acids of Pacific and Atlantic copepods were analyzed to clarify their physiology. The major Pacific copepods (*Neocalanus cristatus*, *Neocalanus flemingeri*, and *Metridia okhotensis*) in the boreal sea were collected near Japan in the North Pacific Ocean, while the Atlantic ones (*Calanus hyperboreus*, *Calanus finmarchicus*, and *Caranoides acutus*) are caught in the Barents Sea and the Antarctic sea in the Atlantic Ocean. Among all the species, high levels of monoene fats in wax esters, which is only the major lipid class, were observed. In particular, the marked high levels of both 20 : 1n-11 and 20 : 1n-9 monoenes have been found in the Pacific copepods, while only 20 : 1n-9 was a major C20 monoene in the Atlantic ones with trace levels of 20 : 1n-11. The difference of the fatty acid and alcohol compositions in the depot wax esters between the two Ocean samples suggests that a physiological difference between the Pacific and the Atlantic samples or an environmental influence of the two Oceans. The enzymatic biosynthesis of the monoene fatty acids in the Pacific copepods might differ from that in the Atlantic copepods.

Key words: chemoecology, fatty acid, marine lipid, copepod, wax ester

P-012 Influence of Environment on the Fatty Acids in Pacific Oyster (*Crassostrea gigas*), Thriving both the Pacific and the Atlantic Oceans

Hiroaki Saito^a and Yanic Marty^b

^a National Research Institute of Fisheries Science, Yokohama 236-8648, Japan

^b University of Western Brittany, Brest 27570, France
hiroakis@affrc.go.jp

The same oyster species *Crassostrea gigas* is known in both the Pacific and the Atlantic Oceans, and the oyster is economically the most important shellfish species both in Japan and France. However, there are little investigations under different environments between the two oceans. We analyzed the lipid and fatty acids of the oyster collected in the two oceans. The lipid classes in both the samples were similar to each other, while the fatty acid compositions between the two samples slightly differed. The difference of 18 : 3n-3 and 22 : 6n-3 (docosahexaenoic acid; DHA) levels in the depot triacylglycerols between the two oceanic samples, suggests the influence of the dietary phytoplankton in the different environments. The high DHA levels both in the phosphatidylethanolamine and phosphatidylcholine, which are the tissue polar lipids, were observed in the Pacific samples, while the lower DHA levels were found in the Atlantic ones. The specific high levels of DHA in the Pacific sample lipids suggests the influence of phytoplankton, such as dinoflagellates and marine fungi, which generally contain high DHA levels.

Key words: chemoecology, fatty acid, marine lipid, bivalve, docosahexaenoic acid

P-013 *cis*-Cinnamoyl Glucosides - Major Plant Growth Inhibitors Contained in *Spiraea thunbergii* and *Spiraea prunifolia*

Syuntaro Hiradate, Sayaka Morita, Hajime Sugie and Yoshiharu Fujii

National Institute for Agro-Environmental Sciences (NIAES), 3-1-3 Kan-nondai, Tsukuba, Ibaraki 305-8604, Japan

hiradate@affrc.go.jp

Spiraea thunbergii and *S. prunifolia* were shown to possess high allelopathic activities. Bioassay-guided isolation yielded two plant-growth-inhibitory compounds: 1-*O*-*cis*-cinnamoyl- β -D-glucopyranose (*cis*-CG) from *S. thunbergii* and *S. prunifolia*, and 6-*O*-(4'-hydroxy-2'-methylenebutyryl)-1-*O*-*cis*-cinnamoyl- β -D-glucopyranose (*cis*-BCG) only from *S. thunbergii*. The plant growth activity (specific activity) of *cis*-CG on lettuce (*Lactuca sativa*) growth was similar to that of *cis*-BCG, and they were more active than that of *cis*-abscisic acid, suggesting that the activity level of *cis*-CG and *cis*-BCG was in the highest range as natural products. It was confirmed that the activities of *cis*-CG and *cis*-BCG were derived from *cis*-cinnamic acid and that their *trans* isomers had very weak activities. Because the content of *cis*-CG plus *cis*-BCG in *S. thunbergii* ($3.59 \mu\text{mol g}^{-1}$ FW) was much smaller than that of *trans* isomers ($47.9 \mu\text{mol g}^{-1}$ FW), it could be possible that the *trans* isomers are the detoxified forms of the allelochemicals for their storage in plants.

References: Hiradate, S. *et al.*, *Phytochemistry* **65**: 731-739 (2004). Hiradate, S. *et al.*, *J. Chem. Ecol.*, **31**: 591-601 (2005). Morita, S., *et al.*, *Plant Growth Regul.*, **46**: 125-131 (2005).

Key words: *cis*-cinnamic acid, *cis*-cinnamoyl glucosides, detoxification, *Spiraea prunifolia*, *Spiraea thunbergii*

P-014 Isolation and Identification of a Plant Growth Inhibitor in Akagi (*Bischofla javanica*)

Hiroko Yamaya^a and Syuntaro Hiradate^a, Hiroshi Araya^b and Yoshiharu Fujii^a

^a National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai Tsukuba 305-8604, Japan

^b Meiji University, 1-1-1 Higashi-mita, Tama-ku, Kawasaki-shi, Kanagawa 214-8571, Japan

hyamaya@affrc.go.jp

There are many indigenous plants in Ogasawara islands, but recently the spread of an invader plant akagi (*Bischofla javanica*) becomes a serious problem. Because akagi showed a high allelopathic activity, the plant growth inhibitor was isolated from the plant leaf under an isolation strategy of "total activity" (Hiradate. 2006), and its chemical structure was identified. The akagi leaves were extracted with EtOH, evaporated with a rotary evaporator *in vacuo*, suspended in water, and extracted with hexane followed by EtOAc. The total activity of each fraction was evaluated on the basis of the plant growth inhibitory on lettuce (*Lactuca sativa*) seedlings, and it was found that a major part of the total activity in the crude extract was transferred into the water fraction. Both of the crude extract and the water fraction showed strong acidity (pH 3), and their total activities significantly decreased when they were neutralized, suggesting that the bioactive component in the plant was proton conjugated with carboxylate(s). Capillary electrophoresis and NMR analyses confirmed that the water fraction contained tartaric acid as a major carboxylic acid. Therefore, we concluded that most of the inhibitory activity in the crude extract of akagi leaf was derived from tartaric acid and its conjugating proton, which could be possible allelochemicals of this species.

Key words: allelochemicals, *Bischofla javanica*, Ogasawara Islands, tartaric acid, total activity

P-015 Momilactone A and B Uptake by *Arabidopsis thaliana* and their Growth Inhibitory Effects

Hiroya Kujime^a, Katsumi Ota^a, Morifumi Hasegawa^b and Hisashi Kato-Noguchi^a

^a Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan

^b Faculty of Agriculture, Ibaraki University, Ami, Ibaraki 300-0393, Japan

Secondary metabolites of rice plants, momilactone A and B, are known to act as growth inhibitory substances. The mode of action of momilactone A and B on the growth inhibition, however, is not fully understood. In this study, effects of momilactone A and B on growth of *Arabidopsis thaliana* seedlings, and uptake levels of momilactone A and B by *A. thaliana* were determined. Momilactone A and B inhibited the root and hypocotyl growth of *A. thaliana* at concentrations greater than 10 and 3 μM , respectively. The inhibitions were increased with increasing concentrations of momilactone A and B. At 10 μM , the lengths of roots and hypocotyls of *A. thaliana* were 66–89% and 23–34% of control lengths for momilactone A and B, respectively. Momilactone B at 30 μM dosage inhibited more than 75% of the root and hypocotyl growth. When *A. thaliana* seedlings were incubated in medium containing 1 μM momilactone A and B for 5 d, concentrations of momilactone A and B in *A. thaliana* were 6.7 and 2.8 pmol plant⁻¹, respectively. At dosage of 10 μM momilactone A and B, concentrations of momilactone A and B in *A. thaliana* were 14.8 and 11.6 pmol plant⁻¹, respectively. It was confirmed that *A. thaliana* itself did not produce momilactone A and B. Therefore, very small amounts of momilactone A and B were absorbed by *A. thaliana* and induced growth inhibitory effects on *A. thaliana*, suggesting that momilactone A and B may involved in early event of the growth inhibiting process in *A. thaliana*.

Key words: *Arabidopsis thaliana*, momilactone A and B, uptake, growth inhibition

P-016 Allelopathic Potential of *Hypnum plumaeforme* L. and its Allelopathic Substances

Kanami Kobayashi^a, Hideyuki Shigemori^b, Hisashi Kato-Noguchi^a

^a Kagawa University, 2393 Miki Kagawa 761-0795, Japan

^b Tsukuba University, Tennodai 1-1-1, Tsukuba 305-8572, Japan

There are a lot of reports the presence of growth and germination inhibitors in various higher plants. However, only several growth regulating substances including abscisic acid have been found in mosses, bryophyte division Musci. Therefore, a search for growth inhibitors in the moss *Hypnum plumaeforme* was undertaken in order to clarify the allelopathic system in *H. plumaeforme*. Methanol extract of *H. plumaeforme* inhibited plant growth of cress, lettuce, alfalfa, *Echinochloa crus-galli* L., *Echinochloa colonum* L., *Digitaria sanguinalis* Scop., and the inhibitions were increased with increasing extract concentrations. The putative two compounds causing growth inhibitory effect of *H. plumaeforme* were isolated from the methanol extract and the chemical structures of these inhibitors were determined by MS, and ¹H- and ¹³C-NMR spectral data as momilactone A and B, which is the first report of the presence of momilactone A and B not only in mosses but in plant kingdom except for *Oryza sativa* L. Momilactone A and B inhibited the growth of cress and *E. crus-galli* at concentrations greater than 30 and 3 μM , respectively. The endogenous concentrations of momilactone A and B in *H. plumaeforme* were 45.6 and 26.2 $\mu\text{g g}^{-1}$ dry weight, respectively. The effectiveness of momilactone A and B on the growth inhibition and the occurrence of these inhibitors in *H. plumaeforme* suggest that momilactone A and B may play an important role in *H. plumaeforme*.

Key words: *Hypnum plumaeforme*, allelopathy, growth inhibitor, momilactone, phytotoxicity

P-017 Screening of Volatile Allelopathic Activity of Alien Plants by Dish Pack Method and Isolation of Isothiocyanate Compounds as Allelochemicals

Mami Sugano^{a, b}, Ken Hashizume^b, Syuntaro Hiradate^a and Yoshiharu Fujii^a

^a National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai Tsukuba 305-8604, Japan

^b Snow Brand Seed Co., Ltd., 634 Naganumahara-cho, Inage-ku, Chiba 263-0001, Japan
sugamami@affrc.go.jp

We have developed a new bioassay named Dish Pack Method for analyzing volatile allelochemicals. In this method, leaves of plants were put into one of the holes in a 6-well-multi-dish. Each side of the multi-dish was sealed and put in incubator kept at 25 degrees Celsius. The growth of test plants was measured after 4 days. It is possible to analyze the internal volatile gas collected from the hole with gas-tight-syringe through the septum set up on the holes. Volatile compounds from these test plants were analyzed by GC-MS. From Leguminosae and Papaveraceae plants, carbonyl compounds (trans-2-hexenal and cis-3-hexenol) were identified as major compounds. From Compositae and Labiatae, monoterpenoids (α -Pinene, Limonene, Myrcene *et al.*) were mainly identified. Spider flower (*Cleome spinosa*) contained strong volatile allelochemical. Methyl isothiocyanate (MITC) was identified in Spider flower by GC-MS. Indian mustard (*Brassica juncea*) showed a strong allelopathic activity and Allyl isothiocyanate (AITC) was detected as a major allelochemical. MITC and AITC were detected about one day after the leaves had been injured, since the synthesis of these compounds needed the enzyme reaction.

Key words: volatile allelochemical, bioassay, Dish Pack Method, isothiocyanate

P-018 Plant-Growth-Inhibitory Activities of Catecholic Allelochemicals as Effects by Soils **Akihiro Furubayashi, Syuntaro Hiradate and Yoshiharu Fujii**

National Institute for Agro-Environmental Sciences (NIAES), 3-1-3 Kan-nondai, Tsukuba, Ibaraki 305-8604, Japan

furuba@affrc.go.jp

3-(3',4'-Dihydroxyphenyl)-L-alanine (L-DOPA), found in velvet bean (*Mucuna pruriens*), inhibits plant growth. Its adsorption and transformation reactions in soils are caused by the catechol moiety in the structure (Furubayashi *et al.*, 2007), resulting in the reduction of L-DOPA plant-growth-inhibitory activity. We hypothesize that many other catecholic allelochemicals may undergo the same reduction, hence in the present study, plant-growth-inhibitory effects of six catecholic compounds (L-DOPA, (+/-)-catechin, caffeic acid, chlorogenic acid, and gallic acid) and for comparison, two non-catecholic compounds (2,4-D and DL-*m*-tyrosine) on root elongation of lettuce were evaluated in the presence of various soil types (volcanic ash soil, calcareous soil, and alluvial soil) to assess the effects of soils on the inhibitory activities. The inhibitory activities of all compounds were reduced by the presence of soils, and the reductions in the catecholic compounds were more obvious than in the non-catecholic compounds. The reduction effects of soils on the plant-growth-inhibitory activities of the catecholic compounds were in the following order: calcareous soil > volcanic ash soil >> alluvial soil. Therefore, we concluded that the concentrations of catecholic allelochemicals in soils will decrease rapidly by means of similar mechanisms responsible for L-DOPA: adsorption and transformation reactions and microbial degradation. The allelopathic phenomena caused by the catecholic compounds may not be observed in many soils.

Key words: catecholic allelochemicals, L-DOPA, soil, plant-growth-inhibition

P-019 Allelopathic Potential of Itchgrass (*Rottboellia exaltata* L. f.) in Soil

Daigo Itaya^a, Pharnuwat Mahatamnuchoke^b, Keiko Yamaji^a and Katsuichiro Kobayashi^a

^a Graduate school of life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8572, Japan

^b Department of Agronomy, Faculty of Agriculture, Kasetsart University, Kamphaeng-Saen Campus, Nakhon-Pathom, 73140, Thailand

kakobab@agbi.tsukuba.ac.jp

Itchgrass (*Rottboellia exaltata* L. f.) is a noxious weed distributing in the tropical and subtropical areas. The farmers in the northern part of Thailand have been traditionally using itchgrass as a mulching material for weed management in their vegetable fields. The root elongation of radish (*Raphanus sativus* L. var. *radicula*) seedling, as a test plant, was inhibited in soil applied with the powder of itchgrass shoot or root in laboratory experiment. Furthermore, in sea sand treated with soil water collected from that soil by centrifugation with double tubes, the root elongation was also inhibited. The result indicates that itchgrass contains phytotoxic compound(s) in both shoot and root. The root elongation of radish seedling was inhibited in soil with growing itchgrass in Wagner's pot in green house. It suggests that itchgrass release phytotoxic compound(s) into soil from the root and the concentration of the compound(s) in the soil water actually induce the phytotoxic activity. Identification of the phytotoxic compound(s) as the allelochemical(s) in itchgrass is under investigation.

Key words: allelopathy, weed, itchgrass, soil water

P-020 Effects of Soils on Plant-Growth-Inhibitory Activities of L-Mimosine, Juglone, and Coumarin

Kenji Ohse, Akihiro Furubayashi, Syuntaro Hiradate and Yoshiharu Fujii

National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba 305-8604, Japan

kenjiouse@affrc.go.jp

It has been reported that allelopathy is one of key factors in invasion of alien plants. Allelopathic activity has been assessed by several bioassay methods correspond to its pathway. Recently, a new method to evaluate allelopathic activity through root exudates in soils, named "rhizosphere soil method" has been established (Furubayashi *et al.* 2002), and it has been suggested that allelopathic activities are strongly affected by the presence of soils. In the present study, plant-growth-inhibitory activities of three allelochemicals were evaluated in the presence of a volcanic ash soil, an alluvial soil, and a calcareous soil, and compared with those in the absence of soil. The allelochemicals were equilibrated with these soils in agar suspension for 24 h at 30°C prior to bioassay at 20°C with an acceptor plant, lettuce. The plant-growth-inhibitory activity of L-mimosine, an allelochemical from *Leucaena leucocephala* and *Mimosa* sp, was extremely weakened in the presence of any soils, although intensive activity was observed in the absence of soil. On the other hand, the presence of soils did not decrease severely the plant-growth-inhibitory activity of juglone, an allelochemical from *Juglans nigra*, and the degrees of activity decrease depended on soil type; the activity was lowest in volcanic ash soil, followed by alluvial soil and calcareous soil. The activity of coumarin was decreased by the soils but the effects of soils were intermediate between L-mimosine and juglone. From these results, it was clarified that allelopathic activity is affected by the presence of soils.

Key words: allelochemicals, bioassay, *Juglans nigra*, *Leucaena leucocephala*, *Mimosa* sp.

P-021 Effects of Soil Chemical Properties on Kudzu Growth

Sayaka Morita, Syuntaro Hiradate, Yoshinobu Kusumoto, Shori Yamamoto and Yoshiharu Fujii

National Institute for Agro-Environmental Sciences (NIAES), 3-1-3 Kan-nondai, Tsukuba, Ibaraki 305-8604, Japan

howan@affrc.go.jp

Kudzu, a Japanese endemic plant, had been planted widely in southeast United States to reduce soil erosion at the first half of the 20th century. Nowadays, kudzu is estimated to dominate 810,000 ha of mesic forest communities in the eastern United States, becoming an invasive alien plant there. In Japan, kudzu is spread in uncultivated fields and in dried riverbed. Because of its high risk in invasion, characteristic of kudzu growth would be an important information for its control. We investigated the relationship between kudzu growth and soil chemical properties, using six types of soils. After 17 days from seeds sowing, germination and root elongation rates of kudzu in each soil were found similar, even in strongly acidic soils. This result indicates that kudzu is tolerant to aluminum toxicity. After 38 days from sowing, significant differences were detected on leaf age and total dry weight of kudzu depending on the soil nutrient conditions, which would correspond to bioavailability of phosphorus and nitrogen, respectively. These results indicate that the soil chemical properties could be important factors for kudzu growth.

Key words: Al tolerance, kudzu, phosphorous bio-availability, plant growth, soil chemical properties

P-022 2'-Epi-orobanchol and Solanacol, Germination Stimulants for Root Parasitic Weeds, Produced by Tobacco

Xiaonan Xie^{a, b}, Dai Kusumoto^a, Kaori Yoneyama^a, Yoichi Yamada^c, Yasutomo Takeuchi^a and Koichi Yoneyama^a

^a Weed Science Center, Utsunomiya University, 350 Mine-machi, Utsunomiya 321-8505, Japan

^b United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

^c Faculty of Education, Utsunomiya University, 350 Mine-machi, Utsunomiya 321-8505, Japan

xmoonlighter@msn.com

Germination stimulants for root holoparasitic weeds broomrapes (*Orobanche* and *Phelipanche* spp.) produced by tobacco (*Nicotiana tabacum* L.) were purified and characterized. The root exudates of tobacco contained at least 5 different stimulants and LC/MS/MS analyses revealed that 4 of them were strigolactones; a tetrahydro-strigol isomer, didehydro-strigol isomer, and two strigol isomers. The two isomers of strigol were identified as (+)-orobanchol and its 2'-epimer by the comparison of NMR and GC- and LC-MS data with synthetic standards. The structure of the tetrahydro-strigol isomer, the most major stimulant of the bright yellow tobacco cultivars, was determined as 4- α -hydroxy-5,8-dimethyl-GR24 [(*E*)-4- α -hydroxy-5,8-dimethyl-3-(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-3a,4-dihydro-3*H*-indeno[1,2-*b*]furan-2(8*bH*)-one] and named solanacol. 2'-*Epi*-orobanchol and solanacol are structurally unique and the first natural strigolactones having a 2'-*epi* stereochemistry and a benzene ring, respectively.

Key words: strigolactone; germination stimulant; parasitic weeds; tobacco; 2'-*epi*-orobanchol; orobanchol; solanacol

P-023 Isolation and Identification of Alectrol as (+)-Orobanchyl Acetate, a Novel Germination Stimulant for Root Parasitic Plants

Xiaonan Xie^{a, b}, Kaori Yoneyama^a, Dai Kusumoto^a, Yoichi Yamada^c, Takao Yokota^d, Yasutomo Takeuchi^a and Koichi Yoneyama^a

^a Weed Science Center, Utsunomiya University, Mine-machi, Utsunomiya 321-8505, Japan

^b United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu 183-8509, Japan

^c Faculty of Education, Utsunomiya University, Mine-machi, Utsunomiya 321-8505, Japan

^d Department of Biosciences, Teikyo University, 1-1 Toyosatodai, Utsunomiya 320-8551, Japan

yoneyama@cc.utsunomiya-u.ac.jp

Alectrol was first isolated as a germination stimulant of *Striga gesnerioides* and *Alectra volgelii* from root exudates of cowpea (*Vigna unguiculata*), and then as a stimulant of *Orobanche minor* from root exudates of red clover (*Trifolium pratense*). The structure originally proposed for alectrol was, however, proven to be incorrect by synthesis. In the present study, alectrol was purified from root exudates of red clover and identified as a novel strigolactone, (+)-orobanchyl acetate [(3a*S*,4*S*,8b*S*,*E*)-8,8-dimethyl-3-(((*R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)methylene)-2-oxo-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-4-yl acetate], by 1D and 2D NMR spectroscopy and ESI- and EI-MS spectrometry. Orobanchyl acetate afforded an [M-42]⁺ ion in EI-MS and thus had been recognized as an isomer of strigol. Orobanchyl acetate was detected in the root exudates of other plant species including soybean (*Glycine max* L.) along with orobanchol.

Key words: alectrol, germination stimulant, orobanchyl acetate, parasitic plants, strigolactones

P-024 Production of Strigolactone, Host Recognition Signals for Root Parasitic Weeds and AM Fungi, and Nutrient Acquisition Strategy of Plants

Kaori Yoneyama^a, Hitoshi Sekimoto^b, Yasutomo Takeuchi^a and Koichi Yoneyama^a

^a Weed Sci. Ctr.

^b Fac. Agric., Utsunomiya Univ., 350 Mine-machi, Utsunomiya 321-8505, Japan
fragrance0917@yahoo.co.jp

Strigolactones are important host recognition signals for both symbiotic arbuscular mycorrhizal (AM) fungi and root parasitic weeds *Striga* and *Orobanche* spp.; strigolactones induce hyphal branching morphogenesis of AM fungi as well as germination of root parasites. Here, we show that in leguminous plants, reduced supply of phosphorus but not of other mineral nutrients significantly promoted the exudation of strigolactones. By contrast, in non-leguminous plants, nitrogen as well as phosphorus deficiency increases the strigolactone exudation. Furthermore, in non-hosts of AM fungi, neither nitrogen nor phosphorus deficiency affects exudation. These results indicate that the regulation of strigolactone exudation may vary with the nutrient acquisition strategy of plants.

Key words: strigolactones, host recognition signal, root parasitic weed, AM fungi, nutrient acquisition strategy

P-025 Characterization of Strigolactones, Host Recognition Signals for Arbuscular Mycorrhizal Fungi and Root Parasitic Plants, Produced by Pea

Yuta Harada^a, Xiaonan Xie^{a, b}, Dai Kusumoto^a, Norio Fusegi^a, Kaori Yoneyama^a, Yasutomo Takeuchi^a, Yoichi Yamada^c, Takao Yokota^d and Koichi Yoneyama^a

^a Weed Science Center, Utsunomiya University, 350 Mine-machi, Utsunomiya 321-8505, Japan

^b United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu 183-8509, Japan

^c Faculty of Education, Utsunomiya University, 350 Mine-machi, Utsunomiya 321-8505, Japan

^d Department of Biosciences, Teikyo University, 1-1 Toyosatodai, Utsunomiya 320-8551, Japan

a043182@cc.utsunomiya-u.ac.jp

Strigolactones are plant secondary metabolites which function as host recognition signals for arbuscular mycorrhizal (AM) fungi and root parasitic plants, *Striga* and *Orobanche*. Plants exude cocktails of strigolactones and their compositions differ with plant species, their growth stages, and growth conditions. In this study, characterization of strigolactones produced by pea (*Pisum sativum* L.), a host of *Orobanche*, was conducted by comparing retention times of germination stimulants on ODS-HPLC with those of synthetic standards and by LC/MS/MS. In the root exudates collected from pea plants grown hydroponically, orobanchol, orobanchyl acetate, and a novel strigolactone were detected as major stimulants. Purification and structural elucidation of the novel strigolactone will be presented.

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

P-026 Inhibitory Activities of Allelochemicals from Dodder (*Cuscuta hygrophilae*)

Tran Dang Xuan^a, Shinkichi Tawata^a, Ill Min Chung^b and Tran Dang Khanh^b

^a Faculty of Agriculture, University of the Ryukyus, Okinawa 903-0213, Japan

^b Department of Applied Life Science, College of Life and Environment Science, Konkuk University, Kwang Jin Ku Hwayang Dong, 143-701 Seoul, Korea

g056003@agr.u-ryukyu.ac.jp

Dodder is a parasitic weed that is troublesome to the growth of many plants. The study shows that this invasive species contains strong allelopathic potential, which exerted strong inhibition against the growth of indicator plants and noxious paddy weeds in bioassay and pot trials. In a greenhouse, incorporation of 0.5 t ha⁻¹ of dried dodder plants to paddy soil reduced spontaneous growth of paddy weeds by about 50%, whereas the 1.5–2 t ha⁻¹ dose suppressed biomass of paddy weeds by more than 75% and completely controlled emergence of barnyardgrass and monochoria. Twenty-two compounds were separated from the dodder and identified by GC-MS, which are belonging to essential oils, long-chain fatty acids, phenols, phenolic acids, and lactone. Among these compounds, 15 substances were quantified and tested for their herbicidal activity. Quantity of cinnamic acid was the highest (37.3 mg g⁻¹), followed by 5,6-dehydrokavain (6.0 mg g⁻¹), myristic acid (3.2 mg g⁻¹), and methyl cinnamate (2.1 mg g⁻¹), whereas the amounts of other compounds were between 0.01–0.1 mg g⁻¹. It is suggested that the content of essential oils exist within dodder, which was in rather high dose (0.41–2.1 mg g⁻¹), correlated to its strength of chemical cues to find host plants. Cinnamic acid, 5,6-dehydrokavain (DDK), methyl cinnamate, and vanillin exerted the most potent herbicidal activities against radish growth. Finding of this study propose that cinnamic acid, DDK, and methyl cinnamate are responsible for its strong phytotoxic action of dodder plants. However, whether these plant growth inhibitors and other compounds detected from the dodder can suppress emergence of their hosts as well as their role to its strong invasiveness need further elucidation.

Key words: Allelopathic potential, allelochemical, dodder, paddy weed, inhibition, weed-suppressing ability, invasiveness

P-027 Defense Chemicals from Camphorweed (*Heterotheca subaxillaris*) against Phytophagous Insects

Masanori Morimoto^{a, c}, Yusuke Fukuda^a, Takahiro Teranishi^b, Charles L. Cantrell^c, Stephen O. Duke^c and Koichiro Komai^a

^a Department of Applied Biological Chemistry, Graduate School of Agriculture, Kinki University 3327-204 Nakamachi Nara 631-8505, Japan

^b Department of Applied Biological Chemistry, School of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan

^c USDA-ARS, Natural Product Utilization Research Unit, University of Mississippi, Oxford, MS, 38677, USA
masanori@nara.kindai.ac.jp

Camphorweed (*Heterotheca subaxillaris* (Lam.) Britton & Rusby) (Asteraceae) is a common annual or biennial weed that grows on sandy fields in the U.S. This species has a characteristic camphor-like smell and does not appear to be subjected to damage from phytophagous insects in the field. Although it has been reported that terpenoids in camphorweed have an effect on phytophagous insect (*Pseudoplusia includens* Walker, Noctuidae) feeding behavior, a systematic bioassay-guided investigation for active compounds present has not been performed¹⁾. We prepared a dichloromethane extract and rinsate from aerial parts for bioassay. The rinsate showed good insect antifeedant activity in a dose-dependent manner against 3rd instar common cutworms (*Spodoptera litura* F. Noctuidae). This rinsate was obtained approx. a 1% yield from plant fresh weight. Constituents in this rinsate were isolated by Si-gel column chromatography and their structures identified based on their spectroscopic data. The monoterpene alcohol borneol was the most dominant compound, and it appears to be responsible for the camphor-like smell of this plant. Monoterpene hydrocarbons, limonene, pinene and myrcene were other significant monoterpenes. Calamenene-type sesquiterpene acids were also major constituents of this rinsate. There were many methylated flavones of which the major flavonoid was hispidulin (6-methoxy-4',5,7-trihydroxyflavone). Insect antifeedant activity of these constituents in the rinsate from camphorweed leaves will be discussed.

1) Charles, A. M. *et. al.*, Inhibition of feeding by a generalist insect due to increased volatile leaf terpenes under nitrate-limiting conditions. *J. Chem. Ecol.* **13**(11), 2059-2067, (1987).

Key words: Camphorweed, *Heterotheca subaxillaris*, Insect antifeedant, allelochemicals

P-028 Screening of Plant Extracts that Induce Systemic Acquired Resistance in the Cucumber

Hidehiro Inagaki, Akira Yamaguchi, Kimihiko Kato, Chizuko Kageyama, and Hiroyuki Iyozumi

Shizuoka Prefectural Reserch Institute of Agriculture and Forestry, 678-1 Tomigaoka Iwata 438-0803, Japan
hidehiro1_inagaki@pref.shizuoka.lg.jp

Several defense mechanisms are known to be induced in plants. Systemic acquired resistance (SAR) is one of these phenomena involved in a plant's defense mechanisms, and it has gained considerable attention in crop production because of its broad-spectrum and long-lasting immunity in noninfected tissues. Generally, SAR can be induced by pathogen attack or treatment with either a biological or a chemical elicitor. In addition, recent studies suggest that SAR can be induced by plant extracts from some plant species, such as giant knotweed, spinach, and rhubarb. In this study, we screened plant extracts from 409 plant species for their ability to induce SAR by bioassay, using cucumber plants and *Colletotrichum lagenarium*. Plant extracts from nine species, including *Cinnamomum verum*, *Campanula punctata*, *Chrysanthemum frutescens*, *Gynura bicolor*, *Lathyrus japonicus*, *Origanum vulgare*, *Portulaca grandiflora*, *Pelargonium graveolens*, and *Senecio cineraria*, controlled symptoms on the upper leaves of cucumber plants. Furthermore, expression of a defense-related gene, peroxidase, was significantly higher when these plant extracts were applied. From these results, we concluded that plant extracts from these nine plant species induce SAR in the cucumber.

Key words: plant extract, systemic acquired resistance, cucumber

P-029 L-DOPA and *m*-Tyrosine Have Similar Chemical Structure, but Different Mode of Action

Mayumi Hachinohe, Hiroaki Shirato and Hiroshi Matsumoto

Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8572, Japan.

mhc@rose.zero.ad.jp

L-DOPA (3,4-dihydroxyphenylalanine) is one of well-known allelochemicals that inhibits plant growth. In the survey of phytotoxicity among structurally related compounds, we found that *m*-tyrosine, a natural compound, has comparable phytotoxic potential with L-DOPA (J. Weed Sci. Tech. 50 Suppl. 204-205, 2005). L-DOPA and *m*-tyrosine (10^{-4} M) inhibited the growth of lettuce root to 20% of non-treated control at 5 DAT. However, *m*-tyrosine did not show selectivity between barnyardgrass and lettuce that was observed in L-DOPA treatment. Our previous study showed that L-DOPA increased reactive oxygen species (ROS) that were generated from the metabolic pathway from L-DOPA to melanin (melanin synthesis pathway). Huge accumulation of melanin and typical oxidative damage by ROS were observed in L-DOPA-treated plant. *m*-Tyrosine also increased lipid peroxide formation in lettuce but did not increase melanin formation. Exogenously-applied antioxidants, ascorbic acid and α -tocopherol, alleviated the phytotoxicity of L-DOPA but did not alleviate that of *m*-tyrosine. Instead, phenylalanine recovered the growth of *m*-tyrosine-treated lettuce root. These results suggest that the phytotoxic action mechanisms are different between L-DOPA and *m*-tyrosine, although they have very similar chemical structures. The phytotoxicity of *m*-tyrosine might be due to misincorporation of *m*-tyrosine for phenylalanine during protein synthesis.

P-030 Allelopathic Activities of Alien Plants by Specific Bioassays: Sandwich Method, Plant Box Method, Dish-pack Method and Demonstration of Dangerous Plants to Biodiversity

Yoshiharu Fujii, Mami Sugano, Fumiko Iino, Pariasca Dolorosa and Syuntaro Hiradate

National Institute for Agro-Environmental Sciences (NIAES), 3-1-3 Kan-nondai, Tsukuba, Ibaraki 305-8604, Japan

yfujii@affrc.go.jp

The allelopathic activities of newly introduced or supposed to be introduced plants were evaluated using sandwich method (for root exudates), plant box method (for leaf leachates), dish-pack method (for volatile chemicals). By sandwich method, about 4000 species were tested and the results obtained using 10 mg of leaves in 10 square cm dish showed normal distribution. Sorghum, Oxalis, and Rosaceae family showed strong inhibitory activities. By plant box method, about 800 species were assayed. The results by dish-pack method did not correlate with the data from sandwich method and plant box method. As a result of these three assays, potentially dangerous alien plants with high allelopathic activities were 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*. The reasons behind each allelopathic activity and the potential allelochemicals will be discussed.

Key words: invasive alien plants, sandwich method, plant box method, dish-pack method, biodiversity

P-031 Allelopathic Effects of Tree Leaf Extracts on Seed Germination and Growth of Wheat and Wild Oats

Khan B. Marwat and M. A. Khan

Department of Weed Science, NWFP Agricultural University Peshawar-25130, Pakistan

kbmarwat@yahoo.com

Experiment was conducted in NWFP Agricultural University Peshawar during January 2006. Grinded leaves of *Prosopis juliflora*, *Eucalyptus camaldulensis* and *Acacia nilotica* were soaked in tap water for 5 hr at room temperature. The concentration of each tree species was 150, 100 and 50 g L⁻¹. Completely randomized design having four repeats was used. Ten seeds of each species were sown in pots and then irrigated with the respective extracts soon after sowing. Results showed that germination %age and plant height of both species were significantly affected by different concentrations. *Prosopis* showed stimulatory effect on germination of both the species. In wheat, maximum germination and plant height of 52.50% and 32.22 cm, respectively was recorded in *Prosopis* treated pots as against 15.00% and 31.50 cm in control however in *Eucalyptus* @150 g L⁻¹ also 15% germination of wheat was recorded. Similarly, for wild oats, maximum germination percentage and plant height of 47.5% and 51.9 cm was recorded in *Prosopis* treated pots. Low concentration of *Prosopis* proved stimulatory as compared to higher concentrations. *Eucalyptus* showed slight negative effect on the species tested. The effect of other concentrations of tree extracts was comparable to each other in the species tested. Hence it can be concluded from the results that allelopathy of trees can be used as viable weed management technique in the future as allelochemicals stimulate the germination of wild oats which give the chance of making soil seed bank weaker.

Key words: allelopathy, *Prosopis*, *Eucalyptus*

P-032 Allelopathy: Problems and Opportunities

Muhammad Azim Khan and Khan Bahadar Marwat

Department of Weed Science, NWFP Agricultural University Peshawar 25130, Pakistan

ahmadzaipk@yahoo.com

Allelopathy refers mostly to the harmful effect of one plant on the other through the release of toxic substances. It is predicted that in the near future the exploration of allelopathy will be used as a weed control strategy. The use of allelopathy against weeds can be used through biotechnological approaches or simple application of plant extracts. Allelopathy is a wonderful field of study which needs to be explored extensively, as many researchers advocate that allelopathy leads to the monoculture and harmful to the biodiversity. Pollens of few allelopathic species can stop fruit setting in many vegetables and fruit trees?. Allelopathic substances could cause soil pollution, inhibit nodulation in legumes, dangerous for fish and other sea animals in aquatic bodies, and adversely affect the physiological functions of certain plants. Detailed knowledge of individual species for allelopathins will help in utilizing weeds against weeds and crops against weeds. Joint efforts of weed scientists, chemists, ecologists, and taxonomists are required to achieve these objectives. Working on this challenge will lead to new discoveries that will keep us excited to learn more, and gain a better understanding of the phenomenon. Equipped with this new knowledge and understanding, we should be able to solve many difficult environmental problems of our time. Thus exploitation of allelopathy provides unlimited opportunities to contribute in the solution of agricultural problems.

P-033 Important Factors of Allelopathy Properties in the Artificial Closed Eco-systems in Space

Kaori Tomita-Yokotani, Yoshiharu Fujii, Hirofumi Hashimoto and Masamichi Yamashita

Graduate school of life and environmental sciences, 1-1-1 Ten-nodai, Tsukuba, Ibaraki 305-8572, Japan

kaboka@sakura.cc.tsukuba.ac.jp

There have been many diverse life forms on the earth. They have individual interactions in each others and live together with certain influence among them as allelopathy. Physical closure of living environment and lack of natural process to decompose allelopathic chemicals or the sink among material circulation are important factors in the biosphere of space. Many organisms and ecological system may behave differently in spacecrafts or on outer planets, based on the modified inter-organisms and -species interactions associated with allelopathy. Gravity is one of the important factors for living plants. For the first step of the basic study on allelopathy and its modification under microgravity, we conducted pseudo microgravity experiment using 3D-clinostat. Biosynthesis, release and sensing process of allelopathic chemicals were examined in details. We have already investigated about this using with some species of plants. We will show the results and discuss the general relationship between allelopathy properties and gravity and the closed eco-systems.

Key words: allelopathy, 3D-clinostat, closed eco-systems, gravity, space utilization

P-034 Phagostimulants in Host Plants against Several Okinawan Danaid Butterfly Larvae

Kazuhito Ogihara, Hiroto Sinyasiki, Kazumi Kamizato, Yoshihito Nagano and Sei-ichi Yogi

Department of Chemistry, Biology and Marin Science, Faculty of Science, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan

kogihara@sci.u-ryukyu.ac.jp

Three danaid butterflies living in Okinawa, such as *Saratula genutia*, *Ideopsis similes*, and *Anosia curisippus* utilized *Cynanchum liukiuense*, *Tylophora Tanakae* and *Asclepias curassavica*, respectively, as host plants. These butterflies have selected their host plants in 15000 species plants in field. Therefore, as a part of studies on feeding factors, we investigated phagostimulants in host plants against these danaid butterfly larvae. MeOH extract from aerial parts of each host plant was individually subjected to several chromatography with bioassay to isolated phagostimulants against each danaid butterfly larva. *S. genutia* larve were found to be stimulated by 4-aminobutyric acid (GABA), *I. similes* and *A. curisippus* larvae were found to be did by a mixture of GABA and L-prolin and myo-inositol, respectively as a key compound. *A. curisippus* larvae begin to feed on young leaves of *C. liukiuense* which is not host plant, when the larvae have fed up the host plants. We investigated the phagostimulants in *C. liukiuense* leaves against *A. curisippus* larvae. In comparison of feeding on young leaves with doing on mature ones, the larvae fed on young leaves by choice and not did on any mature leaves. *A. curisippus* larvae were found to be stimulated by the same constituents as phagostimulants in *A. curassavica*. Moreover, it was suggested that the mature leaves contain antifeeding stimulants against *A. curisippus* larvae.

Key words: chemical ecology, danaid butterflies, phagostimulants, host plants

P-035 Larval Feeding Stimulants for a Rutaceae-Feeding Swallowtail Butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae), in *Citrus unshiu* Leaves

Toshihiro Murata, Naoki Mori and Ritsuo Nishida

Laboratory of Chemical Ecology, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
murata-t@tohoku-pharm.ac.jp

Larvae of a swallowtail butterfly, *Papilio xuthus* L., feed exclusively on the plant family Rutaceae, including *Citrus* crops. Although the specific oviposition stimulants for *P. xuthus* have been elucidated, the chemical basis of the larval feeding stimulants is unknown. The larvae were strongly stimulated to feed on a strip of toilet paper impregnated with ethanolic extracts of host plant leaves. The feeding stimulant in *Citrus unshiu* leaves was found to be composed of multiple chemical factors including sugar components [glucose (1), fructose (2) and sucrose (3)], a betaine [stachydrine (4)], a cyclic peptide [citrusin I (5)], a polymethoxyflavone [isosinensetin (6)] and lipids [1-linoleoylglycerol (7), 1-linolenoylglycerol (8) and 1,2-dilinolenoyl-3-galactosyl-sn-glycerol (MGDG) (9)]. When these components were supplied individually, very few larvae consumed the test strip. However, larvae consumed the test paper strip when some of these compounds were mixed together, indicating that the larval host recognition is controlled by multiple components of a specific chemical composition. The maximum activity was achieved when all of the components (1-9) were combined, close to that of the crude ethanolic extract. We conducted an additional assay to examine the effect of the secondary components (4, 5, 6) on larval feeding activity. The whole mixture (1-9) showed significantly higher activity than that of a mixture of primary components (1, 2, 3, 7, 8 and 9), which suggested the *Citrus*-specific secondary components serve as the factors to control selectivity in host recognition.

P-036 Behavioral and Electrophysiological Analyses of Larval Feeding Stimulants for a Primitive Swallowtail Butterfly, *Sericanus montela*, in the Host Plant, *Aristolochia debilis*

Yoshitsugu Murata^a, Ayako Wada^a, Sachi Matsumoto^a, Mamiko Ozaki^b and Ritsuo Nishida^a

^a Lab of Chemical Ecology, Grad. Sch. Agric., Kyoto Univ., Kyoto, 606-8502, Japan

^b Dept. Biol., Grad. Sch. Sci., Kobe Univ., Kobe, 657-8501, Japan
my@kais.kyoto-u.ac.jp

Larvae of a primitive swallowtail butterfly, *Sericanus montela*, feed exclusively on the pipevine, *Aristolochia debilis* (Aristolochiaceae). The fifth instar larvae readily consumed an agar medium containing an ethanolic extract of the host plant. The feeding stimulant activity was evaluated as determined by an area of thin agar plates consumed by the larvae. A series of sugars [glucose, fructose, sucrose] and a cyclitol [sequoyitol] from the water-soluble fraction, and from the organic layer an alkaloid derivative [aristolochic acid I] and a lipid [1,2-dilinolenoyl-3-galactosyl-sn-glycerol (MGDG)] were identified as the stimulants. None of components was active when presented individually. But the specific feeding response was induced when all of the six compounds were combined. This indicates that the larval host recognition is controlled by multiple compounds of a specific chemical composition. Moreover, using the crude ethanolic extract of *A. debilis* and these feeding stimulants, we recorded electrophysiological gustatory responses from the larval lateral styloconic sensillum and medial styloconic sensillum by a tip-recording method. Each compound elicited characteristic types of impulses from either or both sensilla. When all of the six compounds were combined, the impulses of each sensillum were similar to those induced by the crude extract. The larval host recognition through a gustatory sense seems to be controlled by multiple compounds of a specific chemical composition.

Key words: phagostimulants, host selection, electrophysiology, *Sericanus montela*, *Aristolochia debilis*

P-037 Oviposition and Feeding Stimulants for Okinawan Aristolochiaceae-feeding Swallowtail Butterflies: Pinitol and Aristolochic Acids from *Aristolochia liukiensis* and *Aristolochia zollingeriana*

Hiroto Shinyashiki and Kazuhito Ogiwara

Univ. of Ryukyus Graduate school of Engineering and science chemistry, Senbal 1, Nishihara-Cho, Okinawa 903-0213, Japan

k018553@sci.u-ryukyu.ac.jp

Aristolochia liukiensis and *A. zollingeriana* (Japanese name: ryukyu umanosuzukusa and koushun umanosuzukusa, respectively) belong to Aristolochiae plant and widely distributed in Okinawa archipelago subtropical region in Japan. Two Okinawan Papilionid butterflies, *Atrophaneura alcinous loochoona* and *Pachilioptia aristolochiae* (Japanese name: jakou ageha and benimon ageha, respectively) utilizes *A. liukiensis* and *A. zollingeriana* as host plant exclusively. Aristolochic acid and pinitol which obtained from these host plant distinctly induced oviposition and larval feeding behavior synergistically. In recent twenty years, Okinawan Aristolochie-feeding swallowtails have expanded their distribution range to the northward. As part of studies on chemical ecology between Okinawan butterflies and their host plant, we approached to estimate the distribution range expansion of these butterflies to northward of by means of chemical constituents, possessing ovipositive and feeding activity against the butterflies, in the Aristolochiae plants belong to subtropical and temperate regions of Japan. Consequently, Okinawan Aristolochiae-feeding swallowtails have enough potential to expand their distribution range from subtropical region Okinawa archipelago to temperate region in main Island of Japan. In addition, the progress of global warming probably accelerate such a expansion of the distribution range of Okinawan Aristolochie-feeding swallowtail butterflies.

Key words: Aristolochic acid, pinitol, Aristolochiae plant, Okinawan Aristolochie-feeding swallowtails, distribution range

P-038 Electrophysiological Analysis of Oviposition Stimulants on Tarsal Chemosensilla in a Citrus Swallowtail *Papilio xuthus*

Shintaro Yui^a, Ayako Wada-Katsumata^a, Mamiko Ozaki^b, Ryohei Kanzaki^c, Sawako Niki^c, Naoki Mori^a and Ritsuo Nishida^a

^a Lab of Chemical Ecology, Grad. Sch. Agric., Kyoto Univ., Kyoto, 606-8502, Japan

^b Dept. Biol., Grad. Sch. Sci., Kobe Univ., Kobe, 657-8501, Japan

^c Res. Center for Advanced Sci. & Tech., Univ. of Tokyo, Komaba, Tokyo, 153-8904, Japan

The oviposition behavior of a swallowtail butterfly, *Papilio xuthus*, is induced by perceiving the host plant chemicals via contact with tarsal chemosensilla of the forelegs. In order to evaluate the effect of the leaf components on the chemoreception, we conducted electrophysiological experiments using host plant extracts and fractions. A methanolic extract of *C. unshiu* was partitioned between ether and water. The water layer, containing specific oviposition stimulants, was chromatographed by ODS reverse-phase column into 7 fractions eluting with mixtures of methanol and water. A tip-recording technique was employed to detect the electrophysiological responses of female tarsal chemosensilla using the different fractions as stimuli. The results: (1) three types of impulses [Large (L), Medium (M) and Small (S)] were recorded from sensilla when the fractions were tested; (2) M type impulses were recorded most frequently from 0% and 10% methanol eluates, whereas L type impulses appeared in ODS 0-40% fractions at a greater frequency than in the original water layer or combined fractions (0-100% methanol). These results suggested that a) at least three types of sensory neurons are present within a tarsal sensillum to perceive specific chemicals in *C. unshiu*; and b) a possible involvement of inducers for L, M and S type impulses and a suppressor for an L type impulse in the extract. The oviposition behavior might be controlled by these sensory impetus caused by multiple chemical factors.

Key words: *Papilio xuthus*, host selection, oviposition stimulant, electrophysiology, tarsal chemosensilla

P-039 Oviposition Stimulant Binding Protein in a Butterfly, *Atrophaneura alcinous*
**Kazuko Tsuchihara^a, Tetsuichi Wazawa^b, Toshio Yanagida^b, Ritsuo Nishida^c, Masaji Ishiguro^d,
Kiyoshi Asaoka^e and Fumio Tokunaga^b**

^a Iwaki Meisei University, Department of Pharmacy, Iwaki 970-8551, Japan

^b Osaka University

^c Kyoto University

^d Suntory Institute of Bioorganic Research, Osaka 618-8503

^e National Institute of Agrobiological Sciences, Tsukuba 305-8634

tutihara@iwakimu.ac.jp

The oviposition behavior of butterflies is induced by plant components, which are recognized by the tarsal taste sensilla on their legs. The swallowtail butterfly, *Atrophaneura alcinous*, oviposits on the host plant, *Aristolochia debilis*. A lipophilic aristolochic acid, one of the oviposition stimulants, is required for the oviposition.

We isolated a female-specific protein (Jf23) and cloned its cDNA from female *A. alcinous*. An immunocytochemical study showed that the Jf23 localized in the sensilla. A methanol extract from the host plant, *A. debilis*, evoked neural responses in the tarsal sensilla. The response to the extract was depressed by the presence of Jf23 antiserum. Using highly sensitive fluorescence microscopy, we clarified that Jf23 binds to aristolochic acid. Three-dimensional molecular modeling studies also gave a reasonable structure for the Jf23 and aristolochic acid complex.

These results strongly suggest that Jf23 functions as a binding protein that transfers aristolochic acid to receptors on the chemosensory neurons or activates the receptor molecules by binding to the ligand.

Key words: chemosensory reception, taste sensilla, host plants, binding assay

P-040 Identification of Genes Involved in Perception of Oviposition Regulating Compounds of Swallowtail Butterflies

Katsuhisa Ozaki^a, Hideshi Naka^a, Ai Utoguchi^{a, b}, Ayumi Yamada^{a, b} and Hiroshi Yoshikawa^a

^a JT Biohistory Research Hall, 1-1 Murasaki-cho, Takatsuki, Osaka, 569-1125, Japan

^b Graduate school of science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043, Osaka, Japan

ozaki@brh.co.jp

The perception of oviposition regulators is a key feature in the host selection in phytophagous insects. Swallowtail butterflies select a limited number of plants belonging to a single or a few families as hosts. A possible correlation has been observed between changes in host plants and diversification of species of a butterfly family *Papilionidae*. Although oviposition regulating compounds have been identified for several swallowtail butterfly species from their main host plants, there are no studies on the chemoreception of these compounds at the molecular or gene levels. We performed an EST analysis of female foreleg tarsi including hair sensilla of *Papilio xuthus* in order to discover genes involving in recognition of oviposition regulators. About 10,000 clones were sequenced from both ends, and we identified G protein-coupled receptors (GPCR) gene from the cDNA library that was expressed preferentially in female foreleg tarsi. An extensive phylogenetic analysis of insect chemosensory GPCR showed that the *P. xuthus* GPCR belonged to a gustatory group, suggesting that it is a candidate for an oviposition regulatory receptor. We try to identify function of this gene by calcium imaging method. We identified 19 genes encoding chemosensory proteins (CSP) containing four conserved cysteines from *P. xuthus*. Seventeen of nineteen CSP genes clustered on a restricted region. We identified almost identical synteny in *Bombyx mori*. We assume that CSP gene family diversified in mainly phytophagous insects, and this gene family characterizes their chemoreception behavior.

Key words: chemoreceptor, insects-plants interaction, gustatory perception, function analysis

P-041 Inter- and Intraspecific Variation in Oviposition Regulatory Receptor among *Papilio* Butterflies

Hideshi Naka^a, Ai Utoguchi^{a, b}, Ayumi Yamada^{a, b}, Hiroshi Yoshikawa^a and Katsuhisa Ozaki^a

^a JT Biohistory Research Hall, Murasaki-cho 1-1, Takatsuki 569-1125, Osaka, Japan

^b Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043, Osaka, Japan
chun@wakaba.com

Phytophagous insects recognize their hostplants using multiple sensory modalities, including visual and chemical cues. In butterflies, contact chemical cues from plant leaves are important for the final recognition of host or non-host plants. Female butterflies have the contact chemoreceptors in their foretarsi, and they perceive various allelochemicals in plant leaves. Although oviposition stimulants have been identified for several swallowtail butterflies (Lepidoptera: Papilionidae) from their hostplants, there are no studies on the molecular level chemoreception system. We performed an EST analysis of female foretarsi of a swallowtail butterfly *Papilio xuthus*, and identified a G protein-coupled chemoreceptor (GPCR) as a candidate for receipt oviposition stimulant. In addition, the intraspecific variation of this GPCR was found in *P. xuthus*. With further analysis, we identified homologues of this GPCR from foretarsi in several species of *Papilio* species. Here we show a result of function analysis of these GPCRs.

Key words: insect-plant interactions, gustatory receptor, butterflies, chemoreception, host selection

P-042 Expression Analysis of Genes Involved in Oviposition Behavior of Swallowtail Butterflies

Ai Utoguchi^{a, b}, Ayumi Yamada^{a, b}, Hideshi Naka^b, Hiroshi Yoshikawa^b and Katsuhisa Ozaki^b

^a Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043, Osaka, Japan

^b JT Biohistory Research Hall, Murasaki-cho 1-1, Takatsuki 569-1125, Osaka, Japan
utoguchi@brh.co.jp

Almost phytophagous insects feed on a limited number of hostplants. As for these insects, the recognition of their hostplants in oviposition behavior is important for their life cycles. *Papilio* butterflies use several plant species belonging to one or a few families as their hosts. Female butterflies recognize taste compounds in leaves by hair sensilla located on their foretarsi at the final assessment of hosts and then they lay eggs on actual hostplants. In several *Papilio* butterflies, gustatory oviposition stimulants have been identified, but no studies in molecular or gene level. We identified a G protein-coupled receptor (GPCR) gene and 19 chemosensory protein (CSP) genes from the cDNA library that was made from female foretarsi tissues including hair sensilla of *Papilio xuthus*, those genes are assumed constituting the oviposition regulating compounds perception system working in sensilla. It is known that gustatory type GPCRs are main component of taste signal cascade and CSPs have a role in chemoreception. Temporal or regional expression change of these proteins *in vivo* may have involved in oviposition behavior in female butterflies. We will show results of expression analysis of these genes *in vivo*.

P-043 Genetic Basis of Host-Plant Preference in *Drosophila*

Takashi Matsuo, Jyunichiro Yasukawa, Eriko Harada, Daisuke Haba and Sachiko Tomioka
Tokyo Metropolitan University, 1-1 Minami Osawa, Hachioji 192-0397, Japan
mts@tmu.ac.jp

Drosophila sechellia is a fruit fly endemic to the Seychelles, and known by its exclusive use of *Morinda citrifolia* (Tahitian Noni) as a host plant. The ripe fruit of *M. citrifolia* contains octanoic acid, which is toxic to *D. melanogaster* and *D. simulans*, the most closely related species of *D. sechellia*. In contrast, *D. sechellia* is tolerant of octanoic acid and prefers it. We have found that the two genes encoding odorant-binding proteins, *Obp57d* and *Obp57e*, are involved in the taste perception of octanoic acid. These two genes are responsible for the behavioral difference between species in response to octanoic acid. Using *D. melanogaster* gene-knockout strains for *Obp57d* and *Obp57e*, we examined function of these genes in behavioral response to various fatty acids. The knockout strains were significantly different from the wild type in the oviposition-site preference assay that is based on taste perception. Genomic sequence of the *Obp57d/e* region was determined in 26 species from the *D. melanotaster* species group. Gene number difference between species was observed. Some species lost either of *Obp57d* and *Obp57e*. Expression pattern of the two genes were examined by GFP reporter assay and quantitative RT-PCR analysis. In all of the examined species, the two genes were expressed in legs and proboscis, but not in the antennae. However, the expression level in each tissue was different between species. These differences in gene number and expression level might be involved in the determination of host-plant preference in each species.

Key words: taste perception, behavior evolution, genetic analysis

P-044 Oviposition-Deterring Effect of Several Plant Extracts against *Pieris rapae* L.

Liu Zhongfang and **Yuan Guohui**

Plant Protection College of Henan Agricultural University, Wenhua Road 95, Zhengzhou, China
yguohui@hotmail.com

In this paper, the oviposition-deterring activities of several extracts from stem-leaves of *Tagetes erect*, *Lycopersicon esculentum*, *Chrysanthemum coronarium*, *Foeniculum vulgare*, *Brassica oleracea*, and root of *Tagetes erect* against *Pieris rapae* were studied. The results were summarized as follows: (1) The powder of stem-leaves of *T. erect* and *L. esculentum* had higher oviposition-deterring activities against adult *P. rapae*, the deterring rate were 48.11% and 50.02% respectively 24 hours after treatment. After 72 hours, the powder of stem-leaves of *T. erect*, *L. esculentum*, and *F. vulgare* had higher oviposition-deterring activities, with deterring rate of 45.02%, 45.28%, and 40.80% respectively. (2) In choice tests, the extracts from *T. erect*, *L. esculentum*, *F. vulgare*, and root of *T. erect* had higher oviposition-deterring activities, the deterring rate were 75.88%, 78.93%, 77.32%, and 61.80% respectively after 24 hours, which remained 61.09%, 56.85%, 64.80%, and 66.84% respectively after 72 hours. In non-choice tests, the oviposition deterring rate of extracts from *L. esculentum*, *F. vulgare*, and *T. erect* were 72.04%, 70.06%, and 67.34% respectively after 24 hours, and 60.86%, 49.81%, and 60.10% respectively after 72 hours. (3) The behavioral responses of mated females and males to the powder of stem-leaves of *L. esculentum* were the strongest, and extracts from stem-leaves of *L. esculentum* had more obvious effects than others. (4) There were 52 compounds isolated from extract of stem-leaves of *T. erect* (RT < 50.71) by steam distillation. The main component is piperitone with relative content of 19.18%.

Key words: plant extracts, *Pieris rapae* L., oviposition-deterring activities, behavioral responses

P-045 Phytochemical-mediated Differential Oviposition on four Liliales Plants by a Nymphalid Butterfly, *Kaniska canace*

Hisashi Ômura and Keiichi Honda

Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima 739-8528, Japan
homura@hiroshima-u.ac.jp

Nymphalid butterflies show a wide variety of host selection, where considerable butterfly species often use several hosts belonging to different families. However, it has been identified only a few semiochemicals involved in the adult host selection. *Kaniska canace* mainly feeds on *Smilax china* (Smilacaceae) and also has several minor hosts in the families of Smilacaceae and Liliaceae. We examined the acceptance of four Liliales plants, *S. china*, *S. riparia* (Smilacaceae), *Tricyrtis hirta* (Liliaceae), and *Lilium lancifolium* (Liliaceae), by ovipositing females of the butterfly. Since the foliage of the four plants induced egg-laying from the butterflies, methanolic extracts of the foliage were examined for oviposition-stimulatory activities with plastic leaf models. The extracts of *S. china* and *S. riparia* stimulated 100% and 82% oviposition responses of the butterfly, while those of *T. hirta* and *L. lancifolium* elicited 26% and 36%. It is evident that the two smilacaceous plants are more suitable to the selection by adult females than the two liliaceous plants. Among the three fractions partitioned from the methanolic extract of each plant, the water-soluble fraction showed the highest activity and followed by the isobutanol- and chloroform-soluble ones. Furthermore, from the aqueous fraction of the extract from *S. china* (Sc-3), we attempted to identify oviposition stimulants for *K. canace*. Among the four fractions separated from Sc-3 by porous polymer gel, Sc-3-1 with the highest polarity induced 92% responses, and followed by Sc-3-2 (42%), Sc-3-4 (14%), and Sc-3-3 (6%). The fraction Sc-3-1 was further separated into an acidic, a neutral/amphoteric, and a basic fractions employing cation and/or anion exchange resins. The acidic fraction of Sc-3-1 was significantly active (92%) as well as the original fraction, suggesting the presence of oviposition stimulants.

Key words: Nymphalidae, *Kaniska canace*, oviposition, host selection, Smilacaceae

P-046 Attracting of *Canna edulis* Ker to Oviposition of *Ostrinia furnacalis*

Zhao Guoqiang and Luo Meihao

Plant Protection College of Henan Agricultural University, Wenhua Road 95, Zhengzhou, China
Luomeihao88@163.com

This paper reported the ovipositional responses of *Ostrinia furnacalis* to *Canna edulis* leaf volatiles. The main results are as follows: (1) *C. edulis*, as well as four *Zea mays* varieties used for comparison, were interplanted in the fields. The result demonstrates that the number of *O. furnacalis* eggs deposited on *C. edulis* leaves was 4~10 times that of *Z. mays* varieties. Similar result was also obtained from field cage bioassays indicating the eggs deposited on *C. edulis* leaves was 3~7 times that of *Z. mays* varieties. (2) The leaves of *C. edulis* and four *Z. mays* varieties were extracted via steam distillation respectively, and effects of different leaf extracts on *O. furnacalis* oviposition was tested in the laboratory. The results show that eggs deposited on the glass board coated with *C. edulis* extract was the most among all the extracts at the same concentration, and the ratio of eggs deposited on the glass board coated with *C. edulis* extract ranged from 1.71 to 4.36. The eggs deposited on the ovipositional matrix coated with the leaf extracts of *C. edulis* and "White glutinous maize" were significantly more than that deposited on the other *Z. mays* varieties. (3) The attractiveness of different plant species (varieties) at different concentrations to three groups of *O. furnacalis* adults (virgin females, mated females, and males) was investigated in a selective olfactometer with dichloromethane as the control. The result shows that the extracts of *C. edulis* revealed the strongest attraction to all the three groups of adults, and the attraction ratio of *C. edulis* extracts to *Z. mays* ranged from 2 to 8, showing significant difference by t-test, among which the extracts of *C. edulis* applied at 0.1 g/mL had the highest attraction.

Key words: *Canna edulis*, *Ostrinia furnacalis*, ovipositional attraction

P-047 Host Range of Rice Bug, *Leptocorisa chinensis* and Existence of Chemical Cues in Host Plant Affecting Feeding Behavior

M. Ishizaki, T. Yasuda and T. Watanabe

National Agricultural Research Center, 3-1-1 Kannondai Tsukuba 305-8666, Japan

imami@affrc.go.jp

Leptocorisa chinensis Dallas (Hemiptera: Alydidae), is one of the most important rice insect pest in Japan. The bug inhabits gramineous grass fields, and attacks rice panicles and causes abortive grains or stained grains. To confirm the host range of *L. chinensis*, nymphs were reared on inflorescence of various gramineous and non-gramineous plant species. Nymphs that were reared on some gramineous species including rice, *Setaria viridis*, *Poa annua* could grow to adults, whereas nymphs on other gramineous species including *Eleusine indica*, *Bromus catharticus*, or non-gramineous species could not grow to adults. In the case of plant species on which nymph could grow, many of nymphs settled on and showed frequent feeding behavior on the inflorescence. So existence of chemical factor in host plants which affect feeding behavior was analyzed. When individual nymph was released on a rice panicle in the laboratory, the bug showed a series of feeding behavior. A filter paper strip treated with methanol extracts of rice panicle also elicited similar behavior to that on panicles. This indicates the existence of chemical factor in rice panicles which elicit feeding behavior. Panicles and extracts of various gramineous host and non-host plant species were also examined.

Key words: rice bug, host range, feeding behavior, Heteroptera, Gramineae

P-048 Attractants toward the Olive Weevil (*Dyscerus perforatus*) in their Feces

Shuhei Nakajima, Miho Hosokawa, Ayumi Todo, Tomoko Yamashita and Nomichi Baba

Department of Bioresources Chemistry, Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan
snaka24@cc.okayama-u.ac.jp

The olive weevil [*Dyscerus perforatus*; Coleoptera; Curculionidae] is a native species in Japan and now the most serious pest of the olive tree. Originally, this weevil seemed to colonize on *Ligustrum japonicum* and other trees which belong to the oleacea family, like the olive. However, when olive trees were introduced to Japan and planted on large scale, the weevils immediately attacked the plants and soon preferred them to the former hosts. Unlike in the former hosts where the weevils live in low population densities, it is extraordinary high in the case of the olive tree and the assault thereby becomes seriously damaged for the host plant. During the course of our study on the relationship between the olive tree and the olive weevil, we have been interested in the possible chemical constituents of this plant, that are responsible for host selection and attraction of the olive weevil. Previously, we reported that a secoiridoid glucoside, some lignans and β -sitosteryl-D-glucoside from the olive tree stimulated feeding habit of the weevil.

In this study, the preliminary bioassay by using the olfactometer showed that the olive weevils were attracted by some volatile compounds released from their feces. Therefore, we are trying to isolate and identify the attractive components. Here, the characterization and the activity of such attractants will be discussed.

Key words: the olive weevil, attractants, the olive tree, volatile compounds

P-049 Identification of Feeding Stimulants from *Salix sachalinensis* Leaves for the Willow Leaf Beetle, *Plagioderia versicolora*

Takeshi Matsumoto^{a,*} and Satoshi Tahara^a

^a Laboratory of Ecological Chemistry, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo, 060-8589, Japan

* Present Address: Shikoku Research Center, Forestry and Forest Products Research Institute, 2-915, Asakura-nishimachi, Kochi, 780-8077, Japan

mtakeshi@affrc.go.jp

Willow leaf beetles, *Plagioderia versicolora*, are found on *Salix sachalinensis* as their host plant at Ishikari, Hokkaido Island, Japan. To investigate the characteristics of the host preference of *P. versicolora*, a feeding preference assay using agar disks was performed. The leaves of *S. sachalinensis* are a favorite of *P. versicolora* among the five willow species at Ishikari, while this species is known that the existence of intraspecific chemical variation, and each chemical race (“flavonoid race” and “phenylpropanoid race”) shows a distinctive chemical constituent pattern. We isolated the feeding stimulants to *P. versicolora* adults from leaves of each chemical race of *S. sachalinensis*, and identified them as ampelopsin ((2*R*, 3*R*)-(+)-dihydromyricetin; a kind of flavonoids) from the “flavonoid race” and as glucose-1-cinnamate from the “phenylpropanoid race”. The feeding stimulant activity of each compound was similar to that of the respective crude extracts of the leaves. Both the host plants and the feeding stimulants of *P. versicolora* at Ishikari were different from those reported in other regions in Japan, suggesting that the recognitions and responses of *P. versicolora* to chemical constituents in the host plants depend on the region where they live.

Key words: *Plagioderia versicolora*, *Salix sachalinensis*, feeding stimulants, host preference

P-050 Chemicals Affecting Feeding Preference of Cucurbitaceous Feeding Beetles to Cucurbitaceous Plants

Makoto Abe^a and Kazuhiro Matsuda^b

^a National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan

^b Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-Ku, Sendai 981-8555, Japan
abe.makoto@nies.go.jp

Feeding preference of cucurbitaceous feeding leaf beetle species (*Aulacophora indica*, *A. lewisii* and *A. nigripennis*) and lady beetle species (*Epilachna admirabilis* and *E. boisduvali*) to 18 cucurbitaceous plant species were investigated. Feeding test to these beetles using fresh leaves of cucurbitaceous plants revealed that the feeding preference among these beetle species was quite different. Feeding preferences of *A. lewisii* and *A. nigripennis* were limited compared with other three beetle species. In a feeding test using cucurbitacins which are contained usually in Cucurbitaceae and act as feeding stimulant for cucurbitaceous feeding beetles, genus *Acalymma* and *Diabrotica*, *Aulacophora indica* and *A. lewisii* were stimulated to feed by cucurbitacins B, E, I and E-glucoside and *A. nigripennis* were weakly stimulated by these cucurbitacins. *Epilachna admirabilis* were stimulated to feed by these cucurbitacins and *E. boisduvali* were weakly stimulated by the cucurbitacins. In a feeding test using organic solvent extracts from cucurbitaceous plant leaves, there was similar tendency in feeding preference by these beetle species between fresh leaves and leaf extracts. While Analysis of cucurbitacins revealed that cucurbitacin B was detected in *Sycios angulatus* and *Lagenaria siceraria* leaves and cucurbitacin E-glucoside was in *Cucumis melo* leaf. These results revealed that other chemicals in cucurbitaceous plant leaves as well as cucurbitacins play an important role in feeding preference for these beetle species.

Key words: Cucurbitaceae, Cucurbitacin, leaf beetle, lady beetle, feeding stimulant

P-051 Different Feeding Responses to the Saponin Contained in Spinach due to Different Feeding Experiences in the Tortoise Beetle *Cassida nebulosa* L.

Atsuhiko Nagasawa^a, Hiroyuki Furukawa^b, Hiromasa Kiyota^b and Kazuhiro Matsuda^c

^a National Agriculture and Food Research Organization, National Agricultural Research Center, Hokuriku Research Center, Inada 1-2-1, Joetsu, Niigata 943-0193, Japan

^b Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

^c Laboratory of Insect Science and Bioregulation, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

atsuhiko@affrc.go.jp

Newly emerged adults of the tortoise beetle *Cassida nebulosa* could feed on spinach. However, they rejected spinach after they were reared on *Chenopodium album* var. *centrorubrum*, a main host. The *C. album* var. *centrorubrum*-reared adults were strongly deterred from the methanol extract of spinach. A saponin (hideragenin glycoside) was isolated from spinach extract as a feeding deterrent to *C. nebulosa*. The adults reared on feeding-deterrent-treated *C. album* var. *centrorubrum* consumed more spinach than those reared on only-solvent-treated *C. album* var. *centrorubrum* (control). The feeding deterrent effect of the saponin on the spinach-reared adults was less than that on the *C. album* var. *centrorubrum*-reared adults. These results indicate that exposure to the deterrent reduces the deterrent's effect on *C. nebulosa*. Therefore, alternation in the effect of the deterrent contained in spinach is responsible for the differences in spinach acceptability based on different feeding experiences.

Key words: feeding deterrent, habituation, saponin, spinach, *Cassida nebulosa*

P-052 Host Selection of Cotton Aphids, *Aphis gossypii*

Shin-ichi Tebayashi, Kohei Iwamoto, Kana Nishino and Chul-sa Kim

Department of Bioresources Science, Faculty of Agriculture, Kochi University, B200 Monobe, Nankoku 783-8502, Japan

tebayasi@kochi-u.ac.jp

Cotton aphid, *Aphis gossypii*, is one of serious pests to horticulture in Kochi, Japan. Our studies on their population in the field from 2005–2006 indicated that their occurrence start from female nymphs hatching from eggs on the primary hosts plant, *Hibiscus syriacus* in the early spring. Their population rapidly increased and reached the peak with development of winged aphids in the early May. After dispersing of winged aphids, the population was kept at low density until autumn. During this period, leaves of *H. syriacus* were also collected and extracted with methanol, and the extract was analyzed by HPLC using an ODS column. Eleven peaks were detected among which some of them were synchronized with the change of population. Two synchronized peaks were isolated and identified as fumaric acid and succinic acid. Rearing of *A. gossypii* using an artificial diet containing the identified acids was conducted, and it was revealed that fumaric acid strongly suppressed a reproduction of *A. gossypii*. Thus, it was thought that fumaric acid was one of the factors behind the sudden disappearance of *A. gossypii* in spring and/or keeping a low density of the population in summer on *H. syriacus*.

Key words: chemical ecology, Cotton aphids, *Aphis gossypii*, fumaric acid, succinic acid

P-053 Preliminary Studies on the Repellency Effect of Non-host Plant Extracts to *Myzus persicae*

Liu Shumei, Yuan Guohui and Guo Xianru

Plant Protection College of Henan Agricultural University, Wenhua Road 95, Zhengzhou, China

xrguod@sina.com

This paper reported the repellency of the steam distillation extracts from 9 species of plants against aphid *Myzus persicae* Sulzer, these plants, not host plants of *M. persicae*, include *Cuminum cyminum*, *Toona sinensis* Roem, *Artemisia vulgaris*, *Mozzie buster* L., *Cirsium segetum* Bunge, *Mentha canadaensis*, *Xanthius strumarium* L., *Nerium indicum*, and *Ricinus communis* L. The main results are as follows: (1) All of the candidate distillates showed repellency against *M. persicae* in Y-type olfactometer, among which the repellent rate of extracts from *T. sinensis*, *C. segetum*, *X. strumarium*, *A. vulgaris*, *R. communis* and *N. indicum* against apterous aphids reached 73.33%, 52.38%, 51.52%, 50.00%, 47.37% and 39.53%, respectively. The repellency rate of distillates from *T. sinensis*, *M. buster*, *A. vulgaris*, *C. cyminum* on alate aphid were 45.45%, 41.94%, 36.36%, 31.71% and 26.83%, respectively. (2) EAG responses indicated that the extracts evoking the strongest responses of aphid varied with plants. For alate aphid, the optimal EAG values were evoked by *X. strumarium*, *C. cyminum*, *R. communis*, and *T. sinensis*. For the apterous aphid, *T. sinensis*, *A. vulgaris*, *C. cyminum*, *R. communis*, *N. indicum*, and *Xanthius strumarium* evoked larger EAG value. (3) There are more than 50 compounds isolated from the volatile of *T. sinensis* leaves by Porapak Q adsorption and gas chromatography-mass spectrometer. The main components are β -caryophyllene, farnesol and camphor, with relative contents of 13.02%, 7.57% and 7.55%, respectively.

Key words: non-host plant, *M. persicae* Sulzer, repellency, behavioral response

P-054 A Flavonol Glycoside as a Probing Stimulant of a Cowpea Aphid, *Aphis craccivora*, from *Vicia faba*

Masahiko Ushiro, Naoki Mori and Ritsuo Nishida

Lab of Chemical Ecology, Grad. Sch. Agric., Kyoto Univ., Kyoto 606-8502, Japan

ushiro@kyoto-u.ac.jp

A cowpea aphid, *Aphis craccivora* Koch, feeds selectively on plants in the family Fabaceae and causes serious damages on the bean crops. In order to understand the phytochemical basis of host selection, we investigated the chemical factors controlling the probing behavior of the aphid. The aphid deposited proteinous stylet sheaths on a Parafilm membrane when a solution of host plant extracts were supplied behind the membrane. Significantly more stylet sheaths were formed using extracts from the host plants than those from non-host plants belonging to the same family. Fresh leaves and stems of one of its most favorite host, *Vicia faba*, were extracted with EtOH-H₂O (9 : 1), and applied to an ODS column after defatted with hexane. A chromatographic fraction eluted with MeOH-H₂O (3 : 2) showed the most distinct stylet formation, which was purified further by a preparative HPLC. A single compound (mw 432 for C₂₁H₂₀O₁₀ deduced from LCMS) was isolated as a probing stimulant. From UV and 2D NMR HMBC spectral analyses, the compound was characterized as kaempferol 7-O- α -rhamnopyranoside. This compound in a 1 μ g/ml (65 ppm) solution induced the probing activity close to that of the crude aqueous extracts.

Key words: *Aphis craccivora*, *Vicia faba*, probing stimulant, host selection, flavonol glycoside

P-055 canceled

P-056 The Role of Coleopteran Tarsus in Food Finding

Rei Kakazu, Naoshi Masuoka, Masatoshi Hori and Kazuhiro Matsuda

Graduate School of Agricultural Science, Tohoku University, Tsutsumidori- Amamiyamachi 1-1 Aoba-ku Sendai 981-8555, Japan

hori@bios.tohoku.ac.jp

Some insects, such as Orthoptera, Diptera and Lepidoptera, use tarsi as chemosensory organs in feeding and oviposition. In Coleoptera, while the electrophysiological and morphological properties of the tarsus have been investigated, its precise role as a chemosensory organ remains largely unknown. We examined the role of tarsi as chemosensory organs in Coleoptera using adult strawberry leaf beetles. Gustatory sensilla (sensilla chaetica) were observed on tarsi with a scanning electron microscope. The beetles without maxillary and labial palpi, and/or antennae could discriminate both feeding stimulant, sucrose, and antifeedant, brucine, in choice tests. The beetles without tarsi could also discriminate these. However, ablation of both tarsus and antennae caused a decline in discrimination ability. Ablation of antennae reduced responses to both sucrose and brucine. However, in a previous study, no gustatory organs were found on the antennae by scanning electron microscope. The reduction in response may be caused by a lack of sensitivity to humidity because antennae possess hygrosensitive sensilla. Humidity may be important in gustatory responses. Intact beetles could discriminate leaf surface wax as well as sucrose and brucine. Although the discrimination ability of beetles with only tarsi was lower than that of intact beetles, they could discriminate leaf surface wax. These results suggest that coleopteran insects use tarsi as chemosensory organs in food finding.

Key words: tarsus, Coleoptera, gustatory organs, chemosensory organs, food finding

P-057 Symbiotic Relationship between a Water Lily, *Trapa natans* L. and a Water Strider, *Gerris nepalensis*

Tetsuo Harada, Jun Yamashita, Michinori Imafuku and Tomokazu Miyashita

Laboratory of Environmental Physiology, Faculty of Education, Kochi University, Kochi 780-8520, Japan

Exposure of the water strider, *Gerris nepalensis* to leaves of the water lily, *Trapa natans*, enhanced reproduction by *G. nepalensis* than another floating plant, *Hydrocharis dubia* and mimic leaves made of polystyrene. Exposure of overwintering adult water strider to the water lily in fall caused more shallow diapause and higher fecundity in the next spring than those not exposed to it. Accompanying *T. natans* with *G. nepalensis* seems to increase the individual number of the water strider and their foraging pressure on the lily leaf beetle, *Galerucella nipponensis*. In a behavior experiment, adult *G. nepalensis* made orientation dominantly to leaves of *T. natans* respect to leaves of *H. dubia*. Food shortage and serious damage of leaves by *G. nipponensis* enhanced the orientation to the leaves. Such orientation to the leaves with serious damage occurred even in a “blind” conditions *G. nepalensis*. Adult *G. nepalensis* made orientation to the filter paper (on mimic-leave and partially attached to water film) including the liquid of homogenized lily-leaves with damage by *G. nipponensis*. In another behavior experiment, reproductive females of *G. nepalensis* laid more eggs on leaves of *T. natans* rather than mimic leaves. A “chemical” communication via air or water might be possible to mediate the “triangular” relationship among the water-lily, the herbivorous beetle, and the water strider.

Key words: Triangular relationship, *Trapa natans*, *Gerris nepalensis*, *Galerucella nipponensis*, Chemical from the water lily

P-058 canceled

P-059 Raspberry Flavor or Ginger Pungency? - Synomonal Fragrance of “Fruit Fly Orchids” to Attract Fruit Flies as Pollinators

Ritsuo Nishida^a, Keng-Hong Tan^b, Jaap J. Vermeulen^c and Neville Howcroft^d

^a Lab of Chemical Ecology, Kyoto University, Kyoto, 606-8502 Japan

^b Tan Hak Heng Co., 20 Tan Jit Seng, 11800 Tanjung Bungah, Penang, Malaysia

^c National Herbarium Nederland, 2300 RA Leiden, Netherlands

^d PNG Growers Association, Rabaul, PNG 611, Papua New Guinea

ritz@kais.kyoto-u.ac.jp

Several “fruit fly orchids” in the genus *Bulbophyllum* (Orchidaceae) attract males of *Bactrocera* fruit flies (Tephritidae) by emitting a specific floral fragrance during pollination. The flowers possess a mechanical structure that temporarily traps the flies during pollination. The attractant in *Bu. hahlianum* and *Bu. apertum* was identified as raspberry ketone (RK), which is known to lure melon fly (*B. cucurbitae*) and other RK-sensitive species. Nevertheless, *Bu. patens* and *Bu. baileyi* produce zingerone (ZN), which attracts both RK- and methyl eugenol (ME)-sensitive species, thus, enabling them to entice a wider range of fruit fly pollinator species. The oriental fruit fly (*B. dorsalis*) is a notorious representative of the ME-sensitive species. The male fruit flies of these species effectively help to pollinate flowers and at the same time, by licking the floral tissues, obtain a sex pheromone material (or its precursor) to attract conspecific females. Therefore, the floral volatiles are regarded as synomone, which directly benefits the reproductive systems of both orchids and fruit flies. Interestingly, a subspecies of *Bu. apertum* has two sympatric varieties in the Borneo Island—one (var. apin-apin) was found to produce ZN, while the other (var. temom) RK. The diversion of floral lure ingredients within a species might have occurred in a true mutualistic interaction under a specific fruit fly fauna—possibly via coevolution in a tropical rain forest ecosystem.

Key words: *Bactrocera* fruit fly, *Bulbophyllum* orchid, synomone, pollination, coevolution

P-060 The Role of Methyl Eugenol in the Chemical Ecology of *Bactrocera carambolae* (Diptera: Tephritidae)

Suk-Ling Wee^a, Ritsuo Nishida^b and Keng-Hong Tan^c

^a HortResearch, Gerald Street, Lincoln 7608, Canterbury, New Zealand

^b Lab. of Chemical Ecology, Graduate School of Agriculture, Kyoto University, Japan

^c Tan Hak Heng Co., 20, Jalan Tan Jit Seng, 11200 Tanjung Bungah, Penang, Malaysia

suk_ling_wee@yahoo.com

After pharmacophagy of methyl eugenol (ME), males of *Bactrocera carambolae* (Diptera: Tephritidae) biotransformed ME into (*E*)-coniferyl alcohol (CF). CF was shown to be stored along with its endogenously synthesized pheromonal compounds in the rectal gland; and subsequently released into the air by the ME-fed males only during the courtship period at dusk. CF attracted significantly more males and females than the ME-deprived males in wind tunnel assays. However, earlier onset of sexual attraction and a higher mating success were only observed on the third day post ME feeding, consistent with the temporal accumulation of CF in their rectal glands. Field cage observations on the male-to-male interaction indicated that the ME-deprived males did not form leks but that ME feeding by the males promoted aggregation behavior in *B. carambolae*. ME-deprived males appeared to feed on the anal secretion of the ME-fed males which contained CF along with the endogenous pheromonal compounds. Results obtained for *B. carambolae* were compared to those previously obtained from its sibling species, *B. dorsalis* and discussed in light of species advancement in fruit fly-plant relationship.

Key words: *Bactrocera carambolae*, methyl eugenol, (*E*)-coniferyl alcohol, mating competition

P-061 Development of a Material to Inhibit the Working of Honeybee on Extracting Honey from Plant Leaves

Nan-Hee Ahn, Kwang-Youl Seol, Man-Young Lee, Nam-Jung Kim, Sung-Hyun Kim, and Sung-Jin Hong

Dept. of Agricultural Biology, NIAST, RDA, Suwon, 441-855, Republic of Korea
nanhee79@rda.go.kr

In apiculture, many labors are required on extracting honey. The effect of natural material to inhibit the working of honeybee, *Apis mellifera*, was tested from 5 prunus plants (*Prunus persica*, *P. mume*, *P. serrulata*, *P. tomentosa*, and *P. armenica*). Of those, *P. persica* leaves showed the strongest effect inhibiting the working of honeybee evidently. The major volatile compound of *P. persica* leaves was identified to be benzaldehyde by GC and GC/MS analysis. And then the crushed leaves of *P. persica* were tested to inhibit or not the working of honeybee in hive for the purpose of its practical use, and showed enough inhibitive activity. After removing the crushed leaves on hive, all honeybees repaired within 30 minutes. The crushed leaves of *P. persica* was developed as a material inhibiting the working of honeybee to reduce the bee-keeper's labor on extracting honey through the design of treatment method.

Key words: Apiculture, *Apis mellifera*, *Prunus persica*, Benzaldehyde, Inhibition effect

P-062 Learning of Plant Chemicals for Food Foraging in the Egg-larval Parasitoid *Ascogaster reticulata* Watanabe (Hymenoptera: Braconidae)

Noriko Negishi, Hiroyuki Seino and **Yooichi Kainoh**

Laboratory of Applied Entomology and Zoology, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan
parasite@sakura.cc.tsukuba.ac.jp

We investigated the associative learning of plant chemicals in food foraging by *Ascogaster reticulata* Watanabe (Hymenoptera: Braconidae), the egg-larval parasitoid of the smaller tea tortrix *Adoxophyes honmai* Yasuda (Lepidoptera: Tortricidae). *A. reticulata* females or males learned to associate plant chemicals with honey as a food reward in our previous experiments. In a 9 cm glass Petri dish, the tea-leaf extract was treated in a line on the bottom and a drop of honey was placed in the center. When wasps were released in this arena, they soon approached and started feeding on the honey. The feeding of the wasp was interrupted after 1 min and it was transferred to a container for the next training. After training 3 times, a wasp starved for 6 hr was released in an arena with only the extract treated line. The distance they walked along the line within 3 min was more than 10 cm, but they did not respond to the chemicals after satiation with feeding on honey. If they were put in an empty container and starved for another 6 hr, they became responsive to the chemicals. These results indicate that the wasps learned the chemicals after training with extract and honey, and their responsiveness changed depending on their state of hunger.

Key words: parasitoid, plant chemicals, food foraging, associative learning

P-063 Effect of Learning of Plant Chemicals on Host-searching Behavior of the Egg-larval Parasitoid, *Ascogaster reticulata* Watanabe (Hymenoptera: Braconidae)

Hiroyuki Seino and **Yooichi Kainoh** Laboratory of applied entomology and zoology, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8572, Japan

parasite@sakura.cc.tsukuba.ac.jp

We investigated how associative learning of plant chemicals affect host-searching behavior in *Ascogaster reticulata* Watanabe (Hymenoptera: Braconidae), the egg-larval parasitoid of smaller tea tortrix, *Adoxopyes honmai* Yasuda (Lepidoptera: Tortricidae). *A. reticulata* females can learn to associate plant contact chemicals and plant odors with the host egg mass as host-searching cues. When females learned each 10 plant's ethanol extract (tea, camellia, sasanqua, bayberry, chinquapin, rose, Japanese cedar, fern pine, mulberry and corn), they were able to distinguish tea and other nine plant species except for the closely related species (tea and camellia or tea and sasanqua). Females conditioned with some plant species (chinquapin, rose and Japanese cedar) showed no significantly preferred to conditioned plant species. However, when females exposed to plant extract with no host egg mass as unrewarding process, they were enabled to discriminate closely related species. In other experiment, learning of plant species changed searching behavior in field cage (4 m × 6 m × 2 m). Potted plants of tea and bayberry were placed in cage and 30 females which conditioned with tea shoot were released in the cage. After 10 min, the number of females on tea pot were significantly higher than bayberry pot. Bayberry conditioned females also moved on bayberry pot. Release after 30 min, females gradually left the each conditioned plant species. Decline of learned response is caused by absence of oviposition as unrewarding experience. A series of these experiences suggest that learning of plant chemicals and plant odors contribute to efficient host-searching behavior in *A. reticulata*.

Key words: parasitic wasp, host-searching behavior, plant chemicals, plant odors, associative learning

P-064 Chemical Cues for Host Recognition by the Egg Parasitoid *Aprostocetus fukutai*

Jiquan Li^a, **Youju Jin^b**, **Dazhuang Huang^a**, **Shuxiang Wang^a**, and **Huicang Feng^a**

^a Agricultural University of Hebei, Baoding Hebei 071001, China

^b Beijing Forestry University, Beijing 100083, China

lijqbd@163.com

Aprostocetus fukutai is an egg parasitoid of *Apriona germari*, the stem borer of many trees. The chemical cues used for host recognition by the egg parasitoid were investigated. In non-choice tests, the time for locating healthy host-unit by the wasp was less than that for locating feeding-damaged host-unit, host-unit into which before *A. germari* laying egg or onto which before *A. germari* smearing oviposition secretion, and healthy twig. The searching time on healthy host-unit was longer than that on the other treatments. The n-hexane, dichloromethane and methanol extracts of healthy host-unit were the most attractive, while acetone or distilled water extracts elicited the weakest responses. The attractiveness of n-hexane, dichloromethane and methanol extracts were no significant differences in choice tests. The n-hexane extracts were isolated by silica gel column chromatography. Only n-hexane fraction was attractive to the wasp, but other fractions were not bioactive. (The research was supported by Hebei Natural Science Foundation, C2005000213)

Key words: *Apriona germari*, *Aprostocetus fukutai*, host recognition, chemical cues

P-065 Induced Defensive Effects of Intact Willow Trees in Response to Volatiles from Consppecific Trees Infested by Willow Leaf Beetles

Soichi Kugimiya^a, Kinuyo Yoneya^b and Junji Takabayashi^b

^a National Institute for Agro-Environmental Sciences, Kannondai 3-1-3, Tsukuba 305-8604, Japan

^b Center for Ecological Research, Kyoto University, Hirano 2 509-3, Otsu 520-2113, Japan
kugimiya@affrc.go.jp

Several plants are known to start defensive responses prior to herbivore attack by receiving volatiles emitted from neighboring plants infested by herbivores. Such so-called “plant-plant interactions” have been studied by various approaches of molecular biology, chemistry, ecology, etc. In this study, we examined the plant-plant interaction of the willow tree, *Salix eriocarpa*, in Salicaceae family by measuring performance of the willow leaf beetle, *Plagioderia versicolora*, one of major herbivorous insects that attack Salicaceae plants. GC/MS analysis revealed that *S. eriocarpa* shoots infested by *P. versicolora* larvae emitted volatiles qualitatively and quantitatively different from those that conspecific intact shoots emitted. Under laboratory conditions, downwind intact plants (receiver plants) were exposed to volatiles from upwind plants infested by the larvae (emitter plants) in acrylic chambers. As control, intact plants were exposed to volatiles from intact plants. Then, the newly hatched larvae were introduced to receiver or control plants, respectively. Survival rate and pupal weight of the beetles on receiver plants were significantly lower than those on control plants. Development time of the larvae till pupation on receiver plants was longer than that on control plants, significantly. Damaged area of receiver plants was significantly smaller than that of control plants. From these data, it is supposed that herbivore-induced plant volatiles induced direct defensive response in the receiver plants against *P. versicolora* larvae. On the other hand, the insect performance and the plant damage on previously infested plants were not significantly different from those on intact plants, suggesting that volatile exposure prior to herbivory enhanced the level of induced defense against *P. versicolora* larvae in receiver plants.

P-066 Direct and Indirect Defense of Willow Plants against Herbivores: Comparison of Seven Wild Willow Species in Japan

Kinuyo Yoneya and Junji Takabayashi

Center for Ecological Research, Kyoto University, 2-509-3 Hirano Otsu Shiga 520-2113, Japan

yoneya@ecology.kyoto-u.ac.jp

Plagioderia versicolora is a specialist leaf beetle that feeds on leaves of Salicaceae. In Japan, plural *Salix* species are commonly found in a same field, and they were attacked by the *P. versicolora*. Interestingly, however, some are less damaged by *P. versicolora* than the other. The volatiles emitted from shoots of each of seven *Salix* species (grown in the field along Yasu river in Shiga prefecture, Japan) infested by *P. versicolora* larvae attracted a ladybird beetle, *Aiolocaria hexaspilota*, a dominant predator of *P. versicolora* larvae in spring, over intact shoots of respective species under laboratory conditions. Thus, the distribution of *P. versicolora*-infested plant volatiles would affect the distribution of *A. hexaspilota* in Yasu river fields. Here, we hypothesized that a *Salix* species with low (or no) direct defense against *P. versicolora* attracted more *A. hexaspilota* than a *Salix* species with high direct defense. To test the hypothesis, we conducted the following experiments: (1) relative preference of *A. hexaspilota* to volatiles emitted from plants of seven willow species infested by *P. versicolora* larvae, (2) the comparison of direct defense of seven plant species against *P. versicolora* larvae, and (3) relative preference of *P. versicolora* adults to seven intact *Salix* shoots. The order of preferences of *A. hexaspilota* to tested plant species were negatively correlated with the order of the levels of direct defense of them, and positively correlated with the order of preferences of *P. versicolora* to these willow species.

Key words: herbivore-induced plant volatiles, indirect defense, plant-herbivore-predator interaction

P-067 canceled

P-068 Rice Plants Damaged by Common Armyworms (*Mythimna separata*) Emit Volatiles that Attract a Parasitic wasp *Cotesia kariyai*

Rika Ozawa^a, Kaori Shiojiri^a, Kenji Gomi^a, Masaru Sato^b and Junji Takabayashi^a

^a Center for Ecological Research, Kyoto University, 509-3, 2-chome, Hirano, Otsu, 520-2113, Japan

^b National Agricultural Research Center for Kyushu Okinawa Region, NARO, 2421 Suya, Koshi, Kumamoto, 861-1192, Japan

ozawar@ecology.kyoto-u.ac.jp

Common armyworm (CAW) *Mythimna separata* feeds on many Poaceae including rice and corn plants. It has been reported that corn plants infested with CAW start emitting a blend of volatiles including C6-volatiles and terpenoids, that attracts a specialist parasitic wasp of CAW, *Cotesia kariyai*. The outbreak of CAW gives serious damage to rice plants in Japan. In this study, we report that the volatiles emitted from rice plants infested with CAW also attract *C. kariyai*. The wasps preferred CAW-damaged rice plants over intact plants or over artificial damaged plants in a two-choice test using a choice chamber. Interestingly, volatile compounds emitted from CAW-damaged rice plants were different from those emitted from CAW-damaged corn plants. In rice plants, 6 compounds including 5 terpenoids were induced by CAW infestation.

Key words: tritrophic system, herbivore-induced plant volatiles (HIPV), caterpillar

P-069 Response to Aging Herbivore-damaged Plants in the Parasitoid Fly *Exorista japonica*
Kazushi Hanyu^a, Ryoko Ichiki^b, Satoshi Nakamura^b and Yooichi Kainoh^a

^a Laboratory of Applied Entomology and Zoology, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

^b Japan International Research Center for Agricultural Sciences (JIRCAS), Ohwashi 1-2, Tsukuba, Ibaraki 305-8686, Japan.

parasite@sakura.cc.tsukuba.ac.jp

The tachinid fly, *Exorista japonica*, is a parasitoid of numerous species of lepidopteran larvae. The odor from host (*Mythimna separata*) damaged corn plants attracts naïve females of this fly in a tri-trophic interaction. We investigated the effects of elapsed time on the attraction of the plant after the host stopped feeding. The behavior of the flies within 5 min from introduction was observed in a wind tunnel. In damaged plants, the rate of flies landing on the plants remained high (70%) for 6 h and decreased gradually after 24 h. In contrast, in artificially-damaged plants, the rate of flies reaching the corn plants was high (85%) when tested soon after damage but 40% of flies reached the corn plants 1 h after damage. Systemic induction of volatile release to attract parasitoids occurs in many plants. Therefore, we examined whether undamaged leaves from a damaged plant released volatile compounds to attract this tachinid fly. Undamaged leaves were not attractive when all other leaves on the same plant were damaged even if were damaged for several hours. In addition the undamaged part of damaged leaves was not attractive either. These results indicate that volatiles that attract *E. japonica* are only released from the damaged parts of leaves and they gradually decrease with aging of the damaged plant.

Key words: Tachinidae, tri-trophic interaction, plant volatiles, damaged plant

P-070 EAG Responses of *Nephotettix nigropictus* towards Components of Rice Plant

Jing Li, Tebayashi Shin-ichi and Chul-Sa kim

Faculty of Agriculture, Kochi University, B200Monobe, Nankoku 7838502, Japan

xizukalee0517@yahoo.com

Some of leafhoppers and plant hoppers, such as *Laodelphax striatella*, *Sogatella furcifera*, *Nephotettix cincticeps*, are well known as serious rice plant pests. To elucidate an attractant in a rice plant for these pests, *L. striatella*, *S. furcifera*, *N. virescens*, *N. nigropictus* and *N. cincticeps* which have the similar host plant habits were subject to electrophysiological study using their antennae. Among these insects, *N. nigropictus* displayed strong and stable responses to an ether extract of a rice plant. EAG responses of *N. cincticeps* were also strong to the extract but stability of the responses was weaker. Responses of *N. virescens* to the extract were weaker and it was difficult to detect EAG responses of *L. striatella* and *S. furcifera*. Successive purification of the ether extract by column chromatography using silica gel yielded five EAG active fractions. Of these fractions, the 80% ether in hexane fraction showed the strongest EAG activity towards *N. nigropictus* and this fraction was subjected to a normal phase HPLC yielded three fractions among which two fractions showed strong activities towards *N. nigropictus* by EAG analysis and the other fraction was failed to show any activity. Further purification and structure determination of the active compound(s) in these two active fractions are in progress.

Key words: *Nephotettix nigropictus*, rice plant, attractant, electroantennogram (EAG), column chromatography

P-071 Response of the Asian Ladybird, *Harmonia axyridis* to the Host Infested by the Green Peach Aphid, *Myzus persicae*

Changmann Yoon^a, Dong-Kyu Seo^a, Doo-Jin Noh^a, Ki-Su Ahn^b and Gil-Hah Kim^a

^a Dept. of Plant. Medicine, Chungbuk National University, Cheongju 361-763, Republic of Korea

^b Chungbuk Provincial ARES, Cheongwon, 363-880, Republic of Korea

khkim@chungbuk.ac.kr

Chinese cabbage attacked by green peach aphid, *Myzus persicae* (herbivore) emits gas to attract Asian ladybirds, *Harmonia axyridis* (predator) feeding on *M. persicae*. Using an olfactometer, *H. axyridis* adults and larvae were not attracted to the odor of Chinese cabbage or *M. persicae* alone. In the response to the volatiles from Chinese cabbage infested with the different densities of *M. persicae* adults, *H. axyridis* adult was attracted significantly to the odor of the Chinese cabbage infested with the density of 60 *M. persicae* during 24 hrs. But *H. axyridis* larva was not attracted to the odor of Chinese cabbage infested with the *M. persicae*. Both *H. axyridis* adults and larvae were not attracted to the odor of Chinese cabbage with artificial damage. With the passage of time, *H. axyridis* adults showed highest in response at 24 and 48 hrs after treatment with the density of 60 *M. persicae*, and at 12 hrs after treatment with the density of 90 *M. persicae*. Both *H. axyridis* adults and larvae were not attracted to the odor of the Chinese cabbage infested with Diamondback moth, *Plutella xylostella*.

P-072 Response of *Monochamus saltuarius* (Coleoptera: Cerambycidae) Adults to the Odors of Fresh Pine Tree and Adult-Infested Pine Tree

Min-Ki Kim^a, Changmann Yoon^a, Jeong-Oh Yang^a, Ju-Hwan Han^b and Gil-Hah Kim^a

^a Dept. of Plant Medicine, Chungbuk National University, Cheongju, Republic of Korea

^b Chungbuk Institute of Forest Environment Research, Chongwongun Chungbuk-do, Republic of Korea

khkim@chungbuk.ac.kr

Depending on the maturation age of pine sawyer beetle adult, *Monochamus saltuarius* was investigated the attraction against the odors of host plants. Using a T-tube olfactometer, newly emerged adults (0-3 days old) of *M. saltuarius* preferred the odor of fresh host plants, whereas mature adults (20-30 days old) of *M. saltuarius* preferred the odor of adult-infested host plants. *M. saltuarius* adult preferred the odor from white pine tree than that of pine tree, but did not show the significant difference. Volatile organic compounds of host plants were bioassayed after analyzed by GC and GC-MS. Pine sawyer beetle adult was attracted to the odors of host plants with different depending on the maturation age.

P-073 Induced Plant Defenses against Aphids with Herbivore-induced Volatiles that Attract Parasitic Wasps: Mechanisms Involved in the Induced Volatile Production

Hiroyuki Takemoto^a, John Pickett^b, Wilf Powell^b, and Junji Takabayashi^a

^a Center for Ecological Research, Kyoto University, 2-509-3 Hirano, Otsu, Shiga, 520-2113, Japan

^b Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom.

takemoto@ecology.kyoto-u.ac.jp

Plants defend themselves against herbivores, either directly or indirectly. The production of plant volatiles in response to herbivory (herbivore-induced plant volatiles: HIPV) that attract carnivorous natural enemies of the infesting herbivores can be regarded as ‘induced indirect defenses’. In the production of HIPV, chemical compounds of herbivore origin (so called “elicitors”) have been shown to play an important role. To date, such elicitors have been found in regurgitant of several Lepidopteran insects. HIPV productions in response to aphid damage have also been reported and this production also appears to be initialized by elicitors in aphid saliva. To clarify the chemical nature of saliva, we studied induced volatile productions by broad bean plants *Vicia faba* in response to the pea aphid, *Acyrtosiphon pisum*. We also studied the olfactory response of *Aphidius ervi*, a parasitic wasp that attacks *A. pisum*, to HIPV. We will describe the extraction of aphid saliva and its possible roles in HIPV production by broad bean plants.

Key words: chemical ecology, aphids, induced defenses, tritrophic interaction

P-074 Olfactory Responses of the Predatory Mites *Neoseiulus cucumeris* to two Different Plant Species Infested with the Onion Thrips, *Thrips tabaci*

Takeshi Shimoda^a and Satoshi Tatemoto^b

^a National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan

^b Hiroshima prefecture agriculture Research Center, Hiroshima 739-0151, Japan

oligota@affrc.go.jp

Attraction of carnivorous arthropods to herbivore-induced volatiles from their preferable combinations of plants and prey herbivores has been well documented in the viewpoints of carnivore’s foraging and/or plant’s indirect defense against herbivores. However, the olfactory responses of carnivores to less preferable plant-prey combinations are not well understood. This prompts us an intriguing question as to what responses are elicited by herbivore-induced volatiles from less-preferable plant species in response to infestation damage by preferable prey herbivores. In this study, we investigated olfactory responses of the predatory mites *Neoseiulus cucumeris* to the following two different plant species infested with the onion thrips *Thrips tabaci* in a Y-tube olfactometer. The predators were attracted to odors from infested cucumber leaves without *T. tabaci* and their products, but not attracted to odors from uninfested cucumber leaves, artificially damaged cucumber leaves, or odors from *T. tabaci* plus their products collected from cucumber leaves. These results indicate that they are capable of exploiting herbivore-induced volatiles from *T. tabaci*-infested cucumber leaves as a preferable plant-prey combination. On the contrary, the predators were not attracted to odors from uninfested spring onion plants, infested spring onion plants without *T. tabaci* and their products, or odors from *T. tabaci* plus their products collected from spring onion plants. Indeed, they avoided odors from artificially damaged spring onion plants. Possible explanations for their no-significant olfactory responses to spring onion plants infested with *T. tabaci*, empirically known as a less-preferable plant species for the predators, are discussed.

Key words: herbivore-induced volatiles, *Neoseiulus cucumeris*, *Thrips tabaci*, cucumber, spring onion

P-075 Interactions between Arbuscular Mycorrhizal Fungi and Spider Mites Through Plant Induced Resistance

Takaaki Nishida^a, Yasuyuki Cho^b and Takayuki Ohgushi^a

^a Center for Ecological Research, Kyoto University, 3-509-2 Hirano Ohtsu Shiga 520-2113, Japan

^b Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, 320-1098 SM Kruislaan Amsterdam, Netherlands

taka-n@ecology.kyoto-u.ac.jp

Plant induced resistance to herbivory can alter subsequent interactions between plant and herbivorous arthropods. Plants need to increase uptake of resources for the induced resistance. Most terrestrial plants are associated with arbuscular mycorrhizal fungi (AMF) underground. AMF can improve nutrient absorption of plants and promote plant growth. Therefore, it is more likely that AMF promotes the plant induced resistances with providing nutrients for chemical and physical defensive substances. We conducted a factorial design experiment to test the hypothesis that AMF improve the induced resistance of *Lotus corniculatus* to the spider mite (*Tetranychus urticae*). We set four treatments of plants with AMF (*Gigaspora margarita*) or AMF-free filtrate and with high- and low-P fertilization. Seven weeks after sowing, the plants were infested by adult female mites for 3 days. To evaluate effects of AMF on the plant resistance to the spider mites, we took leaves one day before mite introduction, 1, 3, 5, 8 days following mite introduction. We provided each leaf to one female mite for 3 days and recorded number of eggs laid. AMF increased plant growth and phosphorus contents in low-P fertilization, but did not affect in high-P fertilization. However, AMF induced earlier reduction of the number of eggs following herbivory both in high- and low-P fertilization. Therefore, it is concluded that AMF enhanced the induced resistance to the spider mites independent of AMF effects on plant growth.

Key words: plant induced resistance, arbuscular mycorrhizal fungi, spider mites, chemical defense, indirect effect

P-076 Analyses of Plant Response to Thrips Feeding Using Arabidopsis System

Hiroshi Abe^a, Jun Ohnishi^b, Mari Narusaka^c, Shigemi Seo^d, Yoshihiro Narusaka^c, Shinya Tsuda^b and Masatomo Kobayashi^a

^a RIKEN BioResource Center, Tsukuba 305-0074, Japan

^b National Agricultural Research Center, Tsukuba 305-8666, Japan

^c Research Institute for Biological Sciences Okayama, Kaga 716-1241, Japan; ^dNational Institute of Agrobiological Sciences, Tsukuba 305-8666, Japan

ahiroshi@rtc.riken.jp

Plants are exposed to many types of abiotic or biotic stresses. Many researchers have analyzed the mechanism of these stress responses using Arabidopsis plants as a model system in detail. On the other hand, insect damage is very serious problem that decrease the crop yields. However, the mechanism of plant response to feeding damage has not been well understood.

Western flower thrips (*Frankliniella occidentalis*) is one of the most important insect herbivores and is an exotic pest of greenhouse production in many countries. Thrips are cell content feeding insect that penetrate single cells with stylet to suck out the contents. In addition, thrips transmit the virus from plant to plant. Because the thigmokinetic behavior of western flower thrips and the emergence of insecticidal resistance, it is difficult to control this species with insecticides. Therefore, elucidation of the molecular mechanism of the plant response to the feeding of western flower thrips is important for the development of new methods to prevent damage. We performed the GeneChip analyses (Affymetrix: ATH1 chip) using Arabidopsis plants attacked by thrips. We report here the function of Jasmonic acid (JA), Ethylene (ET) and Salicylic acid (SA) in thrips feeding. In addition, we introduce the Arabidopsis genome resources that are distributed from RIKEN BioResource Center (BRC).

Key words: Arabidopsis, western flower thrips, insect feeding

P-077 Absolute Configuration of Volicitin from the Regurgitant of Lepidopteran Caterpillars and Biological Activity of Volicitin-Related Compounds

Yoshitsugu Sawada, Naoko Yoshinaga, Ritsuo Nishida, Yasumasa Kuwahara and Naoki Mori
Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo Kyoto 606-8502, Japan
mokurin@kais.kyoto-u.ac.jp.

Volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] and *N*-linolenoyl-L-glutamine are known as insect-produced plant volatile elicitors. The absolute configuration of the hydroxylinolenoyl moiety of volicitin from three noctuid species, *Helicoverpa armigera*, *Mythimna separata* and *Spodoptera litura*, was determined to be all 17*S* in high enantiomeric excess. When treated with 30 pmol of (17*S*)- and (17*R*)-volicitin, corn seedlings were induced to release volatiles. There was no significant difference in the amount released between the two isomers. On the other hand, *N*-linolenoyl-L-glutamine was only about 30% as active as volicitin. Among several synthesized *N*-linolenoylamino acid conjugates, only the L-glutamine conjugate induced the emission of volatile organic compounds. These results show that the L-glutamine moiety of volicitin played a more critical role than the hydroxyl moiety, although both moieties affected the elicitor activity inducing the release of volatiles.

Key words: insect-produced elicitor, induced plant resistance, *Helicoverpa armigera*, *Mythimna separata*, *Spodoptera litura*

P-078 Volicitin Biosynthesis and Nitrogen Metabolism in *Spodoptera litura* Larvae
Naoko Yoshinaga, Ritsuo Nishida and Naoki Mori

Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo Kyoto 606-8502, Japan
yoshinaga100@hotmail.com

Volicitin and related compounds (fatty acid amides, FAAs), which have been found in the larval gut lumen of many lepidopteran species, are unique compounds as elicitors for plant volatile emission, although the role(s) for caterpillars are still unknown. Focusing on the glutamine moiety as a common chemical structure of these FAAs, the physiological role(s) in caterpillar nitrogen metabolism has been studied. *Spodoptera litura* larvae fed on artificial diets containing ¹⁵N-ammonia and glutamic acid, were placed in a sample tube for ¹⁵N-NMR analysis. ¹⁵N-labeled volicitin-related compound(s) along with γ -¹⁵N-glutamine, α -¹⁵N-glutamine and ¹⁵N-glutamic acid were detected as the metabolites. Chemical shift values indicated that only γ -¹⁵N-glutamine was incorporated into volicitin-related compounds. These results suggest that volicitin-related compounds might play some role in glutamine synthetase-mediated ammonia assimilation in caterpillars.

Key words: Volicitin, plant volatile emission, nitrogen metabolism, *Spodoptera litura*

P-079 Efficient Incorporation of Unsaturated Fatty Acids to the Fatty Acid-Amides in *Spodoptera litura*

Takako Aboshi, Naoko Yoshinaga, Koji Noge, Ritsuo Nishida and Naoki Mori

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
mokurin@kyoto-u.ac.jp

Volicitin and its related compounds have been identified in the oral secretion of *Spodoptera litura*. Volicitin causes plants to release volatiles, which in turn work as chemical cues for natural enemies such as parasitic wasps. In vitro selective incorporation of glutamine into fatty acid-amides (FAAs) by caterpillar gut tissue is already reported. In this study we examined the selectivity of fatty acids in the biosynthesis of FAAs in *S. litura*.

In lettuce leaves, almost equal amounts of linolenic, linoleic and palmitic acids were detected by GC analyses. LC/MS analyses of gut contents isolated from *S. litura* fed on lettuce, however, detected volicitine, *N*-linolenoyl-L-glutamine, *N*-(17-hydroxylinoleoyl)-L-glutamine, and *N*-linoleoyl-L-glutamine. Furthermore, incubation of sodium salts of these fatty acids each with glutamine and gut tissues showed that the production of linolenoyl- and linoleoyl-L-glutamine were more than three times higher than that of palmitoyl-L-glutamine. These results indicated that unsaturated fatty acids were efficiently coupled with glutamine in the FAAs biosynthesis.

Key words: volicitin, plant defense signal, fatty acids, *Spodoptera litura*

P-080 Salivary Laccase of the Green Rice Leafhopper, *Nephotettix cincticeps* and its Possible Functions in Feeding Activity

Makoto Hattori^a, Hirosato Konishi^a, Yasumori Tamura^a, Kotaro Konno^a and Kazushige Sogawa^b

^a National Institute of Agrobiological Sciences, Ohwashi 1-2, Tsukuba 305-8634, Japan

^b Kamiyokoba, Tsukuba 305-0854, Japan

hatto@affrc.go.jp

The saliva of leafhoppers is important in feeding on the rice plant and is considered to play a physiological role in detoxifying toxic plant substances or in ingesting sap. We have found that *Nephotettix cincticeps* has a laccase type of phenoloxidase in the salivary glands and secretes it in watery saliva. To date, insect laccase has been enzymatically detected in the cuticles of many species, but no report in salivary glands. Nonreducing SDS-PAGE of salivary gland homogenates with staining by 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) at pH 5 revealed a band at molecular masses of 85 kDa. IEF analysis stained with ABTS showed a band at PI of 4.8. The salivary laccase had a pH optimum of 4.75-5.0 in citrate-phosphate buffer, suggesting that it play a role in the oxidation of phenolics in plant tissues other than the sieve elements, pH of which is about 8.0. Laccase activity was histochemically localized in V-cells of the posterior lobe of the salivary glands. It was detected in a sucrose solution that was fed on through a membrane for 16 h by leafhoppers and in stylet sheaths. One proposed function for *N. cincticeps* salivary laccase may promote polymerization of monolignols to prevent accumulation of quinone methides, which are potent alkylating agents of protein and toxic to insects. Another possible function of salivary laccase is the promotion of rapid oxidative gelling of proteinaceous sheath saliva by quinone tanning reaction.

P-081 Gall induction by a Leafhopper *Cicadulina bipunctata*: So-called “Pseudogaller” as a Model for Presuming Evolution of Gall-inducing Ability in Insects

Keiichiro Matsukura^a, Masaya Matsumura^a and Makoto Tokuda^b

^a National Agricultural Research Center for Kyushu Okinawa Region, 2421 Suya Koshi Kumamoto 861-1192, Japan

^b National Institute of Advanced Industrial Science and Technology, Azuma 1-1-1, Tsukuba Ibaraki 305-8566, Japan

mtkr@affrc.go.jp

Many biologists have been paying special attentions to sophisticated gall-inducing ability in insects and the adaptive significance of galling habit. The maize orange leafhopper *Cicadulina bipunctata* (Homoptera: Cicadellidae) is considered one of the most suitable materials for presuming these evolutionary processes, because of its evolutionary state so-called “pseudogaller”. We examined the relationship between the feeding site and gall-inducing site of *C. bipunctata* on maize and dose-dependency in the degree of gall induction. As a result, *C. bipunctata* did not induced galls on the feeding leaves but on other leaves situated at more distal positions, strongly suggesting that the galls are induced by chemical stimulus alone. Significant correlation of the degrees of gall induction and growth stunting with the infestation density and period indicated that the gall induction is a dose-dependent reaction. An experiment of continuous feeding suggested the existence of threshold limit of chemical dose in the gall induction. These results will contribute to further understandings of gall-inducing mechanism and evolutionary process of galling habit in insects.

Key words: gall induction, galling habit, *Cicadulina bipunctata*, pseudogall, insect-plant interactions

P-082 Tolerance of *Drosophila* Flies to Ibotenic Acid Poisons in Mushrooms

Nobuko Tuno^a, Kazuo H. Takahashi^b, Hiroshi Yamashita^c, Naoya Osawa^c and Chihiro Tanaka^c

^a Graduate School of Natural Science, Kanazawa University, Kanazawa 920-1192, Japan

^b Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan

^c Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502 Japan

tunobuko@gmail.com

The mushroom genus *Amanita* has a spectrum of chemical compounds affecting survival and performance of animals. Ibotenic acid is one of such compounds found in some *Amanita mushrooms*. We studied the effects of ibotenic acid and its derivative, muscimol, on egg-to-pupa survival, pupation time, and pupal size in five *Drosophila* species (Diptera: Drosophilidae), *D. bizonata*, *D. angularis*, *D. brachynephros*, *D. immigrans*, and *D. melanogaster*. The first three species are mycophagous and use a wide range of mushrooms for breeding, whereas *D. immigrans* and *D. melanogaster* are frugivorous. We reared fly larvae on artificial medium with 500, 250, 125, and 62.5 $\mu\text{g}/\text{ml}$ of ibotenic acid and/or muscimol. The three mycophagous species were not susceptible to ibotenic acid, whereas the two frugivorous species were affected. In experiments with *D. melanogaster*, muscimol was revealed less toxic than ibotenic acid.

P-083 Identification of DIMBOA, MBOA Glucosides in Noctuid Caterpillars

Kenjiro Murakami, Masahiro Ishida, Hiroaki, Sasai, Naoko Tadokoro, Atsushi Ishihara, Ritsuo Nishida and Naoki Mori

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
mokurin@kais.kyoto-u.ac.jp

DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is one of the benzoxazinoids (BXs), which are well-known as defense substances of graminaceous plants, such as maize, wheat and rye. DIMBOA is stored in the vacuole as a glucoside, and when the tissue is damaged by insect feeding, DIMBOA is released from the glucoside with β -glucosidase present in the plastid. When *Spodoptera litura* and *Mythimna separata* larvae were fed on artificial diets containing DIMBOA, three kinds of glucosides [DIMBOA-2-O- β -Glc, HMBOA (2-hydroxy-7-methoxy-1,4-benzoxazin-3-one)-2-O- β -Glc and MBOA (6-methoxy-2-benzoxazolinone)-N- β -Glc] were identified from their frass by LCMS and NMR analyses. Furthermore, when the midgut tissues of *S. litura* or *M. separata* were incubated with UDP-glucose and DIMBOA or MBOA, these glucosides were detected during the incubation. These results strongly suggest that *S. litura* and *M. separata* larvae metabolize DIMBOA analogues by glucosyltransferase(s) with UDP-glucose as a glucosyl donor.

Key words: glucosylation, DIMBOA, MBOA, *Spodoptera litura*, *Mythimna separata*

P-084 Toxicity of Citrus Essential Oils against *Callosobruchus maculatus* (F.) Adults

G. Moravvej^a, M. Azizi^a and S. Abbar^b

^a Assistant Professor, Faculty of Agriculture, Ferdowsi University of Mashad, Mashad, Iran

^b Agricultural Entomology M.Sc. student, Faculty of Agriculture, Ferdowsi University of Mashad, Mashad, Iran
azizi@um.ac.ir

Insecticidal activity of many plants against several insect pests has been documented. In the present study, the effects of volatile components of *Citrus paradise*, *C. aurantium*, *C. limonium*, and *C. sinensis* peel essential oils were investigated on the cowpea adult bruchid, *Callosobruchus maculatus* (F.). The oils were extracted from the fruit peel using hydrodistillation. The results indicated that the citrus oils had high fumigant activity against adult beetles. The mortality of 1–2 day-old adults increased with concentration and exposure time from 3 to 24 h. The oil of *C. paradise* was more effective than those of *C. aurantium* and *C. limonium* (The LC₅₀s were 127.9, 147.6 and 237.6 μ l/l at 24 h, respectively). The oil of *C. sinensis* proved to be least toxic (LC₅₀=262.4 μ l/l). The results suggested that citrus peel oils can be used as potential control measure against cowpea beetles.

Key words: *Citrus paradise*, *C. aurantium*, *C. limonium*, *C. sinensis*, *Callosobruchus maculatus*, botanical insecticides, fumigant toxicity

P-085 Insecticidal Activity of Some Medicinal Plants Essential Oils against *Oryzaephilus surinamensis* L. and *Tribolium castaneum* Hbst

M. Azizi^a, E. Rabbani^b and M. Modarres^b

^a Assistant Professor, Faculty of Agriculture, Department of Horticulture, Ferdowsi University of Mashad, Mashad, Iran

^b Faculty of Agriculture, Department of Entomology, Ferdowsi University of Mashad, Mashad, Iran
Azizi@um.ac.ir

Insecticidal and repellent activity of some medicinal plants essential oils against several insect pests has been documented. In the present study, the effects of essential oils of Rosmary (*Rosmarinus officinalis*), Thyme (*Thymus vulgaris*), Peppermint (*Mentha piperita*), Eucalyptus (*Eucalyptus globules*) and Green cumin (*Cuminum cyminum*) were investigated on two important pest of rice during storage (*Oryzaephilus surinamensis* L. and *Tribolium castaneum* Hbst). The oils were extracted from dried mentioned plant using hydrodistillation. The treatments were different concentration of the essential oils (0.04, 0.2 and 0.4 ppm) as fumigation in a 25 ml glass jar with 8 adult insects. The experiment was conducted in Randomized Complete Block Design with four replications. LC50 and LC90 of each treatment were calculated and analyzed by Probit Software. The results indicated that toxicity of Rosmary and Green Cumin was higher than others on the pests. There are not significant differences between the plants as LC50 and LC90 on *Oryzaephilus surinamensis* as concerned (LC50=0.0042 ppm and LC90=0.309 ppm). *Rosmarinus officinalis* essential oils affect faster than *Cuminum cyminum* followed by Eucalyptus (LC50=0.419 and LC90=0.0915), Peppermint and Thyme (LC50=0.37 and LC90=4.69). Our results In *Tribolium castaneum* proved that oil of Rosmary and Green Cumin are least toxic and after 16 hour exposure mortality reach to 100%. In conclusion Rosmary and Green Cumin essential oils are the best candidates and safe for control of *Oryzaephilus surinamensis* L. and *Tribolium castaneum* Hbst. and further research need on the formulation of the essential oils.

Key words: essential oils, fumigant toxicity, medicinal plants, *Oryzaephilus surinamensis*, *Tribolium castaneum*

P-086 Physiological and Behavioral Countermeasures against Acorn Tannins in the Japanese Wood Mouse *Apodemus speciosus*

Takuya Shimada^a, Akiko Takahashi^b, Takashi Saitoh^c and Ro Osawa^d

^a Tohoku Research Ctr., Forestry & Forest Products Research Inst., Nabeyashiki 92-25, Shimo- Kuriyagawa, Morioka 020-0123, Japan

^b Lab. Forest Biology, Kyoto Univ., Oiwake-cho, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan

^c Field Science Ctr., Hokkaido Univ., N11, W10, Sapporo 060-0811, Japan

^d Graduate School of Agriculture, Kobe Univ., Rokko-dai, Nada-ku, Kobe 657-8501, Japan
tshmd@affrc.go.jp

Acorns (seeds of oak trees) are staple resource for forest-dwelling wildlife, but they contain high level of tannins and cause negative effects on consumers. In order to elucidate the defense mechanisms against tannins in the Japanese wood mouse, which heavily rely on acorns during fall and winter, we investigated the role of physiological acclimation against tannins and selective foraging decreasing tannin intake. First, we allocated 26 wood mice into two groups: the acclimated group and the nonacclimated group. Of the 14 mice in the non-acclimated group, 8 died, whereas only 1 of the 12 mice in the acclimated group died. Mean body weight change was -17.9% in the non-acclimated group and -2.5% in the acclimated group. These results indicate that wood mice can mitigate the negative effects of tannins by acclimation. Path analysis revealed that increased secretion of tannin-binding salivary proteins (TBSPs) and abundance of tannase-producing enterobacteria (TPB) may be main elements of the mechanism of acclimation to tannins. Second, we examined the selective foraging against tannins by the wood mouse and revealed that wood mice can selectively consume acorns with low tannin content and avoid ones with high tannin content. These findings indicate that the wood mouse can consume tannin-rich acorns effectively with these physiological and behavioral defense mechanisms against tannins.

Key words: acclimation, defense mechanisms against tannins, tannin-binding salivary proteins, tannase-producing enterobacteria

P-087 Chemical Interaction between Brassicaceae Plants and Rhizospheric Fungi

Hiroshi Ishimoto

Active Ingredients Unit, Agrochemicals laboratory, Development Center, Advanced Chemicals Business Sector, 1144, Togo, Mobarashi, Chiba 297-0017, Japan
hiroshi1.ishimoto@mitsui-chem.co.jp

From the rhizospheres of Brassicaceae plants, *Fusarium* and *Rhizopus* fungi were isolated frequently as dominant fungal genera. These fungi from rhizospheres of Brassicaceae plants were more tolerant to antifungal compounds of Brassicaceae plants than fungi isolated from non-Brassicaceae plants. Antifungal compounds of Brassicaceae plants may be a selection pressure for those rhizospheric fungi.

Two *Fusarium* strains, isolated from rhizosphere of Brassicaceae plants, induced the resistance in *Lepidium sativum* against pathogenic fungi, *Pythium ultimum*. These *Fusarium* strains caused the increase of the content of antifungal compounds and its precursor, isothiocyanate and glucosinolate, in roots of the host plants. The resistance of *L. sativum* against *P. ultimum* may be due to the increase of isothiocyanate content induced by the inoculation of *Fusarium* fungi, and this increase seemed to be a defense response of the host plant.

One *Fusarium* isolate, which showed myrosinase activity, inhibited the growth of *P. ultimum* on agar medium containing glucosinolate. This strain would hydrolyze glucosinolate to generate isothiocyanate in tissue or rhizosphere of host plant and inhibit the growth of pathogenic fungi consequentially. This isolate may have another protection mechanism in rhizosphere of *L. sativum*, in addition to the ability to cause the increase of antifungal compounds in the roots of host plant.

Key words: Brassicaceae plants, Non-pathogenic *Fusarium*, *Rhizopus*, induced resistance, glucosinolate

P-088 Antifungal Compounds of Seeds Influence Early Mycoflora in the Seedling Rhizosphere of *Thujaopsis dolabrata* var. *hondai*

Keiko Yamaji^a, Shigeta Mori^b, Masaru Akiyama^c and Atsushi Kato^d and Tadakazu Nakashima^d

^a Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8572, Japan

^b Tohoku Research Center, Forestry and Forest Product Research Institute

^c Horonobe Research Institute for the Subsurface Environment, Northern Advancement Center for Science and Technology

^d Forestry and Forest Products Research Institute

yamajik@sakura.cc.tsukuba.ac.jp

Thujaopsis dolabrata SIEB. et ZUCC. var. *hondai* MAKINO is an endemic tree species in Japan. The seeds contain several terpenoids. We hypothesized antifungal terpenoids in seeds might influence early fungal mycoflora in the seedling rhizosphere, that is, 1) antifungal compounds in seeds influence seed fungi and soil fungi and 2) fungi tolerant of antifungal compounds may grow in the seedling rhizosphere. We examined 1) rhizosphere fungi of seedlings growing in Kanuma pumice (a model mineral soil) and nursery soil, and each fungal detection frequency, 2) seed fungi, 3) soil fungi, and 4) tolerance of seed fungi and soil fungi to the seed extract and antifungal compounds. We then calculated correlations between the fungal detection frequency in the rhizosphere and the fungal tolerance of the seed extract and antifungal compounds. Fungal detection frequency in the rhizosphere in both soils significantly and positively correlated with fungal tolerance of the seed extract. That is, fungi tolerant of the seed extract can grow in the seedling rhizosphere. A similar correlation was found between the fungal detection frequency in the rhizosphere and the fungal tolerance of antifungal compounds, totarol and eudesmol in seeds. We conclude antifungal compounds of seeds influence seed fungi and soil fungi, and early mycoflora in the seedling rhizosphere will grow.

Key words: *T. dolabrata* var. *hondai*, rhizosphere fungi, totarol, eudesmol

P-089 Damping-off of Current-Year *Fagus crenata* Seedlings under Different Illuminations—Temporal Change of Antifungal Production and Periderm Formation in Hypocotyls—

Yu Ichihara^a and Keiko Yamaji^b

^a Tohoku Research Center, Forestry and Forest Product Research Institute, 92-25, Nabeyashiki, Shimo-kuriyagawa, Morioka, 020-0123, Japan

^b Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, 305-8572, Japan

ichiyu@fpri.affrc.go.jp

The mortality of current-year seedlings of *Fagus crenata* by damping-off is affected by light intensity on forest floor. To clarify the factors affecting the difference of the mortality under different light conditions, chemical and histological features of hypocotyls were compared between seedlings growing in closed stand and forest edge. Damping-off of current-year seedlings was observed mainly from the end of June to July, and the surviving rate in forest edge was higher than closed stand. The causal pathogens were recognized to be *Colletotrichum dematium* and *Fusarium* sp. by an inoculation test. In the beginning of July, seedling hypocotyls in forest edge clearly formed periderm, on the contrary, those in the closed stand did not. In the middle of June, seedling hypocotyls in forest edge also produced 4 times higher amount of total phenols than closed stand. Ethyl acetate phase of methanol extract and a single fraction of hypocotyls collected from forest edge had antifungal activity on the pathogenic fungi. HPLC-DAD analysis clarified that the fraction mainly included catechin and epicatechin, which had antifungal activity. The concentrations of these substances were higher in the hypocotyls of forest edge than closed stand. These results indicate that seedlings in forest edge seem to defend against pathogenic attack, forming periderm and increasing phenolic compounds.

Key words: *Fagus crenata*, damping-off, light intensity, periderm, antifungal substance

P-090 Induced Response of Oak Trees to *Raffaelea quercivora* as a Defense against a Vector Ambrosia Beetle *Platypus quercivorus*

Naoto Kamata^a, Hisahito Oana^b, Miwa Kasai^c, Kenryu Kato^b, Tohru Mitsunaga^d, Nobuko Kakiuchi^b, Kojiro Esaki^c, Masayuki Mikage^b and Shin-Ichiro Ito^c

^a Graduate School of Agricultural and Life Sciences, the University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-8657, Japan

^b Kanazawa University

^c Mie University,

^d Gifu University

^e Ishikawa Forest Experiment Station

kamatan@uf.a.u-tokyo.ac.jp

A fungus, *Raffaelea quercivora*, vectored by an ambrosia beetle, *Platypus quercivorus*, is the causal agent of Japanese oak wilt (JOW), which causes >40% of mortalities for a deciduous oak, *Quercus crispula*. Necrosis in sapwood, which has been attributed to *R. quercivora*, stops water conductance. A tree dies when necrosis completely blocks any cross-section of the tree. Polyphenol analyses showed that hydrolyzable tannin was contained in healthy sapwood but diseased sapwood contained large quantities of ellagic acid and lesser amounts of gallic acid. Tannase and laccase activities were identified from *R. quercivora*. Purpogallincarboxylic acid bio-converted with laccase from gallic acid was contained in diseased sapwood. We conclude that the discoloration of sapwood is caused by the biological oxidation of wood extractives, especially polyphenolic compounds. *P. quercivorus* males avoided such necrosis when they tunneled into trees that had been attacked in the previous year. A laboratory experiment proved that *P. quercivorus* adults avoided the sapwood with high concentrations of gallic acid or ellagic acid. It is estimated that 0.0456% of gallic acid (c. 25 times as dense as in necrosis) and 0.0260% of ellagic acid (c. 1/2 as dense as in necrosis) completely prevent insect tunneling. These tannic acids thus have some potential as control tools against Japanese oak wilt.

P-091 Isolation of Biodegradable Plastic-Degrading Microorganisms from Alimentary Canals and Body Surfaces of Stag Beetles

Hironori Sakamoto^a, Hiroko K. Kitamoto^a, Motoo Koitabashi^a, Ken Suzuki^a, Jun Tabata^a, Atsushi Mochizuki^a, Toshiaki Nakajima-Kambe^b, Takeshi Fujii^a and Seiya Tsushima^a

^a National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba 305-8604, Japan

^b University of Tsukuba, 1-1-1 Tennoudai, Tsukuba 305-8571, Japan

sakahiro@affrc.go.jp

Microorganisms which can degrade biodegradable plastic are powerful tools for controlling the degradation of biodegradable plastics used in the agricultural field. Such biodegradable plastic-degrading microorganisms are expected to inhabit within the alimentary canals of the insects which eat high molecular compounds with ester bond-rich structures such as lignin. We tried to isolate such microorganisms from the larvae of three species of stag beetles, *Aegus laevicollis*, *Aesalus asiaticus*, and *Ceruchus lignarius* (Coleoptera: Lucanidae) which prefer the lignin-rich (brown-rotted) dead woods strikingly. From these larvae, midguts, hindguts and integuments were separated, and microorganisms from each tissue were cultivated using the medium containing emulsion of poly-butylene succinate/agipate (PBSA) in an upper-layer on the nutrient agar (NA) under the aerobic condition to investigate the biodegradable plastic-degrading ability. Several isolated bacteria and fungi degrade PBSA. An isolated fungus degrades poly-butylene succinate (PBS) which is more persistent than PBSA against microorganism's attack.

Key words: biodegradable plastic, degrading bacteria, degrading fungi, PBS, PBSA

P-092 Molecular Identification of *Wolbachia* in *Aprostocetus prolixus*

Dazhuang Huang and Fangfang Yuan

Agricultural University of Hebei, Baoding Hebei 071000, China

huangdazhuang@sohu.com

Wolbachia is a common and widespread group of bacteria found in reproductive tissues of arthropods. *Aprostocetus prolixus* is a new species found in 1984, it has inhibitory action to *Apriona germari*. And inducing *Aprostocetus prolixus* parthenogenesis with *Wolbachia* is an effective way to improve bio-control efficiency of *Aprostocetus prolixus*. Firstly, Based on the amplification of part of the *wsp*, *ftsZ* and 16S rDNA gene, we expanded the fragment of the 600 bp, 1000 bp and 900 bp respectively in the DNA of a laboratory population of *Corcyra cephalonica*, which proved the *Corcyra cephalonica* infected with the *Wolbachia*; But we did not expand any fragment in the DNA of the *Aprostocetus prolixus*. Secondly, we studied its parthenogenesis, the offspring were almost male. Next, we plan to use microinjection to horizontally transfer *Wolbachia* from *Trichogramma cacoeciae* to *Aprostocetus prolixus*.

Key words: *Wolbachia*, *Aprostocetus prolixus*, *Corcyra cephalonica*, microinjection

P-093 The Role of Cuticular Hydrocarbons in Ant-Aphid Mutualism: Chemical Marking and Mimicry

Shintaro Endo^a and Takao Itino^b

^a Department of Mountain and Environmental Science, Shinshu University, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan

^b Department of Biology, Faculty of Science, Shinshu University, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan

s07t404@shinshu-u.ac.jp

The association of aphids and ants is one of the most well-known examples of mutualism. However, ants not only defend aphids but also they prey on aphids. This resembles human breeding of domestic animals in that the owners keep and control the population of the target animals.

Sakata (1994) investigated what kind of aphids were less preyed on, and showed that the aphids attended by the ants from the same nest (nestmates) were less preyed. This observation may mean that ants put some marks to their tending aphids. Here, to confirm whether ants really put marks on the aphids, we analyzed cuticular hydrocarbons (CHCs) of *Dendrolasius* ants which are used as nestmate recognition pheromon, and compared the profiles with those of tended *Stomaphis* aphids.

Gas chromatography analysis showed that *Stomaphis yanonisi* and attending *Dendrolasius* ants shared almost all components of the CHCs. Then, to see the origin of aphid CHC components, we compared the CHC profiles among several aphids which are attended by different species of *Dendrolasius* ants. The aphid CHC components are divided into two groups. Some are originated from ants, and others are produced by aphids themselves. These results suggest that ants put chemical marks to aphids, and also that aphids produce ant-mimicking CHCs probably to avoid predation by ants.

Key words: chemical mimicry, cuticular hydrocarbons, *Dendrolasius*, semiochemical, *Stomaphis*

P-094 Intracolony Chemical Mimicry in Ant Parasitic Inquiline *Niphanda fusca* (Lepidoptera: Lycaenidae)

Masaru K. Hojo^a, Ayako Wada-Katsumata^b, Toshiharu Akino^a, Susumu Yamaguchi^c, Mamiko Ozaki^d and Ryohei Yamaoka^a

^a Graduate School of Science and Technology, Kyoto Institute of Technology, Kyoto, 606-8585, Japan

^b Graduate School of Agriculture, Kyoto University, 606-8502, Japan

^c Wakamiko, Sutama-cho, Hokuto city, Yamanashi, 408-0112, Japan

^d Graduate school of Science, Kobe University, Kobe, 657- 8501, Japan

mjo03@kit.ac.jp

Caterpillars of lycaenid butterfly *Niphanda fusca* develop as parasites inside the host ant colonies. In the host ant nest, it is thought that the caterpillars chemically mimic to the host larvae because they are attended and fed by the host workers at brood chambers. In our bioassay, the host workers attended the glass dummies treated with cuticular chemicals of the *N. fusca* caterpillars as much as dummies with cuticular chemicals of the larvae and the males of the host ants. The chemical analyses revealed that cuticular chemicals of the *N. fusca* were mainly constituted of hydrocarbons. In their host ant nest, the hydrocarbon composition of *N. fusca* caterpillars closely matched to that of the host ants. The multivariate analysis revealed that cuticular hydrocarbon profiles of the *N. fusca* caterpillars differed from that of the ant larvae. Furthermore, the *N. fusca* caterpillars closely resembled the ant males in the relative proportions of cuticular hydrocarbons. Our results suggest that male-like hydrocarbon profiles may play an important role to the *N. fusca* caterpillars in receiving care by the host workers.

Key words: Lycaenidae, *Camponotus* ants, parasitism, chemical mimicry, cuticular hydrocarbons

P-095 Significance of Minor Alarm Pheromone Components in Major Five Japanese *Camponotus* Ants

Nao Fujiwara-Tsujii^a and Ryohei Yamaoka^b

^a Venture Laboratory, Graduate school of Science and Technology, Kyoto Institute of Technology, Matsugasaki Gogyokaido Kyoto 606-8585, Japan

^b Chemical Ecology laboratory, Graduate school of Science and Technology, Kyoto Institute of Technology, Matsugasaki Gogyokaido Kyoto 606-8585, Japan.

naoki99@kit.ac.jp

High similarities in alarm pheromone compositions have been reported in the genus *Camponotus*, but no report has documented the chemical composition of exocrine glands of *Camponotus* ants from Japan. Here, alarm pheromones of five major Japanese *Camponotus* (Formicinae) ants were identified and quantified by gas chromatography (GC) and gas chromatograph-mass spectrometry (GC-MS). Similarity of alarm pheromone profiles among individual samples was transformed by using chord-normalized expected species shared (CNESS) distances, followed by visualization of the data with nonmetric multidimensional scaling (NMDS). The result of NMDS using CNESS_m=1 indicates that five major Japanese *Camponotus* have similar chemical compositions. In case of CNESS_m=27 that is sensitive equally to major and trace components, alarm pheromone profiles could be divided into each species, but only two species, *C. obscuripes* and *C. devestivus* could not be separated. Only when CNESS_m=9999 (trace compound emphasized maximally), alarm pheromone of the five species grouped by species.

Together with these results and our previous works on the alarm communication steps of *C. obscuripes*, significance of trace components and its role will be discussed.

Key words: alarm pheromones, genus *Camponotus*, species specificity

P-096 Recognition System in Grooming Behavior against Entomopathogenic Fungi of the Termite, *Coptotermes formosanus* Shiraki

Aya Yanagawa^{a, b}, Fumio Yokohari^c, Kazuhiro Iiyama^a, Chisa Aoki^a and Susumu Shimizu^a

^a Institute of Biological Control, Graduate School of Bioenvironmental Science, Kyushu University; Fukuoka 812-8581, Japan

^b Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists

^c Division of Biology, Department of Earth Science, Faculty of Science, Fukuoka University

a-yana@grt.kyushu-u.ac.jp

Our research has showed that termites, *Coptotermes formosanus* Shiraki, protect themselves from entomopathogenic fungi by mutual grooming behavior. The termites remove foreign organisms, such as fungal conidia, from the body surface by glossa. Consequently, the termite populations hardly suffer from fungal epizootics owing to mutual grooming, even though termites live in high-density populations and high humidity habitats. We examined in this study the role of antennae related with grooming behavior using electrophysiological methods. The experimental results indicated that antennae serve the termites in recognition of fungal conidia and in elongation of mutual grooming duration. For resistance of termites, roles of antennae are quite important in protection against fungal infection.

Key words: *Coptotermes formosanus* Shiraki, mutual grooming behavior, entomopathogenic fungi, antennae, electrophysiological response

P-097 Is Octenol a Non-Host Signal or an Old Host Signal for Scolytid Beetles (Coleoptera: Scolytidae)?

Akira Ueda

Hokkaido Research Center, Forestry and Forest Products Research Institute, 7 Hitsujigaoka, Toyohira Sapporo 062-8516, Japan
akira@ffpri.affrc.go.jp

Some coniferophagous scolytid beetles are known to avoid the volatiles from old hosts and angiosperm non-hosts. The old host volatile is verbenone that is thought to be produced by microorganisms dwelling in the walls of galleries that the beetles have bored earlier. The non-host volatiles are green leaf alcohols (C6-alcohols such as 1-hexanol) and bark volatiles such as *trans*-conophthorin and C8-alcohols including octenol (1-octen-3-ol). However, C8-alcohols volatilize also from many kinds of mushrooms (fungi) growing in old dead woods that are unsuitable for the reproduction of most scolytid beetles. Thus, there is a possibility that some scolytid beetles respond to C8-alcohols as old host signals. If so, C8-alcohols can be old host signals also for the scolytid beetles that attack broad leaf trees, despite the fact that these alcohols volatilize from barks of their hosts. To answer this question, I collected scolytid beetles with flight intercept traps using ethanol as bait, and compared the catch by the traps using both ethanol and octenol with the catch by the traps using only ethanol. The catches of *Scolytoplatus tycon* and *Xylosandrus germanus*, both of which attack broad leaf trees, were considerably lower in the traps with octenol. The two species would recognize octenol as a volatile from old hosts infected with fungi. If further studies reveal similar responses in many other scolytid beetles, the avoidance of C8-alcohols as old host signals may be common in scolytid beetles including coniferophagous species.

P-098 Novel Compounds in the Metathoracic Gland of the Predatory Stink Bug, *Eocanthecona concinna* (Walker)

Ho, Hsiao-Yung

Institution of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan, ROC
shine@gate.sinica.edu.tw

Volatile compounds from the metathoracic gland (MTG), dorsal abdominal glands (DAG) and sternal glands (SG) of the generalist predatory stink bug, *Eocanthecona concinna* (Walker) in Taiwan, are studied by gas chromatography-mass spectrometry. The major compound in the MTG of both male and female bugs is (*E*)-2-octenal. Other minor components include two compounds with the novel functional groups of methylthio and aldehyde. These two compounds are 3-(methylthio)hexanal and 3-(methylthio)octanal and they were identified by mass spectra and confirmed by synthesis. The amount of compounds found in MTG of female bugs is higher than those found in MTG of male bugs. The DAG contents of nymphs were also analyzed, with 4-oxo-(*E*)-2-hexenal, 4-oxo-(*E*)-2-octenal, tridecane, and tetradecanal as major components. Male bugs possess sternal setae but not females, and 6,10,13-trimethyltetradecyl isovalerate was detected only from some of the male sternal setae. The contents in the glands of *E. concinna* are compared with the contents found in the glands of another predatory stink bug, *Eocanthecona furcellata*.

Key words: metathoracic gland, Pentatomidae, *Eocanthecona concinna*

P-099 Chemical Ecological Studies on *Platypus koryoensis* (Coleoptera: Platypodidae) I

Junheon Kim, Sang-Gil Lee, Sang-Chul Shin and Il-Kwon Park

Division of Forest Insect Pests and Diseases, Korea Forest Research Institute (KFRI), 207 Cheongnyangni 2, Dongdaemun, Seoul 130-712, Republic of Korea.

junheon@gmail.com

Mass mortality of oak was firstly reported on 2004 in Kyunggi province, Korea. Since then, the number of dead oak, especially *Quercus mongolica*, has increased every year. The mass mortality of oak is considered to result from ambrosia fungi, which tentatively identified as *Raffaelea* sp. *Platypus koryoensis* is a critical vector of the fungi which female beetle carrying the fungi on her mycangia.

In some platypodid species, mass attack on their host and the existence of the aggregation pheromone in male-produced frass (saw-dust) were reported. In case of *P. koryoensis*, a small number of males attacked a particular tree (*Q. mongolica*) firstly and then a large number of the other males concentratively bored the tree. This phenomenon suggests the presence of semiochemicals that make *P. koryoensis* aggregate.

For integrated management of this pest, it is needed to understand the chemical ecological aspects of *P. koryoensis*. For this aim, male and female body extracts and beetle-produced frass (saw-dust) were analyzed on GC and GC/MS. Here we reported the result of the analysis.

Key words: *Platypus koryoensis*, *Quercus mongolica*, GC/MS analysis

P-100 Diet-Induced Chemical Phytomimesis by Twig-like Caterpillars of *Biston robustum* Butler (Lepidoptera: Geometridae)

Toshiharu Akino

Chemical Ecology Laboratory, Kyoto Institute of Technology, Matsugasaki 606-8585, Japan.

toshiakino@gmail.com

Polyphagous caterpillars of the giant geometer *Biston robustum* resemble the twigs of their respective food sources in color and shape. Common predatory ants, including *Lasius* and *Formica*, were often observed to freely prowl directly on caterpillars' bodies, even after antennal contact. This suggests that the cuticular chemicals of the caterpillars resemble those of the twigs of the foodplants, so we analyzed both by GC and GC-MS. The chemical compositions differed among caterpillars fed on a cherry, *Prunus yedoensis*, a chinquapin *Castanopsis cuspidata*, and a camellia *Camellia japonica*. The cuticular chemicals of the caterpillars resembled those of their corresponding food sources. When the caterpillar diets were switched from the cherry to camellia or chinquapin at the 4th instars, the caterpillars' cuticular chemicals changed after molting to resemble those of their respective foods. Caterpillars also changed their cuticular chemicals when they perched on cherry twigs and fed on camellia or chinquapin leaves, but not when they perched on camellia or chinquapin twigs and fed on cherry leaves. The chemical similarities between the caterpillars and the twigs were due to the digestion of host leaves, which indicates that this is a diet-induced adaptation.

P-101 Sequestration and Metabolism of Host-Plant Flavonoids by the Pale Grass Blue, *Pseudozizeeria maha* (Lepidoptera: Lycaenidae)

Hiroki Mizokami and Kunijiro Yoshitama

Graduate School of Science and Technology Kumamoto University, 2-39-1 Kurokami Kumamoto 860-8555, Japan

063d9005@gsst.stud.kumamoto-u.ac.jp

Many butterflies belonging to the family Lycaenidae stored dietary flavonoids in their wings from the hostplants at larva stages. It has been suggested that the flavonoids contribute to the UV-absorbing wings pattern and visual communication. Flavonoids, exhibiting a variety of functions in plant-herbivore interactions, are phenolic secondary metabolites in vascular plants and insects are unable to synthesize these compounds. So far very little is known about sequestration and the metabolic processes of flavonoids by insects. In this study, we analyzed the flavonoids in the leaves of hostplant (*Oxalis corniculata*) and also those in the mature larvae, pupae and adult butterflies of *P. maha*, to investigate the uptake and the metabolism of flavonoids in the insects. The three flavonoids in the leaves were identified as 6-*C*-glucosyl luteolin (isorientin), 6-*C*-glucosyl apigenin (isovitexin) and isovitexin 7-methyl ether (swertisin). Isovitexin was the major flavonoid detected in *P. maha* pupae and imagines, but not in the larva; the major one in the larva was isovitexin 7-*O*-glucoside (saponarin). Saponarin, which is *O*-glucosylated compound by larvae biotransformation, is converted again into isovitexin at pupae stages. Within the imagines wings, there was no significant difference in the amount of flavonoid between males and females, whereas female butterflies show a higher flavonoid concentration than males in their bodies.

Key words: *Oxalis corniculata*, *Pseudozizeeria maha*, *C*-glycosylflavones, selective uptake

P-102 Hydrocarbons with a 1,3,6,9-, 3,6,9,11-, or 6,9,11-Polyene System: Sex Pheromone Candidates of Lepidopteran Insects in Highly Evolved Groups

Masanobu Yamamoto, Rei Yamakawa, Toshiya Oga, Hidekazu Obara and Tetsu Ando

Graduate School of Bio-Applications and Systems Engineering (BASE), Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

ymanob@cc.tuat.ac.jp

Lepidopteran Type II sex pheromones composed of 3,6,9-trienes, 6,9-dienes, and their epoxy derivatives were biosynthesized from linolenic and linoleic acids in the species of highly evolved families. Since diversity of an insect species indicates further structural modification, such as the introduction of an extra double bond, we synthesized 1,3,6,9- and 3,6,9,11-tetraenes and 6,9,11-trienes with C₁₇-C₂₁ chains to accumulate new information for the pheromone research. Namely, using the Wittig reaction, (*Z,Z,Z*)-1,3,6,9-tetraenes were synthesized from 3-hexyn-1,6-diol, and (*Z,Z,Z,E*)-3,6,9,11-tetraenes and (*Z,Z,E*)-6,9,11-trienes were synthesized from (*E*)-2-alkenals with appropriate carbon chains. GC-MS analysis of each synthetic polyene, whose chemical structure was confirmed by ¹H- and ¹³C-NMR, revealed some characteristic fragment ions reflecting the positions of the double bonds; *i.e.*, *m/z* 79, 106, and M-54 of 1,3,6,9-tetraenes, *m/z* 79, M-56, and M-82 of 3,6,9,11-tetraenes, and *m/z* 57, 79, and 163 of 6,9,11-trienes. Because the determination of the unsaturated positions is difficult to accomplish by chemical derivatization with a limited amount of natural pheromones, these diagnostic ions found in authentic samples would help identify the hydrocarbons in a pheromone extract. Furthermore, we carried out field screening tests of these polyenes to discover new sex attractants of lepidopteran insects in forests in Tokyo and Iriomote Island in Japan. To date, the attraction of four geometrid species in Tokyo and one noctuid species in Iriomote Island was observed.

Key words: Lepidoptera, pheromone synthesis, diagnostic ion, GC-MS analysis, sex attractant

P-103 Synthesis and Characterization of 2,13- and 3,13-Octadecadienals for the Identification of the Sex Pheromone Secreted by a Clearwing Moth

Masanobu Yamamoto^a, M. D. A. Islam^a, Mieko Sugie^a, Hideshi Naka^b, Jun Tabata^c, Yutaka Arita^d and Tetsu Ando^a

^a Graduate School of BASE, Tokyo University of Agriculture and Technology, Tokyo, Japan

^b JT Biohistory Research Hall, Osaka, Japan

^c National Institute for Agro-Environmental Sciences, Tsukuba, Japan

^d Meijo University, Tempaku-ku, Nagoya, Japan

antetsu@cc.tuat.ac.jp

In addition to 2,13- and 3,13-octadecadien-1-ols and their acetates, an aldehyde analogs has been identified from lepidopteran species in the family Sesiidae. In order to establish a reliable analytical method for determining the positions and configurations of the two double bonds in natural pheromone components, all geometric isomers of the 2,13- and 3,13-octadecadienals were synthesized by Dess-Martin oxidation of the corresponding alcohols. Their ¹H and ¹³C NMR data indicated successful preparation with quite limited isomerization of the double bond at the 2- or 3-position. GC-MS analysis of these aldehydes showed isomerization of (*Z*)-2-, (*Z*)-3-, and (*E*)-3-double bonds to an (*E*)-2-double bond, even though a cool on-column injector was used. In contrast, HPLC analysis with an ODS column was accomplished without isomerization. The 2,13-dienal with a conjugated system was detectable in nanogram amount using a UV detector at 235 nm. Whereas the detection of 3,13-dienals was difficult, a highly sensitive analysis was achieved after derivatization with 2,4-dinitrophenylhydrazine. LC-MS with atmospheric pressure chemical ionization, showed a strong [M-1]⁻ at *m/z* 443 for the derivatives. Based on these analytical data, a pheromone extract of a sesiid moth, *Macroscelesia japona*, was examined by HPLC and LC-MS, and it was confirmed that the octadecadienal tentatively identified by a previous GC-MS analysis had the 2*E*,13*Z* configuration.

P-104 7,11,13-Hexadecatrienal Identified from Female Moths of the Citrus Leafminer as a New Sex Pheromone Component: Synthesis and Field Evaluation in Japan and Vietnam

Le Van Vang^{a, b}, M. D. A. Islam^a, Nguyen Duc Do^a, Tran Van Hai^b, Shinji Koyano^c, Nobuo Ohbayashi^d, Masanobu Yamamoto^a and Tetsu Ando^a

^a Graduate School of BASE, Tokyo University of Agriculture and Technology, Tokyo, Japan

^b Cantho University, Cantho City, Vietnam

^c Tokyo Metropolitan Agriculture and Forestry Research Center, Tokyo, Japan

^d Ehime University; Matsuyama, Japan

antetsu@cc.tuat.ac.jp

(7*Z*,11*Z*)-7,11-Hexadecadienal (Z7,Z11-16 : Ald), which has been identified from female moths of the citrus leafminer (*Phyllocnistis citrella*), strongly attracts males in Japan. Recently, in addition to the dienyl aldehyde, a trienyl derivative, (7*Z*,11*Z*,13*E*)-7,11,13-hexadecatrienal (Z7,Z11,E13-16 : Ald), was found as another sex pheromone component of females collected in California and Brazil. Thus, we synthesized Z7,Z11,E13-16 : Ald and its isomer (Z7,E11,E13-16 : Ald) to evaluate their effects on the males inhabiting Asia. Starting from 1,7-heptanediol, alcohols with the trienyl structures were prepared by applying the Wittig-coupling reaction twice and oxidized to yield the objective aldehydes after separation by HPLC with a ODS column. At citrus orchards in Ogasawara Islands and Ehime Prefecture of Japan, neither of the trienyl aldehydes attracted males and showed a synergistic effect on the male capture by Z7,Z11-16 : Ald. Far from being reinforced, attractant activity of dienyl aldehyde was diminished by mixing of Z7,Z11,E13-16 : Ald. At an orchard in Cantho City of Vietnam, males could not be caught by a lure baited only with Z7,Z11-16 : Ald, but they were successfully attracted with the 1 : 3 mixture of Z7,Z11-16 : Ald and Z7,Z11,E13-16 : Ald. These results indicated that the sex pheromones of the Vietnam and American strains were similar; however, the Japanese strain established a different communication system from those of the foreign strains.

P-105 Synthesis of (8E,10Z)-8,10-Tetradecadien-1-al the Sex Pheromone of Horse Chestnut Leaf Mines *Cameraria ohridella* Descha-Dimic Species

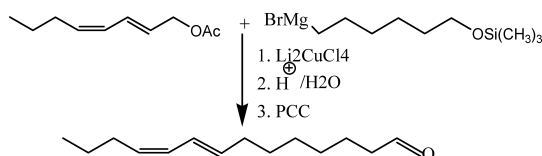
Lucia Gânscă, Sanda Maxim, Irina Ciotlăuș and Ioan Oprean

Institute for Research in Chemistry "Raluca Ripan", 400294 Fântânele 30, Cluj-Napoca, Romania

ganscalucia@yahoo.com

The biocenotic balance of the ornamental chestnut tree was perturbed by the presence of a new pest—the leaves mining moth *Cameraria ohridella*.

The synthesis of (8E,10Z)-8,10-tetradecadiene-1-al, the sex pheromone of *Cameraria ohridella* species was based on a $C_3 + C_3 + C_2 = C_8$ and $C_8 + C_6 = C_{14}$ coupling scheme, starting from 2-propin-1-ol, using Grignard-Schlosser cross-coupling.



A mixture was obtained through synthesis of the four geometrical isomers, which presented a very good attractivity and selectivity.

Key words: chemical ecology, *Cameraria ohridella*, pheromones

P-106 Synthetic Studies on Decaturins

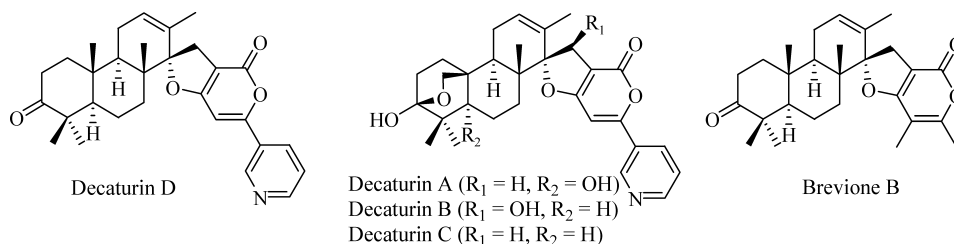
Hirosato Takikawa, Kojiro Hayashi, Takashi Hashimoto and Mitsuru Sasaki

Graduate School of Agricultural Science, Kobe University, Rokkodai 1-1, Nada-ku, Kobe 657-8501, Japan
takikawa@kobe-u.ac.jp

Decaturins are antiinsectant diterpenoid derivatives isolated from *Penicillium* spp. These compounds are structurally unique natural products consisting of diterpene, polyketide and nicotinate subunits. Especially, the spiro linkage between diterpene and polyketide subunits is characteristic.

We have already accomplished the first synthesis of brevione B, a closely related diterpenoid isolated from *Penicillium* sp. as an allelochemical, by employing our original spiro-cyclization as the key step. We thus initiated synthetic studies on decaturins. As a result, the first synthesis of (+)-decaturin D was accomplished based on our original methodology, and the diterpene-model compounds of decaturins were also synthesized. Further studies toward the total synthesis of decaturins A and C are in progress.

Key words: diterpenoid, antiinsectant, spiro-cyclization, allelochemical



P-107 Direct Determination of the Stereoisomeric Compositions by the Ohrui-Akasaka Method and Stereochemistry-Pheromone Activity Relationships of the Pheromones of Azuki and Cowpea Weevil

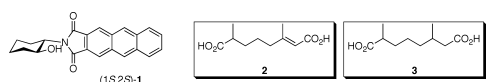
Arata Yajima^a, Kazukaki Akasaka^b, Tomonori Nakai^a, Masamichi Yamamoto^a, Tomoo Nukada^a and Goro Yabuta^a

^a Department of Fermentation Science, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagayaku, Tokyo 156-8502, Japan

^b Graduate School of Life Sciences, Tohoku University, Tsutsumidori- Amamiya 1-1, Aoba-ku, Sendai 981-8555, Japan

ayaji@nodai.ac.jp

The development of an effective analytical method is important to determine the absolute configuration in insect pheromone chemistry. We presumed that the Ohrui-Akasaka method might become a powerful tool for insect pheromone chemistry owing to its high sensitivity. The azuki bean weevil, *Callosobruchus chinensis* L. and the cowpea weevil, *C. maculatus*, are serious cosmopolitan pests of stored products. The structures of the copulation release pheromones were proposed to be as **2** and **3**. However, it was impossible to determine the absolute



configurations because of the scarcity of the materials and the incorporation of other acids. We reisolated the natural pheromones, synthesized optically active **2** and **3** via Evans asymmetric alkylation

as a key step and determined stereoisomer constitutions of **2** and **3** by 2D-HPLC analyses of the corresponding Ohrui-Akasaka esters. The stereochemistry-pheromone activity relationships will be reported.

Key words: pheromone, *Callosobruchus chinensis*, *Callosobruchus maculatus*, Ohrui-Akasaka method

P-108 NMR Determination of Absolute Configuration of Organic Compounds by Use of Axially Chiral Reagents — Axial Chirality Methods —

Yukiharu Fukushi and Satoshi Tahara

Laboratory of Ecological Chemistry, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

y-fuku@abs.agr.hokudai.ac.jp

A chiral molecule has an isomer of mirror image structure that is not superposed. The absolute stereochemistry and enantiomeric purity of it must, therefore, be clarified on discussing its bioactivity and biological function. In this work, “Axial Chirality Methods”, a set of chiral derivatizing methods to establish the absolute configurations of organic compounds, are presented. The methods can be applicable to chiral secondary alcohols, β -chiral primary alcohols, α -chiral primary amines, α -chiral carboxylic acids, and chiral alkenes. In the derivatives, NOEs are observed between the protons of the reagent's moiety and those of substrate's one. The absolute configuration of substrate can be unambiguously determined by the NOE correlation. Conformational correlation models for these derivatives are presented. Most diastereomeric derivatives obtained by the methods were separated on silica-gel TLC plates. Interaction models between the solutes and silica-gel stationary phase are also proposed. Therefore, these methods can be used for optical resolution of racemates as well as for configurational analyses. The diastereomeric derivatives of ginnol (10-nonacosanol) were separated on silica-gel TLC plate. Versatility and limitation of the methods are discussed.

Key words: Axial Chirality Methods, absolute configuration, NMR, NOE

P-109 Enantio-Differential Approach to the Receptor Protein Concerning Nyctinasty of *Albizzia* Plants

Yoko Nakamura, Ryoji Miyatake, Sho Inomata and Minoru Ueda

Department of Chemistry, Tohoku University, Sendai 980-8578, Japan

nakamura-org@mail.tains.tohoku.ac.jp

Albizzia plants close their leaves in the evening, as if to sleep, and open them in the morning according to the circadian rhythm. Potassium β -D-glucopyranosyl-12-hydroxyjasmonate was isolated as leafclosing factor (LCF) of *Albizzia saman*. We developed molecular probes consisting of modified LOF in order to identify its mode of action.

We used a pair of enantiomers of FITC-labeled LCF for the determination of target cell for LCF in the plant body. Comparing the results using both enantiomers, it was clearly shown that target cell of LCF is the motor cells. The results demonstrated the involvement of a receptor in the motor cell, which recognizes the stereochemistry of LCF. Similarly, we synthesized enantio pair-type photoaffinity-labeling probes, and used them for photoaffinity labeling of receptor protein for LCF. By using protoplast of motor cell, we found 35 kDa membrane protein strictly recognizes the stereochemistry of LCF, and it is highly likely that the protein is the specific receptor for LOF.

Key words: nyctinasty, jasmonate glucoside, receptor

P-110 Development of Pd Catalyzed Stereoselective Cyclization and its Application for Synthesis of Natural Products

Yasunao Hattori^{a, b}, Shin-ichi Furuhata^c, Yoshihiro Mohri^c and Hidefumi Makabe^{a, c}

^a Interdisciplinary Graduate School of Science and Technology, Shinshu University, 8304, Minami-minowa, Kami-ina, Nagano 399-4598, Japan

^b Satellite Venture Business Laboratory, Shinshu University, 3-15-1, Tokida, Ueda, Nagano 386-8567, Japan

^c Sciences of Functional Foods, Graduate School of Agriculture, Shinshu University, 8304, Minami-minowa, Kami-ina, Nagano 399-4598, Japan.

makabeh@shinshu-u.ac.jp

Palladium-catalyzed stereoselective cyclization of alkenylamine and alkenylalcohol is very important methodologies for the stereoselective construction of nitrogen and oxygen hetero alicycles. In this symposium, we wish to present Pd-catalyzed diastereoselective *N*- and *O*-alkylation and their application for natural products. A number of the piperidine alkaloids, especially 2,6-disubstituted piperidin-3-ols, have been found abundantly in nature, and many of them show interesting pharmacological activities. We have developed the Pd catalyzed cyclization and applied synthesis of (–)-cassine (**1**). Recently, spectamine A (**2**) was isolated from an African legume, *Cassia spectabilis*, as a novel piperidine alkaloid. Herein we wish to report the progress toward synthesis of **2** using Pd catalyzed stereoselective cyclization to give 2,6-*trans*-piperidine ring **4a**. Total synthesis of **2** is currently underway.

Annonaceous acetogenins, that have been isolated from a number of Annonaceae, have attracted due to a wide variety of biological activities. Synthetic studies on pyranicin (**3**), isolated from *Goniothalamus giganteus* as a one of the rare THP acetogenins, is described. The THP ring moiety was constructed using Pd catalyzed stereoselective cyclization. Total synthesis of **3** is now underway.

Key words: piperidine alkaloid, annonaceous acetogenin, stereoselective cyclization, natural product

P-111 More Efficient Open Column Chromatography for Bioactive Natural Products Isolation

Nobutoshi Yokoi, Nanri Fujita, Shuhei Enjo, Masashi Sato and Toru Yamasaki

Department of Biological Science, Kagawa University, Miki-Ikenobe 2393, Kagawa 761-0795, Japan

yamasaki@ag.kagawa-u.ac.jp

Liquid-liquid (LL) extraction or silica gel column chromatography (CC) followed by reversed phase (RP)-CC is in wide use for preparative purposes. However, such traditional methods have limitations. In the LL extraction methods, the range of the solvent choice is limited, and it is emphasized here that RP-CC, which has been developed under the impetus from the high resolution achieved on GC columns, has been found to be most effective in separating relatively hydrophobic and low- or medium-molecular weight natural products. In addition, in the case of high performance liquid chromatography (HPLC), the applicable sample sizes are too small for preparative separation. Nevertheless, discussions on the preparative isolation methods are inactive. A great number of polar and relatively high-molecular weight compounds occur in nature. Various types of stationary phases, including Sephadex LH 20 and MCI GEL CHP 20P, therefore have been applied, and found to be more effective than the traditional phases. They are mostly highly porous matrices and/or have three-dimensional network; as a consequence, their hydrophobic/hydrophilic interaction sites are increased in number, giving rise to high resolution. With the stationary phases liquid-liquid extraction is not necessarily required. Elution with water and/or aqueous media (e.g., acetone and EtOH) is performed. RP-HPLC may be employed at times. The aim of this presentation is to review the isolation of active, polar or lipophilic compounds by open CC from pine (*Pinus densiflora*), tea plant (*Camellia sinensis*) or a medicinal plant (*Smilax roxburghiana*) native to the tropical Himalayas.

Key words: natural products, open column chromatography, polymeric columns, interaction sites

P-112 Novel Sex Pheromone Components from a Lithosiinae Moth, *Lyclene dharma dharma*, in the Family of Arctiidae

Masanobu Yamamoto^a, Nguyen Duc Do^a, Tomoya Kamata^a, Yasushi Adachi^a, Masakatsu Kinjo^b and Tetsu Ando^{a,*}

^a Graduate School of BASE, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan

^b Tropical Biosphere Research Center, University of the Ryukyus, Okinawa 907-1541, Japan

*antetsu@cc.tuat.ac.jp

The family of Arctiidae is mainly divided into two subfamilies, Arctiinae and Lithosiinae. From some species in Arctiinae, Type II sex pheromones composed of polyunsaturated hydrocarbons and/or the epoxy derivatives have been identified. In Lithosiinae, however, neither the pheromones nor the male attractants have been reported, which suggests that their mating communication systems depend on different types of chemicals. In order to understand the diversity of lepidopteran pheromones, we caught several Lithosiinae species in the Iriomote Islands using a black-light trap and found novel compounds in a pheromone extract of *Lyclene dharma dharma* females. The GC-EAD analysis showed that the extract includes three EAG-active components (Comps. I-III) at a ratio of about 4 : 3 : 2. On a column chromatography with Florisil, these EAG-active components were eluted together with 10% ether in hexane, indicating that these components were more polar than a Type II epoxy pheromone and less polar than a Type I alcohol pheromone. The GC-MS analysis revealed the structure of Comps. I-III as ketones without a C=C bond; *i.e.*, methyl-2-octadecanones for Comps. I and II, and dimethyl-2-octadecanone for Comp. III. We estimated the positions of the methyl groups by Wolff-Kishner reduction of the natural pheromone and are going to synthesize candidates to confirm the structures.

Key words: Lepidoptera, arctiid moth, sex pheromone of moth, methyl-branched ketone, identification

P-113 Sex Pheromone of the Larch Caterpillar Moth, *Dendrolimus superans* from Northeastern China

Xiang-Bo Kong^{a, b}, Cheng-Hua Zhao^b and Zhang zhen^a

^a Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China

^b Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China
kongxbo@hotmail.com

The larch caterpillar moth, *Dendrolimus superans* Butler (Lepidoptera: Lasiocampidae), is a serious pest in the northeastern part of China. (5Z,7E)-5,7-Dodecadienal, (5Z,7E)-5,7-dodecadienol, (Z)-5-dodecenol, and (Z)-5-dodecenal (100:95:75:71) were identified by gas chromatography and coupled gas chromatography-mass spectrometry in extracts of pheromone glands of female *D. superans*. However, only (5Z,7E)-5,7-dodecadienal elicited strong responses from conspecific male antennae in coupled gas chromatography-electroantennography studies. Field tests with synthetic compounds indicated that baits containing (5Z,7E)-5,7-dodecadienal as a single component attracted male *D. superans* moths, whereas addition of one, two, or all three of the possible minor components did not increase the attractiveness of lures. (5Z,7E)-5,7-Dodecadienyl acetate, one of the pheromone components in sympatric *Dendrolimus* species was antagonistic, but the analog (5Z,7E)-5,7-dodecadienyl propionate was not. Pheromone traps baited with (5Z,7E)-5,7-dodecadienal have been used effectively for monitoring populations of *D. superans* in the northeastern part China.

Key words: Lasiocampidae, Lepidoptera, sex pheromone, (5Z,7E)-5,7-dodecadienal, population monitoring

P-114 Sex Pheromone for the Population Suppressing of Sawfly, *Diprion jingyuanensis* Xiao et Zhang (Hym., Diprionidae)

Zhen Zhang^a, Erik Hedenstrom^b, Hongbin Wang^a, Xiangbo Kong^a and Olle Anderbrant^c

^a Research Institute of Forest Protection, Chinese Academy of Forestry, Beijing, P. R. China

^b Department of Chemistry and Process Technology, Mid Sweden University, Sundsvall, Sweden

^c Department of Ecology, Lund University, Lund, Sweden

Zhangzhen@caf.ac.cn

The pine sawfly, *Diprion jingyuanensis* Xiao et Zhang, is a serious pest of Chinese pine (*Pinus tabulaeformis* Carr.) in the northern parts of China. In this study both mass-trapping and mating disruption were tested on the isolated stands using the three four-isomer mixture containing the active (1S,2R,6R)-1,2,6-trimethyldodecyl propionate. The result showed that the damages in mass-trapping plots were 4% and 20% compared to 86% and 68% in the control plots. The larva population density decreased by 92.4%. Compared with the number of males caught in control area, there were 97.5% and 98.2% decreasing in mating disruption plot I and plot II respectively. The damages were 5% and 3% compared to 86% in the control plot. The larva population densities decreased by 92% and 91.6% respectively. Though both mass-trapping and mating disruption can effectively reduce the sawfly damage, mass-trapping is more economical and practical especially in developing countries.

Key words: *Diprion jingyuanensis*, sex pheromone, mass-trapping, mating disruption

P-115 Attractiveness of Synthetic Sex Pheromone to Males of the Oriental Tea Tortrix Moth, *Homona magnanima* Diakonoff (Lepidoptera: Tortricidae) in China

Jian-yu Deng^a, Shi-Ying Wu^b, Yong-Ping Huang^c, Jie-xia Jiang^a and Yan Wang^d

^a Research Institute of Plant Protection, Shanghai Academy of Agricultural Sciences, Shanghai 201106, China

^b Pudong New District Administration of Round-the-city Greenbelt Construction, Shanghai 201203, China

^c Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

^d Shanghai Forestry Station, Shanghai 200072, China

jydeng70@yahoo.com.cn

The attractiveness of synthetic sex pheromones of the oriental tea tortrix moth, *Homona magnanima* was evaluated in the field in China, using a three-component synthetic sex pheromone consisting of (*Z*)-11-tetradecen-1-yl acetate, (*Z*)-9-dodecen-1-yl acetate, and 11-dodecenyl acetate. When the three-component synthetic sex pheromone was blended in different ratios on rubber septa, maximum catch was observed in a ratio of 80 : 10 : 10. Lures captured more males baited with the three-component synthetic sex pheromones at a dose of 4.8 mg than at other doses tested. Trap height had a significant effect on moth catch, with a height of 1.8 m capturing significantly more males than 0.6 m and 1.2 m from the ground. Rubber septum dispensers were shown to have an active exposure time of up to 17 days and in water pan traps to be capable of monitoring a population of *H. magnanima* in Shanghai.

Key words: *Homona magnanima*, synthetic sex pheromone, field attraction, monitoring, in China

P-116 Attractiveness of the Synthetic Sex Pheromone to the Citrus Flower Moth (*Prays citri* Milliere) in the Mekong Delta of Vietnam

Le Van Vang^a, Nguyen Duc Do^{a, b}, Nguyen Le Quynh Thien^a and Tetsu Ando^b

^a Dept. of Plant Protection, Cantho University, Cantho City, Vietnam

^b Graduate School of BASE, Tokyo University of Agriculture and Technology, Tokyo, Japan

lvvang@ctu.edu.vn

The citrus flower moth, *Prays citri* (Yponomeutidae), is a widespread pest of citrus. Meanwhile the *P. citri* larvae attack citrus flowers, especially lemon flowers, in many countries, this species mostly attacks primarily pomelo fruits in the Mekong delta of Vietnam. From a strain cultured in Israel, the sex pheromone composed of one component, (*Z*)-7-tetradecenal (*Z*7-14 : Ald), has already been characterized [Nesbitt et al., 1977 (*Insect Biochem.*, 7: 355-359)]. Therefore, we synthesized *Z*7-14 : Ald and the related compounds and examined the attractiveness at a pomelo orchard in Vinh Long province, which is located near the center of the Mekong delta. A trap baited with a rubber septum including *Z*7-14 : Ald (0.5 mg) captured more *P. citri* males than one baited with three virgin females. In the dose-response examination, the one mg-dose of *Z*7-14 : Ald attracted the *P. citri* males the most strongly among five tested doses (0, 0.1, 0.3, 0.5, and 1 mg). These results showed that the synthetic lure was useful as a monitoring tool. (*Z*)-7-Tetradecen-1-ol strongly inhibited the attractiveness of *Z*7-14 : Ald, but the addition of (*Z*)-7-tetradecenyl acetate slightly increased the number of males captured by *Z*7-14 : Ald, indicating that the acetate was a minor pheromone component. In a future study, we will analyze pheromone components of *P. citri* distributed in Vietnam.

Key words: Lepidoptera, sex pheromone, male attractant, monitoring system, field test

P-117 GC-EAD Detection of Novel Aggregation Pheromone, (1S,4R)-p-Menth-2-en-1-ol of The Ambrosia Beetle, *Platypus quercivorus* (Coleoptera: Platypodidae)

Masahiko Tokoro^a, Masahide Kobayashi^b, Shoichi Saito^c, Haruo Kinuura^d, Tadakazu Nakashima^a, Etsuko Shoda-Kagaya^a, Takehiro Kashiwagi^e, Shin-ichi Tebayashi^e, Chul-Sa Kim^e and Kenji Mori^f

^a Department of Forest Entomology, Forestry and Forest Products Research Institute (FFPRI) 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

^b Kyoto Prefectural Forestry Experimental Station

^c Yamagata Prefectural Forest Research and Instruction Center

^d Kansai Research Center, Forestry and Forest Products Research Institute

^e Department of Bioresources Science, Faculty of Agriculture, Kochi University

^f Toyo Gosei Co., Ltd., Photosensitive Materials Research Center

tokoro@affrc.go.jp

The ambrosia beetle *Platypus quercivorus* (Maruyama) (Coleoptera: Platypodidae) is the most serious pest of Japanese oak tree, recently. We identified an aggregation pheromone of *P. quercivorus* using volatiles from the boring frass of an unmated male. GC-EAD showed an EAD active component from the volatiles. GC-MS data and comparison of the retention time of stereospecific synthetic results of the optical isomers on chiral GC column showed that the chemical structure of the EAD active component was (1S,4R)-p-menth-2-en-1-ol (quercivorol). A field trapping tests using quercivorol and its isomers demonstrated that the EAD active quercivorol is main component of aggregation pheromone of *P. quercivorus*.

Key words: *Platypus quercivorus*, aggregation pheromone, (1S,4R)-p-menth-2-en-1-ol, quercivorol, ambrosia beetle

P-118 Attractance of the Synthetic Aggregation Pheromone of the Brown-Winged Green Bug, *Plautia crossota stali* SCOTT, to Two Stink Bugs, *Halyomorpha halys* STAL and *Glaucias subpunctatus* WALKER

Koji Mishiro^a and Yoshio Ohira^b

^a National Institute of Fruit Tree Science, 2-1 Fujimoto, tsukuba, Ibaraki 305-8605, Japan

^b Kuchinotsu Citrus Research Station, National Institute of Fruit Tree Science, 954 Otsu, Kuchinotsu, Minami-Shimabara, Nagasaki 859-2501, Japan

mishiro@affrc.go.jp

The synthetic aggregation pheromone of the major fruit injury stink bug, *Plautia crossota stali*, attracts its parasitoids and other fruit injury stink bugs; i.e. *Halyomorpha halys* and *Glaucias subpunctatus*. But attractive trait to these stink bugs has never been clarified. Then, attractance of the synthetic aggregation pheromone (SAP) to other two fruit injury stink bugs was investigated from 2000 to 2004. Sticky traps with the SAP lures were placed in conifer forests, which are the major reproductive area of these stink bugs, and citrus orchards, which are not their reproductive area. Seasonal prevalence of stink bugs was researched using pheromone traps (PT) in each area. In 2004, seasonal prevalence was compared between PT and light traps (LT). Response to SAP was also compared between *H. halys* and *G. subpunctatus*. The seasonal prevalence of *G. subpunctatus* indicated two peaks in May to June and October in every area in every year. That of *H. halys* indicated different patterns in each area and the number of catches in citrus orchard was smaller than that in conifer forests. The seasonal prevalence of *G. subpunctatus* trapped by LT was similar to that by PT from July to September, when night temperature was relatively high. That of *H. halys* was different between PT and LT; i.e. at some periods, this bug was attracted by LT but not by PT. This suggests that response of these stink bugs to SAP may be different.

Key words: synthetic aggregation pheromone, seasonal prevalence, fruit injury stink bugs

P-119 Male-produced Aggregation Pheromones for the Lucerne Weevil, *Sitona discoideus* (Coleoptera: Curculionidae)

Suk Ling Wee^a, Mark McNeill^b, Barry Bunn^c and David Maxwell Suckling^a

^a HortResearch

^b Agresearch, Lincoln 7608, Canterbury, New Zealand

^c Palmerston North, Tennent Drive, Palmerston North 4474, New Zealand

suk_ling_wee@yahoo.com

The lucerne weevil, *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) became established in lucerne crops throughout New Zealand in 1974, causing considerable crop losses through larval feeding on the nitrogen-fixing root nodules. Laboratory bioassay demonstrated positive attraction of males and females to the male source. Using static and dynamic headspace sampling over the weevils, two male-specific compounds, 4-methyl-3,5-heptanedione (major) and 3-hydroxy-4,5-heptanone (minor), were identified for the mature males during autumnal post-aestivatory flight period coincided with the time when cohort of females were reproductively mature. Topical application of synthetic juvenile hormone (JH III) onto the immature males induced the production of these pheromonal compounds. Electroantennogram (EAG) showed a positive linear dose-response relationship of male and female antennal response to the two compounds. Field trial using the identified pheromones is underway.

Key words: lucerne pest, *Sitona discoideus*, aggregation pheromone, 4-methyl-3,5-heptanedione, 3-hydroxy-4-methyl-5-heptanone

P-120 Synthetic Sexual Pheromone Used for Monitoring Quarantine Pest Eastern Corn Rootworm *Diabrotica virgifera virgifera* in Romania, Transylvania area

Maria Pojar-Fenesan, Ana Balea and Ioan Oprean

Institute for Research in Chemistry "Raluca Ripan"30 Fantanele str.400294 Cluj-Napoca, Romania

mfenesan@yahoo.com

The Western Corn Rootworm (WCR) *Diabrotica v. virgifera* (Coleoptera, Chrysomelidae) is a dangerous corn pest, whose soil-inhabiting larvae can seriously damage roots of maize (*Zea mays*) and lead to yield losses. In Romania WCR was first detected in 1996 and started to spread at a rather rapid pace in Transylvania and Danube basin. The control of this corn pest requires that multiple management strategies be developed for. Crop rotation is a primary non-insecticide control strategy. However, some entomologists in the USA (EDWARDS, 1997) have observed economic WCR larval damage in corn following other crops such as soybean. In Romania the pest was monitored using synthetic pheromone obtained in our lab. The key reactions of the synthesis are a cross-coupling of two bromoderivates catalyzed by Cu^I salts, then a secondary alcoholic group obtained by coupling of aldehydic derivative with bromomethyl-magnesium and a final esterification.

In Romania, Turda area, using sticky traps, baits—racemic 1,7-dimethyl-nonanyl propanoate, from June until September, year 2005, was caught 1601 males WCR, 100–248 WCR adults/trap/week. In the climatic condition of year 2006 was counted 1327 WCR males, 101 WCR/week/trap average. This result shows the rapid spread of WCR in Romanian corn fields.

Key words: *Diabrotica virgifera*, synthesis, pheromones, maize

P-121 2-Ethyl-1,6-dioxaspiro [4,4]-nonane — The Main Component of the Spruce Bark Beetle's Pheromone *Pityogenes chalcographus*, Synthesis and Biological Tests

Ana Balea, Maria Pojar-Fenesan and Ioan Oprean

Institute for Research in Chemistry "Raluca Ripan" Cluj-Napoca, RO-400294 Cluj-Napoca, 30 Fantanele street, ROMANIA, Phone: +40-264-580165, Fax: +40-264-420441

Balea.ani@personal.ro

Synthesis, by oxidative cyclisation, of chalcogran—a major component of the aggregation pheromone—is briefly described. The proposed synthesis path has 4 stages and uses 1,6-hexanediol as a starting substance. It is an original reaction path except for the last stage, where the reaction conditions used by Cekovic, Z and Bosnjak, J (1985) at the cyclisation of 1,7-nonanediol were modified.

In the reaction products mixture, besides the main product, were obtained side-products too, identified by GC-MS. The main compound obtained, having two asymmetric centers, has 2 pairs of optical diastereoisomers: **A** (2R,5S; 2R,5R) and **B** (2S,5S; 2S,5R). All the four diastereoisomers were obtained, by synthesis and the biological tests indicate for the two non-natural isomers an intermediary activity between that of the natural ones. The biological activity, respectively the efficiency of the pheromone is tested in comparison with other imported compound. All the tests was obtained in Brasov area (Romania) between 2001–2005.

Key words: synthesis, pheromones, *Pityogenes chalcographus*

P-122 Female Sex Pheromone Components of Allium Leafminer *Acrolepiopsis sapporensis*: Identification and Field Attraction

Nobuhiro Shimizu and Yasumasa Kuwahara

Laboratory of Bioorganic Chemistry, Department of Bioscience and Biotechnology, Faculty of Bioenvironmental Science, Kyoto-Gakuen University, 1-1 Nanjo, Sogabe, Kameoka, Kyoto 621-8555, Japan
shimizu@kyotogakuen.ac.jp

The pheromone gland extract from female moths of allium leafminer, *Acrolepiopsis sapporensis* (Matsumura) (Lepidoptera: Plutellidae) was analyzed by gas chromatography-mass spectrometry (GC-MS) in order to identify the female sex pheromone of the species and evaluate its biological activity in field bioassays. Three female-specific compounds were identified and the most abundant of the three compounds was (*Z*)-11-hexadecenyl acetate (Z11-16 : Ac). The other two compounds, which were present in minor amounts, were identified as (*Z*)-11-hexadecenal (Z11-16 : Ald) and (*Z*)-11-hexadecenol (Z11-16 : OH). For determining the double bond position, an extract derived from 12 females was reacted with dimethyldisulfide and the adducts were analyzed by GC-MS. The geometric isomerism of three compounds was determined by comparing GLC retention times of *Z*- and *E*-isomers. *E*-Isomers were synthesized in 4 or 5 steps starting from 10-bromo-1-decanol. Two components, Z11-16 : Ald and Z11-16 : Ac constitute the female sex pheromone of the diamondback moth, *Plutella xylostella* (L.) which is closely related to *A. sapporensis*, and its male attraction property increase by addition of Z11-16 : OH. In field bioassays conducted at Okabe, Fukushima in 1977 and 1978, the male moths of *A. sapporensis* were captured with traps baited with a 5 : 5 : 1 blend of Z11-16 : Ac, Z11-16 : Ald and Z11-16 : OH. In our field bioassays, male moths of *A. sapporensis* were also attracted to a mixture of these three components.

Key words: female sex pheromone, *Acrolepiopsis sapporensis*, (*Z*)-11-hexadecenyl acetate, (*Z*)-11-hexadecenal, (*Z*)-11-hexadecenol

P-123 Sex Pheromone Components of *Callosobruchus rhodesianus*

Kenji Shimomura, Kamada Koichi, Shunsuke Yajima and Kanju Ohsawa

Department of Bioscience, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156-8502, Japan.

nodai101@nodai.ac.jp

Callosobruchus spp. (Coleoptera: Bruchidae) are major pests of several stored legumes and widespread across tropical and subtropical area. The damage by the weevils is serious problem for people who eat the legumes as the key dietary protein. *C. rhodesianus* is one of the stored pests of the *Callosobruchus* spp. found in South, East and West Africa and infests mainly cowpea¹⁾. In our preliminary experiment, we observed a short-range attractancy against the non-mated males using the extracts collected from virgin females. Therefore, we expected the existence of the sex pheromone and have tried its identification. We collected a crude pheromone sample from virgin females, and purified it by a silica gel column chromatography, normal phase HPLC and GC. Screening by a gas chromatography coupled electroantennogram detector (GC-EAD) showed two peaks induced by depolarization. Based on the structural analysis of these components by GC-MS and ¹H-NMR, we suggested that these structures had terpenoid skeletons. To confirm the putative structures, currently, we are trying to synthesize them.

1) Subramanyam B. and Hagstrum D. W., (eds.). Integrated management of insects in stored products. pp 1- 40 (1996)

P-124 Individual Variation of the Male Bean Bug, *Riptortus pedestris* (Heteroptera: Alydidae) on its Attractiveness to the Same Species

Nobuo Mizutani, Tetsuya Yasuda, Takuhiro Yamaguchi and Seiichi Moriya

National Agricultural Research Center, Kannondai 3-1-1, Tsukuba 305-8666, Japan

nobuo@affrc.go.jp

The individual variation of *R. pedestris* (*R. clavatus*) males on their attractiveness to the same species and the amounts of five pheromone components were examined in field experiments and GC-MS analysis in 2005 and 2006. The number of adults attracted by a single male varied greatly among individuals tested. The number of adults attracted per day was 0.92 ± 0.12 and 1.21 ± 0.13 in 2005 and 2006 (mean \pm SE), respectively. Of 142 males (64 males in 2005 and 78 males in 2006) examined one by one, seventy percent of them attracted 0.5 or more adults per day, especially, six percent of them (9/142) attracted 3 or more adults per day. On the other hand, twelve percent of them attracted only 0.5 adults or fewer per day and eighteen percent of them attracted no adults.

Proportion of five pheromone components also differed greatly among those males. As for tetradecyl isobutyrate (14:iBu), which is thought to be an essential component of the pheromone, it was apparently detected from seventy-five percent of the males. Ten percent of them had only a few amount of 14:iBu (below 0.1 μ g). Sixteen percent of them had no 14:iBu. Some of the males had 14:iBu without having (*E*)-2-hexenyl (*E*)-2-hexenoate (E2-6:E2Hx) and (*E*)-2-hexenyl (*Z*)-3-hexenoate (E2-6:Z3Hx), which are synergistic components of the pheromone, and the rest of those had E2-6:E2Hx and/or E2-6:Z3Hx without having 14:iBu. The amount of 14:iBu or octadecyl isobutyrate (the fourth pheromone component) showed a positive and significant co-relationship to the numbers of attracted adults.

Key words: *Riptortus pedestris*, *R. clavatus*, aggregation pheromone, tetradecyl isobutyrate, soybean

P-125 Effect of Adult Age on Pheromone Production and Emission Ratio in Soybean Stink Bug, *Piezodorus hybneri* (Heteroptera: Pentatomidae)

Nobuyuki Endo^a, Tetsuya Yasuda^b, Takashi Wada^a, Shin-etsu Muto^c and Rikiya Sasaki^c

^a National Agricultural Research Center for Kyushu Okinawa Region, 2421 Suya Koshi 861-1192, Japan

^b National Agricultural Research Center, 3-1-1 Kannondai Tsukuba 305-8666, Japan

^c Fuji Flavor Co., Ltd., 3-5-8 Midorigaoka Hamura 205-8503, Japan.

enobu@affrc.go.jp

The stink bug, *Piezodorus hybneri* (Heteroptera: Pentatomidae) is an important soybean pest in southern Japan. Male-produced pheromone system of the species has been reported to be a mixture of β -sesquiphellandrene, (*R*)-15-hexadecanolide, and methyl (*Z*)-8-hexadecenoate. When we investigated the relationship between adult age and pheromone production of the males, the average amount of β -sesquiphellandrene in whole-body extracts increased steadily until day 30 after adult emergence, while the other two components became maximal at day 10 and then somewhat decreased. As a result, the proportion of β -sesquiphellandrene increased from ca. 5% (at day 3) to 50% (at days 15 to 30). These findings suggest that the pheromone component ratio emitted by males may differ according to age. However, pheromone emission may not coincide with the pheromone production, we investigated the actual pheromone emission from individual males with the glass-beaker method. Repeated volatile collections of 10 males over a period of 15 consecutive days showed that the average amount of β -sesquiphellandrene emitted by males was less than that of the other two components until day 9 and thereafter pheromone emission of β -sesquiphellandrene increased and more than the others. These results agree well with those of pheromone production, indicating that the pheromone emission ratio of *P. hybneri*, especially proportion of β -sesquiphellandrene changes according to adult age. This change may influence the functions of the pheromone.

Key words: *Piezodorus hybneri*, pheromone ratio, adult age, soybean bug

P-126 Components of the Androconial Secretion of a Danaid Butterfly, *Ideopsis similis* (Lepidoptera: Danaidae): Their Origin and Sex-pheromonal Activity

Wataru Yagi^a, Keiichi Honda^a, Hisashi Ômura^a, and Hiroshi Honda^b

^a Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima 739-8528, Japan

^b Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan
honce@hiroshima-u.ac.jp (K. Honda)

Male adults of *Ideopsis similis* have two types of scent-producing organs (androconia), *i.e.* a pair of abdominal hairbrushes (hairpencil) and alar patch glands (sex brand). We have previously reported that wild males secrete hydroxydanaidal (**1**) from the hairpencils, and danaidone (**2**) from the sex brands. Upon further investigation of the androconial secretion of field-caught males, we have newly identified a trace amount of viridiflorine β -lactone (**3**) from the sex brand. Since laboratory-reared males entirely lacked all these compounds, which were suspected to be derived from pyrrolizidine alkaloids (PAs) acquired as an adult, oral administration tests with indoor males were conducted of five PAs (a 4 : 1 mixture of intermedine/lycopsamine (I/L), heliotrine, monocrotaline, retronecine, and heliotridine). While only males fed with I/L secreted all the components (**1-3**), those fed with retronecine secreted **1** only and other PAs afforded none of them. Feeding responses to the PAs triggered by stimulating tarsi and/or proboscis also revealed that I/L had the highest feeding-stimulatory activity. These results indicate that *I. similis* males depend on particular PA(s) for the efficient biosynthesis of secretion components. Behavioral experiments in an outdoor cage to test the activity of the androconial secretion as the sex pheromone showed that males fed I/L mated far more successfully than unfed males (43 : 16). The results, combined with GC-EAG data of the secretion, indicate that the three compounds (**1-3**) serve as the male sex pheromones of *I. similis*. These findings will be discussed in relation to the aphrodisiac of other danaid butterflies.

Key words: androconial secretion, *Ideopsis similis*, pyrrolizidine alkaloids, biosynthesis, sex pheromone

P-127 Male Hair-Pencil Volatiles and their Functions For Reproductive Isolation in Sympatric Sibling Pyralid Moths

Hiroshi Honda, Takayoshi Kimura and Yuko Yoshihara

Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8572, Japan.

hhonda@sakura.cc.tsukuba.ac.jp

The yellow peach moth *Conogethes punctiferalis* GUENEE, *Conogethes* sp., the cotton leaf-roller *Notarcha derogate* FAB and *N. basipunctalis* BREM are well known as morphologically similar sympatric pyralid pests in Japan. The two species of *Conogethes* have the same sex-pheromone system, (*E*)-10-16 : Ald and (*Z*)-10-16 : Ald at ratios of 95.5 and 4.5, resulting in a high cross-attraction among male moths. However, interspecific mating rarely occurs between these species. The male hair-pencil structures are also very similar, but volatile tiglic acid has only been found in *C. punctiferalis*. GC-EAD analyses revealed high responsiveness to tiglic acid in female antennal receptors of both species. In behavioral assays, mating success of male *C. punctiferalis* significantly decreased when the hair-pencils were surgically removed or chemically modified. The female sex pheromones of *N. derogate* and *N. basipunctalis* have been shown to consist of (*Z,E*)-10,12-16 : Ald and (*E,E*)-10,12-16 : Ald at significantly different ratios between the two species. Males of both species have developed very similar hair-pencils, but they release different volatile chemicals: β -ocimene by *N. derogate* and benzaldehyde and 3-octanone by *N. basipunctalis*. In addition, surgical and chemical modification of the hair-pencils led to significant reduction in male mating success for both species. GC-EAD analyses showed that female antennal receptors are responsive to these volatiles. These results reveal that highly sympatric sibling species that have the same or very similar female sex-pheromone systems may depend on the male hair-pencil volatiles to make their final decision for reception of mating partners. Male hair-pencil volatiles may function as allelochemicals as well as pheromones.

Key words: hair-pencil, volatiles, sibling species, reproductive isolation, pheromones, allelochemicals

P-128 Synergistic Lure Effect of Crude Extraction from Cracked Wheat and Insect Pheromone on Stored Product Insects and Analysis its Chemical Compounds

Yujie Lu, Xingkui Li and Jianfeng Zhong

Food and oil college, Henan University of Technology Henan Province, Zhengzhou, 450052 China

Luyjhaut@yahoo.com.cn

Plant volatiles could influence insect behaviors such as finding host plant, feeding and finding fitting oviposition position. Plant volatiles have significant synergistic effect with insect pheromone. The synergistic interaction of crude extraction from cracked wheat and major stored grain pest pheromones were studied in this paper and the chemical compounds were analyzed by GC and GC-MS. The trapping effects of various cracked wheat within 24 hours in different amounts and granularities were compared by bioassay in laboratory. The result showed that the variety, granularity and amount of wheat being used all, to some extent, affect the trapping effect. Wenmai No. 6 claims optimal effect of the total while Yumai No. 70 is the worst. The experiments of combining extracts of cracked wheat with insect pheromones in the ratio of 1 : 1 and trapping various insects show that a combination can not only enhance the trapping effects on insects of same kind, but also have good effects on other insects. By the analysis of GC-MS, there are n-hexadecanoic acid, (*Z,Z*)-9,12-octadecadienoic acid and n-icosane, and each of them has its particular components and the relative percentages of chemicals in the extracts are greatly different with (*Z,Z*) 9,12-octadecadienoic acid and n-hexadecanoic acid taking up 70.23% and 18.82% respectively in Wenmai No. 6 as against 40.80% and 32.11% in Yumai No. 70.

Key words: synergistic lure effect, cracked wheat, insect pheromone, chemical compounds

P-129 Search for Host-Plant Volatiles from Young Peach Shoots Attractive for Oriental Fruit Moth *Grapholita molesta* Busck (Lepidoptera: Tortricidae)

Alexandre L. IL'Ichev^a, Soichi Kugimiya^b, Junji Takabayashi^c and David G. Williams^a

^a Primary Industries Research Victoria, Department of Primary Industries, Tatura Centre, Victoria, 3616, Australia

^b Biodiversity Division, National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604, Japan

^c Center for Ecological Research, Kyoto University, Otsu, 520-2113, Shiga, Japan

alex.ilichev@dpi.vic.gov.au

Oriental fruit moth *Grapholita molesta* Busck (Lepidoptera: Tortricidae) (OFM) is one of the most important pest of commercial fruit orchards worldwide. Stone fruit are considered to be the primary OFM host-plants. Newly planted peach trees in particular, can be very attractive for mated OFM females for oviposition, where initially OFM larvae damage young shoot tips. Samples of air-borne host-plant volatiles from intact (1–2 cm long) young peach shoot tips vs. older leaves of the same potted plants were collected by passive absorption into special devices “Twisters”. More than 20 chemical components were identified by GS-MS from all air-borne samples including aliphatic and aromatic esters, aldehydes, monoterpene, homomonoterpene and sesquiterpenes. The most promising chemicals present in young shoot tips were determined by discounting any chemicals that were also present in older leaves. Different doses of identified chemicals were tested individually and in various combinations in replicated field trials during OFM flights. Moth capture in traps with synthetic mixtures was compared to that of the standard TA food trap (terpinyl acetate and fermenting brown sugar solution) for OFM and butyl hexanoate. A 1 mg dose of 1 : 2 : 2 mixture of Z-3-hexenyl acetate, ocimene and farnesene combined with 10 mg of butyl hexanoate was the most attractive combination (c.a. 72.88 OFM/trap/week), followed by a 100 mg of 1 : 2 : 2 mixture of Z-3-hexenyl acetate, ocimene and farnesene without butyl hexanoate (c.a. 45.75 OFM/trap/week). Butyl hexanoate alone was not very attractive for OFM at all (c.a. 4.88 OFM/trap/week), when TA food trap caught c.a. 43.88 OFM/trap/week. Unfortunately all mixtures and combinations of chemicals attracted only OFM males when TA food trap attracted also mated OFM females. The possible use of identified attractants for OFM monitoring will be discussed.

Key words: semiochemicals, host-plant attractants, oriental fruit moth, bioassay

P-130 Intraspecific Communication in the White-spotted Longicorn Beetle by Host Plant Chemicals

Hiroe Yasui^a, Tetsuya Yasuda^a, Midori Fukaya^a, Toshiharu Akino^a, Sadao Wakamura^a, and Hiroshi Ono^b

^a National Institute of Agrobiological Sciences, 1-2, Ohwashi Tsukuba 305-0851, Japan

^b National Food Research Institute, Kannondai, Tsukuba 305-8642, Japan.

yasui@affrc.go.jp

The white-spotted longicorn beetle *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae: Lamiinae) is a serious pest of willow and horticultural crops such as citrus, apple, pear, and maple, and mating behavior has been intensively studied in the beetles fed on citrus. The elytra extract of female beetles fed on *Citrus unshiu* bark evoked male mate location before direct contact, but that fed on an artificial diet containing no citrus did not. The active volatiles were found both in the elytra extracts and in *C. unshiu* bark extracts. The volatiles were isolated by preparative GC, analyzed by NMR and identified to be sesquiterpene hydrocarbons; β -elemene, β -caryophyllene, α -humulene, and α -farnesene. Those sesquiterpenes in the elytra should be acquired from *C. unshiu* by feeding, contact, and/or adsorption. The sesquiterpenes cause not only male but also female mate location, and evoke the long- and short-distance mate location in this species. Therefore, it is considered that the sesquiterpenes serve as chemical signals for intra-specific communication on this species.

Key words: mate location, intraspecific communication, host plant chemicals, sesquiterpenes

P-131 Comparative Chemical Ecology of Volatile Components Emitted from Labial Glands of Male Bumblebees (*Bombus* spp.)

Ryohei Kubo and Masato Ono

Unit of Insect Technology and Sociochemistry, Division of Applied Entomology and Zoology, Graduate School of Agriculture, Tamagawa University, Machida, Tokyo 194-8610, Japan

We investigated the labial gland secretions from males of eight Japanese bumblebee species and one allopatric bumblebee species (*Bombus terrestris*). Volatile chemicals from crushed labial glands were captured by SPME and analyzed by GC/MS. Ethyl dodecanoate (*B. hypocrita hypocrita* and *B. h. sapporoensis*); 2,3-Dihydrofarnesal and 2,3-Dihydrofarnesol (*B. ignitus*); Citronellol (*B. ardens ardens*); and *E,E*-Farnesol (*B. diversus diversus* and *B. d. tersatus*) were detected. Major components differed between species. The data strongly suggest that these chemicals aid reproductive isolation between closely related sympatric species.

However, Ethyl dodecanoate, 2,3-Dihydrofarnesal and 2,3-Dihydrofarnesol were also detected from the labial glands of the allopatric bumblebee species *B. terrestris*. These components are the same as the volatile chemicals detected from *B. hypocrita hypocrita*, *B. h. sapporoensis* and *B. ignitus*, suggesting a risk of crossbreeding between sympatric and allopatric bumblebees.

Key words: Male bumblebee, labial gland, sex pheromone, reproductive isolation, SPME

P-132 Gorse Pathogenic Fungus, *Fusarium tumidum* Uptake and Carry by *Epiphyas postvittana* (Lepidoptera: Tortricidae)

Alvin KW Hee^{a, b}, Emmanuel Yamoah^b, Monika Walter^a, E. Eirian, Jones^b, Graeme W. Bourdot^c, David M. Suckling^a and Alison Stewart^b

^a HortResearch, PO Box 51, Lincoln 7640, Canterbury, New Zealand

^b National Centre for Advanced Bio-Protection Technologies, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand

^c AgResearch, PO Box 60, Lincoln 7640, Canterbury, New Zealand

alvin_hee@yahoo.com

Gorse (*Ulex europaeus*) (Fabaceae) is a highly invasive weed that occurs widely in North America, Hawaii, Chile, Australia and New Zealand. Several gorse-associated insect herbivores such as the seed weevil (*Exapion ulicis*), thrip (*Sericothrips staphylinus*) and tortricid moths (*Cydia succedana* and *Epiphyas postvittana*) were investigated for their ability to pick up *Fusarium tumidum*, a naturally occurring gorse pathogenic fungus. In laboratory agar assays, *E. postvittana* demonstrated greater capacity for *F. tumidum* pick up following exposure to the fungus and thus, it was selected to be evaluated for its conidia-carrying ability in trials using pheromone attractants. In wind tunnel assays, male moths that had flown to two different media containing *F. tumidum*, respectively showed that, upon initial landing, the conidia uptake from a conidia-lined moist filter paper was significantly higher compared with inocula grown on an oatmeal agar. Field cage results also revealed that male moths that had visited the pheromone baited-station containing fluorescent dye (as conidia surrogate) picked up significantly more dye on their ventral abdomen compared with other parts such as the legs, thoraces and wings. Wind tunnel assays further showed that at a range of different conidia loadings (750–350,000 conidia), moths shed a high proportion of the initial loadings (87–99%) from the time of inoculation followed by first flight to, and landing on a pheromone source. This study demonstrates that although conidia loss is high following inoculation on the moths, it is still possible for moths to carry and shed the conidia during flight. These results are discussed in the light of developing an attractant-based system to lure insects to fungal inoculum for spore pick up, thereafter dispersing and infecting susceptible host weeds that offer a novel concept to augment existing weed biocontrol strategies.

Key words: *Epiphyas postvittana*, *Fusarium tumidum*, *Ulex europaeus*, pheromone, weed biocontrol

P-133 Control of the Cherry Tree Borer, *Synanthedon hector*, Occurring on a Steep Slope by Means of Mating Disruption with a Synthetic Sex Attractants

Kazuma Matsumoto^a, Kiyoshi Nakamuta^b and Tadakazu Nakashima^b

^a Tama Forest Science Garden, Forestry and Forest Products Research Institute, 1833 Todoricho, Hachioji, Tokyo 193-0843 Japan

^b Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba 305-8687, Japan
kazuma@fpri.affrc.go.jp

Cherry Tree Forest of Tama Forest Science Garden, Hachioji, Tokyo, has been suffering attacks by the cherry tree borer, *Synanthedon hector* (Butler). A mixture of (*Z,Z*)-3,13-octadecadienyl acetate and (*E,Z*)-3, 13-octadecadienyl acetate is known to be a potent sex attractant for the male moth of *S. hector*, and the sex attractant (Sukashiba-kon[®], Shin-Etsu Chemical Co. Ltd., Tokyo) has been used for practical control of *S. hector* in orchards of Japanese by mating disruption. However, the Cherry Tree Forest is on steep slopes where mating disruption may be inconsistent because pheromones would likely be diluted by the wind or the pheromone plume may fall to the lower part of the slope and would not be able to cover the entire area of the forest. To test the efficiency of the method on slopes, we conducted the mating disruption for six years. No male adult moths have been captured in traps baited with the synthetic attractant since the commencement of the control. The incidence of attack decreased to a low level in the third year and has remained low thereafter, indicating the effectiveness of mating disruption in controlling the borer population.

Key words: pheromones, mating disruption, *Synanthedon hector*, cherry tree, slope

P-134 Mating Disruption of the Carpenter Moth, *Cossus insularis*

Tomoaki Nakanishi^a, Kiyoshi Nakamuta^b and Fumiaki Mochizuki^c

^a Fruit Tree Research Institute, Tokushima Agriculture, Forestry and Fisheries Technology Support Center, Nue, Katuura, Tokushima 771-4301, Japan

^b Forestry & Forest Products Research Institute, Tsukuba 305-8687, Japan; ^c Shin-Etsu Chemical Co., Ltd., Specialty Chemicals Research Center, 28-1, Nishifukushima, Kubiki-ku, Joetsu, Niigata 942-8601, Japan

The carpenter moth, *Cossus insularis* (Staudinger) (Lepidoptera: Cossidae) is found in Honshu, Kyushu and Tsushima Island of Japan. Although host plants of this species have not been well known, it causes damages to fruit trees and broad-leaved trees such as apple, pear, willows, etc. The first incidence of *C. insularis* larvae in Japanese pear was recorded recently. Aggregations of larvae were observed to bore into woody stems, causing significant damage and frequent mortality of the trees. Sex pheromone of *C. insularis* is identified as a mixture of *E*3-14: Ac and *Z*3-14: Ac and the synthetic *E*3-14: Ac attracts lots of male moths. We, therefore, tested the effect of mating disruption on the mating of this species and the damage of pears in a Japanese pear orchard in Tokushima prefecture, Japan. Mating disruption was conducted for three years since 2004. Only a few male adult moths have been captured in traps baited with synthetic sex pheromone in pheromone-treated orchards since the commencement of the treatment, although many male moths have been captured in untreated orchards. When tethered females were placed in a treated orchard, no female mated during one night, although about 50% of tethered females mated in an untreated orchard, demonstrating that mating was disrupted by synthetic pheromones permeated into the environment. The incidence of attack decreased in 2006 in a treated orchard, while it increased in a control orchard, indicating the effectiveness of mating disruption in controlling the borer population.

P-135 Mating Disruption of the Persimmon Fruit Moth, *Stathmopoda masinissa*, by the Synthetic Sex Pheromone

Toshiro Suzuki^a, Tetsu Ando^b, Masanobu Yamamoto^b, Hideshi Naka^c, Koji Tsuchida^d, Fumiaki Mochizuki^e and Takehiko Fukumoto^e

^a Agricultural Technology Institute of Gifu Prefecture, Gifu, Gifu 501-1152, Japan

^b BASE, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

^c JT Biohistory Research Hall, 1-1 Murasaki-cho, Takatsuki, Osaka 569-1125 Japan

^d Laboratory of Insect Ecology, Faculty of Applied Biological Sciences, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan

^e Specialty Chemical Research Center, Shin-Etsu Chemical Co. Ltd., Kubiki, Niigata 942-8601, Japan
suzuki-toshiro@pref.gifu.lg.jp

The persimmon fruit moth, *Stathmopoda masinissa* (Oecophoridae), is a well-known harmful pest of persimmon fruits in Japan. We identified the sex pheromone of *S. masinissa* females and developed a method for the mating disruption. GC-EAD and GC-MS analyses found three EAG active components with a 4,6-diene system in a C₁₆ chain; i.e. (4*E*,6*Z*)-4,6-hexadecadienyl acetate (E4,Z6-16:OAc) and the corresponding derivatives, aldehyde (E4,Z6-16:Ald) and alcohol (E4,Z6-16:OH). Meanwhile the role of E4,Z6-16:OH in the mating communication has not been clarified, lures baited with a mixture of E4,Z6-16:OAc and E4,Z6-16:Ald (10:1) in fields. The number of males attracted to both artificial pheromone lures and tethered virgin females were strongly decreased in the fields with mass-application of the sex pheromone, comparing to the control fields. Consequently, we successfully decreased the damage of the fruits using the sex pheromone.

Key words: persimmon fruit moth, sex pheromone, mating disruption, disruptant, pest control

P-136 Resistance to Mating Disruption in the Smaller Tea Tortrix, *Adoxophyes honmai* Yasuda

Jun Tabata^a, Hiroshi Noguchi^a, Fumiaki Mochizuki^b, Yooichi Kainoh^c, and Hajime Sugie^a

^a National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai Tsukuba 305-8604, Japan

^b Shin-Etsu Chemical Co. Ltd., 28-1 Nishifukushima Kubiki-mura Nakakubiki-gun Niigata Pref. 942-8601, Japan

^c University of Tsukuba, 1-1-1 Tennohdai Tsukuba 305-8572, Japan

jtabata@affrc.go.jp

Mating disruption is an environmentally safe plant protection strategy that uses a synthetic copy of an insect pheromone to interfere with sexual communication and hence reproduction. Recently, the first example of resistance to mating disruption was documented in one of the major tea pests in Japan, the smaller tea leafroller moth, *Adoxophyes honmai* Yasuda. To avoid other such cases, it is important to elucidate the mechanism(s) by which the disruptant lost its effectiveness. To this end, we imposed further selection by rearing field-collected resistant insects with a synthetic pheromone in the laboratory. After more than 70 generations of selection, a strain with quite strong resistance was established, males of which could find and copulate with their mates even in the presence of 1 mg/l of disruptant. In this strain, male response to the pheromone blend was markedly broadened so that resistant males could locate a synthetic pheromone source even when it lacked a pheromone component that is normally necessary for attraction. Males capable of locking onto off-ratio pheromone blends may be better able to find calling females in pheromone-treated environments than narrowly-tuned males because of greater capability of overcoming sensory imbalance.

Key words: mating disruption, resistance, sensory imbalance, asymmetric tracking

P-137 Some Information About the Sex Pheromone Trap of the Japanese Mealybug, *Planococcus kraunhiae* (Kuwana)

Yutaka Narai^a, Nobuo Sawamura^a and Hajime Sugie^b

^a Shimane Agricultural Technology Center, 2440 Ashiwata, Izumo, shimane 693-0035, Japan

^b National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan
narai-yutaka@pref.shimane.lg.jp

The Japanese mealybug, *Planococcus kraunhiae* (Kuwana), is a euryphagous pest of fruit trees. Recently, its sex pheromone component was isolated and identified. In the present study, we report on the form of sex pheromone trap for monitoring the seasonal prevalence of occurrence and the setting angle of sticky paper board for catching the pest males. We examined seven kinds of sticky traps from May to June 2006 in a persimmon field. From the result, we concluded that five of the commercially available traps, which had openings to the outside in two or four directions, could be utilized for monitoring. Furthermore, we evaluated the relationship between the number of males caught in the traps and the setting angle of sticky board. The setting angle of sticky board, 0, 45, 90, 135 and 180 degrees were examined from Nov. to Dec. 2006 in a greenhouse. Although the ranking of the caught male number at each setting angle wasn't significant by Freidman test at 5% levels, we observed that the ranking of the caught male number tended to increase as the angle became larger. And we also observed that more males were caught on the upper part of a sticky board from the setting point of the lure when the angles of sticky boards were 45, 90 and 135 degrees. Attraction of males to the lure was observed. In the case of 0 degrees angle (horizontally), males landed on the lure but didn't in the case of other angles. The males may directly approach to the lure from above the lure, although they don't fly straightly. These results will be useful for making monitoring traps of the Japanese mealybug males.

Key words: *Planococcus kraunhiae*, sex pheromone, monitoring trap, behavior of male

P-138 Pheromone Trap Monitoring of San Jose Scale *Quadraspidiotus perniciosus* Adult Males and Prediction of Crawler Occurrence

Tomonori Arai^a, Osamu Narita^b and Fumio Ihara^a

^a Apple Research Station, NIFTS, 92-24 Nabeyashiki, Shimokuriyagawa, Morioka, Iwate 020-0123, Japan

^b Kennan Fruit Tree Research Center, Apple Experiment Station Aomori Prefectural Agriculture and Forestry Research Center, Ogita, Gonohe, Sannohe-gun, Aomori 039-1527, Japan
gaityuu@affrc.go.jp

Accumulated temperature required for attaining the peak of San Jose scale adult male emergence and crawler occurrence were investigated from 2004 to 2006 in an apple orchard of Morioka in Iwate Prefecture. Adult male emergence of the overwintering and the first generation peaked when accumulated temperature reached from 80 to 154 (126 on average) and from 789 to 831 (815 on average) degree days above 10.5°C from March 1, respectively. When accumulated temperature reached from 302 to 305 (303 on average) degree-days above 10.5°C from the peak of adult emergence of the overwintering generation, crawler occurrence of the first generation peaked. Crawler occurrence of the second generation peaked when accumulated temperature reached from 296 to 393 (340 on average) degree-days above 10.5°C from the peak of adult emergence of the first generation. We will show the result of prediction of crawler occurrence in an apple orchard from degree days, peak of adult male emergence, and temperature in 2007.

Key words: *Quadraspidiotus perniciosus*, pheromone trap, crawler occurrence, degree-days, apple

P-139 Detect Propagative Stage juveniles of *Bursaphelenchus xylophilus* by a Trapping Tube

Lilin Zhao, Wei Wei and Jianghua Sun

State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, the Chinese Academy of Sciences, Beijing 100080, P. R. China

sunjh@ioz.ac.cn

The pinewood nematode, *Bursaphelenchus xylophilus*, is the causal agent of the destructive pine wilt disease. It has caused irreparable damage to forested ecosystems in China, South Korea and Japan. The quarantine and population monitoring of nematode are important in the forest detection. The traditional sampling method is removing a larger piece of sapwood from the trunk or branches with a hatchet in wilted or killed pine and the nematodes are extracted by the Baermann-funnel extract techniques. The process is laborious, not rapid and injures or alters the plant product, which delay the detection of pinewood nematodes. We applied the chemotaxis of pinewood nematode to developing a new rapid sampling method. The results indicated that a trapping tube with attractants could effectively capture propagative pinewood nematodes from symptomatic pines. The chemical attraction technique is selective, simple, effective and rapid and should assist greatly identification efforts of pinewood nematodes by quarantine officials at field detection.

Key words: *Bursaphelenchus xylophilus*, attractants, sampling, population monitoring

P-140 Monitoring of the Cabbage Looper, *Trichoplusia ni*, Using a Pheromone Trap in Japan

Hajime Sugie^a, Tadasi Matsunaga^b and Junya Yase^c

^a National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba, 305-8604, Japan

^b Sankei Chemical CO., LTD, 2-9 Nanei, Kagoshima, 891-0122, Japan

^c Hyogo Agricultural Research Center, 1533 Minamino-oka Kou, Befu, Kasai, 679-0198, Japan

hsugie@affrc.go.jp

The cabbage looper has not been recognized to be one of serious pests in vegetable fields in Japan. At autumn in 2000, Yase collected many larvae in a cabbage field at Hyogo Prefecture and reared them to identify species. Unexpectedly, they are the cabbage looper. After this year, occurrence of this species was reported from many places in southern part of Japan. The sex pheromone components of the cabbage looper have been already reported to be Z7-12:Ac, Z5-12:Ac, 12:Ac, 11-12:Ac, Z7-14:Ac, and Z9-14:Ac (1000, 77, 47, 29, 4, 3 μg, respectively). We evaluated the necessity of these compounds in a field at Kagoshima Prefecture to make an efficient lure for the cabbage looper distributed in Japan. Six-component lure or one of six kinds of five-component lure was attached to a corn trap or a funnel trap. A five-component lure without Z7-12:Ac attracted small number of males (6% of that of the six-component lure). Five-component Lures without one of Z5-12:Ac and 12:Ac attracted smaller number of males than the six-component lure (41%, 37%, respectively). The caught numbers by five-component lures without one of 11-12:Ac, Z7-14:Ac and Z9-14:Ac were more than half of that of the six-component lure (54%, 62%, 55%, respectively). As the necessity of these compounds was confirmed, we decided to use the six-component lure for monitoring. Now, the occurrence of the cabbage looper can be successively monitored by using the pheromone trap equipped with the lure.

Key words: sex pheromone, monitoring, *Trichoplusia ni*, cabbage looper, trap

P-141 Monitoring and Mating Disruption Using the Sex Pheromone of the Rice Leaf Bug, *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae)

Masashi Kakizaki

Hokkaido Dohnan Agricultural Experiment Station, 680 Hon-cho, Hokuto 041-1201, Japan

kakizams@agri.pref.hokkaido.jp

The rice leaf bug, *Trigonotylus caelestialium*, is distributed in Japan, China, Japan, Europe, Russia, and North America. This bug is one of major pests causing a pecky rice, which deteriorates a grade of rice, and also injure wheat, maize, and gramineous forage grasses in the northern part of Japan. *T. caelestialium* females attract conspecific males, and the female sex pheromone was identified as a mixture of *n*-hexyl *n*-hexanoate, (*E*)-2-hexenyl *n*-hexanoate, and *n*-octyl *n*-butyrate in the ratio of 100 : 40 : 3 (Kakizaki and Sugie, 2001). The sex pheromone is useful to monitor and to control a population in an IPM program in this plant bug. (1) Monitoring: A 'Sticky Net Cylinder (SNC) trap' (5-mm mesh black colored net, 6 cm diam. and 30 cm long, set vertically on the ground, lure at 30 cm ht), which is able to capture 3.8 to 5.7 times more males than the water-pan trap, was used for monitoring. This sex pheromone trap captured 2 to 5 times more males than net sweeping method (20 times sweeping), and was able to investigate clearly seasonal occurrences of *T. caelestialium* adults in a paddy fields. Then, a control threshold for protecting a pecky rice would be established by a number captured by the sex pheromone trap. (2) Mating Disruption: In Italian rye grass field experiments, which were treated 200 dispensers each containing 300 mg of the 3-component sex pheromone in an area of 10,000 m², the male catches by traps in the treated fields reduced to 15.1–3.5% of those in the untreated fields, and the population densities in the treated fields reduced to 45.8–46.7% (adults) and 0–8.7% (nymphs) of those in the untreated fields (Kakizaki, 2004). However, a control effect by the 3-component sex pheromone is not enough in paddy field, it might be necessary an addition of new sex pheromone component.

P-142 Foraging Disruption of the Argentine Ant (Hymenoptera: Formicidae) by Synthetic Trail Pheromone: Potential Control Strategy of Pest Ants

Eiriki Sunamura^a, Koji Nishisue^a, Yasutoshi Tanaka^a, Hironori Sakamoto^b, Mamoru Terayama^a, Takehiko Fukumoto^c and Sadahiro Tatsuki^a

^a The University of Tokyo, Tokyo 113-8657, Japan

^b National Institute for Agro-Environmental Sciences, Ibaraki 305-8604, Japan

^c Shin-Etsu Chemical Co., Ltd., Tokyo 100-0004, Japan

aa66011@mail.ecc.u-tokyo.ac.jp

The control of pest ants has been conducted mainly with insecticides or baits with toxicants. Here we present a novel potential strategy to control them that exploits their trail pheromones. Following the concept of mating disruption, we hypothesized that high concentration of synthetic trail pheromone would disrupt the trails of target ant species, leading to its reduced foraging efficiency. The Argentine ant, *Linepithema humile*, is one of the world's most notorious invasive species. No one strategy has proven entirely successful in controlling it. The species uses Z9-16 : Ald as the major component of its trail pheromone. Utilizing synthetic Z9-16 : Ald, we obtained three lines of evidence that support the above hypothesis. First, Argentine ants did not walk efficiently on artificial trails drawn with excess concentrations of Z9-16 : Ald. Second, natural trails of Argentine ants were dramatically disturbed when polyethylene tube dispensers (Shin-Etsu Chemical Co., Ltd.) evaporating Z9-16 : Ald at a steady rate were placed nearby. Third, short-term field treatment of the dispensers decreased the Argentine ant recruitment to baits. Our study suggests that long-term, as well as areawide treatment of Z9-16 : Ald might make Argentine ants short of foods and therefore decrease their densities.

Key words: Argentine ant, *Linepithema humile*, control, trail pheromone, disruption

P-143 Female's Specific Gustatory Perception of the Nuptial Gift in the German Cockroach
Ayako Wada-Katsumata^a, Mamiko Ozaki^b and Ritsuo Nishida^a

^a Lab of Chemical Ecology, Grad. Sch. Agric., Kyoto Univ., Kyoto 606-8502, Japan

^b Dept. Biol., Grad. Sch. Sci., Kobe Univ., Kobe, 657-8501, Japan

awada@kais.kyoto-u.ac.jp

A Nuptial food gift given by a male to a female is an integral trait of the mating systems in a wide variety of insects. Males of the German cockroach, *Blattella germanica*, use a glandular gift in its sequential courtship behavior. The female's feeding on the secretions from male's eighth tergal glands (TG-8) triggers the extrusion of male's genitalia. Thus, the nuptial feeding is an indispensable step for the successful copulation.

We investigated the gustatory effects of TG-8 extracts and its major ingredients, maltotriose (MT) and phosphatidylcholine (PC) on the behavioral or the electrophysiological response in both sexes. As a result, TG-8 extracts induced significantly higher feeding response in females than males. A gustatory sensillum, when stimulated with TG-8 extracts, showed three types of impulses in both sexes, but the sensillar responsiveness was significantly higher in female than male, regardless of impulse types. MT also induced three types of impulses from the sensillum, and the sensillar responsiveness was no significant difference between the sexes. In many cases, PC elicited no response in both sexes. However, the sensillum, when stimulated with mixtures of MT and PC, showed significantly higher response in female than male.

These results suggest that there is sexual difference in the chemosensory mechanism to perceive a mixture of MT and PC through the gustatory sensillum. Female's specific gustatory perception regulated by the synergistic effect of PC on MT may be one of the crucial factors to induce a nuptial feeding in its courtship behavior.

Key words: electrophysiology, cockroach, nuptial gifts, gustatory perception, phagostimulants

P-144 Odor Receptor Swap between Two Sensory Neurons Reverses Male Moth Preference for Pheromone Blend

Teun Dekker^a, Zsolt Karpati^{a, b} and Bill Hansson^{a, c}

^a Division of Chemical Ecology, Swedish University of Agricultural Sciences, PO Box 44, SE-230 53, Sweden

^b Plant Protection Institute of Hungarian Academy of Sciences, PO Box 102, H-1525, Budapest, Hungary

^c Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology

Hans-Knoell-Strasse 8, D-07745 Jena, Germany

The European corn borer *Ostrinia nubilalis* (Hubner) is a model of evolution of sexual communication in insects. Two pheromone strains produce and respond to opposite ratios of the two pheromone components, E11 and Z11-tetradecenylacetate. The E-strain uses a ratio of 99 : 1 of E11 : Z11 tetradecenylacetate, whereas the Z-strain uses a ratio of 3 : 97. Extensive physiological and morphological analyses of the corn borer's primary olfactory relay center, the antennal lobe revealed a reversed innervation pattern by neurons detecting and relaying pheromone component information. The data are thus coherent for both antennal lobe input and output. The most parsimonious explanation for this single-gene mediated reversed sensitivity is a swap of olfactory receptors (ORs) in the olfactory receptor neurons (ORNs), which co-inhabit the same sensillum. Such 'simple' swaps could represent a route of olfactory specialization and evolution unique to insects.

P-145 Characterization of Sex Pheromone Receptor Genes Isolated from Four Moth Species
**Hidefumi Mitsuno^a, Takeshi Sakurai^a, Masatoshi Ichida^b, Tetsuya Yasuda^c, Soichi Kugimiya^d,
Rika Ozawa^e, Junji Takabayashi^e and Takaaki Nishioka^f**

^a Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo 153-8904, Japan

^b Center for Bioresource Field Science, Kyoto Institute of Technology, Kyoto 616-8354, Japan

^c National Agricultural Research Center, Ibaraki 305-8666, Japan

^d National Institute for Agro-Environmental Sciences, Ibaraki 305-8604, Japan

^e Center for Ecological Research, Kyoto University, Shiga 520-2113, Japan

^f Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
mitsuno@brain.imi.i.u-tokyo.ac.jp

Although sex pheromones have been identified in various insect species, their receptors have not been identified except silkworm. Here we report on the cloning and characterization of sex pheromone receptor genes from *Plutella xylostella*, *Samia cynthia*, *Mythimna separata*, and *Diaphania indica*. By using degenerate primers based on the sex pheromone receptor genes of *B. mori* we cloned one candidate sex pheromone receptor gene, *OR1*, from each moth species. Double-labeling *in situ* hybridization with pheromone binding protein (PBP) RNA probes showed that OR1 expressing cells in male moths are surrounded by PBP expressing cells, confirming that OR1s are expressed in the olfactory receptor neurons that are responsible for the sex pheromone reception. In addition, the *Xenopus* oocytes expressing OR1 with an OR83b ortholog specifically and dose-dependently responded to only one of the sex pheromone components of the species that *OR1* was isolated from. Sequence analysis of the OR1s suggests that sex pheromone receptors of Lepidoptera species might have randomly evolved from several ancestral odorant receptors expressing only in the male moths.

Key words: sex pheromone, olfactory receptors, *Xenopus oocyte*, evolution

**P-146 Axonal Projections of Pheromone Receptor Neurons to the Antennal Lobe
Macroglomerular Complex in the Silkworm, *Bombyx mori***

Takeshi Sakurai^a, Keiro Uchino^b, Hideki Sezutsu^b, Toshiki Tamura^b and Ryohei Kanzaki^a

^a Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku,
Tokyo 153-8904, Japan

^b National Institute of Agrobiological Sciences, 1-2 Owashi, Tsukuba, 305-8634, Japan
sakurai@brain.imi.i.u-tokyo.ac.jp

Pheromonal information detected by pheromone receptor neurons in the antennae of male moth is transmitted to the macroglomerular complex (MGC) in the antennal lobe. The *Bombyx mori* MGC consists of three subdivisions the toroid, cumulus, and horseshoe. In the present study, we aimed to clarify how the input of pheromonal information is represented in the *B. mori* MGC. In *B. mori*, BmOR1 and BmOR3 encode the pheromone receptors for bombykol and bombykal, respectively. To trace axonal projection of bombykol and bombykal receptor neurons separately, we used transgenic moths that express green fluorescent protein (GFP) under the control of putative promoters of BmOR1 or BmOR3. In the male antennae of the both lines, GFP fluorescence was detected in receptor neurons that innervated pheromone sensitive trichoid sensilla. Confocal microscopic analyses combined with anti-GFP antibody staining of the brain revealed that almost all BmOR1 expressing neurons projected to the toroid, while BmOR3 expressing neurons to the cumulus, indicating that bombykol and bombykal information is conveyed exclusively to toroid and cumulus, respectively. This discrete axonal projection will provide a basis of segregated processing pathway between bombykol and bombykal in the silkworm brain. Supported by JSPS (Scientific Research (B) 18370028).

Key words: *Bombyx mori*, pheromone receptor, antennal lobe, macroglomerular complex, transgenic silkworm

P-147 Morphological Investigation of Aggressive Center in the Antennal Lobe of *Camponotus japonicus*

Kentaro Ishiura^a, Yuji Satoji^b, Hitoshi Aonuma^c and Mamiko Ozaki^a

^a Kobe University, 1-1 Rokkodaicho, Nada, Kobe 657-8501, Japan

^b Kyoto Institute of Technology, Matsugasaki-Hashigamicho, Sakyo, Kyoto 606-8585, Japan

^c Hokkaido University Research Institute for Electron Science, Kita 12 Nishi 6, Sapporo 060-0812, Japan
ishiura@stu.kobe-u.ac.jp

It is important for ants to recognize the congeneric encounterers as nestmates or non-nestmates. In *Camponotus japonicus* 18 cuticular hydrocarbons (CHCs) were identified and the CHC blend pattern is colony-specific to act as nestmate recognition factor. CHC sensilla on the antennae respond only to non-nestmate CHCs but not to nestmate CHCs, so that the ants aggressively reject non-nestmate. Thus, the projection region of sensory nerves in the CHC sensilla is considered to be primary center of the nestmate recognition and aggressive behavior. We conducted the anterograde staining from antenna, and counted 433 glomeruli in an antennal lobe stained. When we followed the sensory nerves in a single CHC sensillum, 136 glomeruli in the ventro-median (VM) region were stained, although, when we followed other sensilla, other than VM region was stained. We also investigated the localization of nitric oxide (NO) synthase with a histochemical staining of NADPH diaphorase activity. Almost all antennal lobe region were stained except for the VM region. Consequently, we suggested that VM region identified as the primary center of aggressiveness does not use NO as the signal transmitter to higher brain.

Key words: aggressive behavior, brain, cuticular hydrocarbon, nestmate recognition, nitric oxide

P-148 Evolution of Sex Pheromone Communication Systems in the Genus *Ostrinia*

Yukio Ishikawa^a, Yongping Huang^b, Takuma Takanashi^a, Xiaoyan Fu^a, Suguru Ohno^a, Jun Tabata^a, Mai Fukuzawa^a, Sugihiko Hoshizaki^a and Sadahiro Tatsuki^a

^a Laboratory of Applied Entomology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

^b Shanghai Institute of Plant Physiology & Ecology, The Chinese Academy of Sciences, Shanghai 200032, China
ayucky@mail.ecc.u-tokyo.ac.jp

The genus *Ostrinia* (Lepidoptera; Crambidae) contains 21 closely related species with diverse host plant ranges, providing an excellent system for studies in evolutionary biology. Among all *Ostrinia*, eight species, *i.e.*, *O. furnacalis* (Asian corn borer), *O. orientalis*, *O. scapularis*, *O. zealis*, *O. zaguliaevi*, *O. palustralis*, *O. latipennis* and *O. ovalipennis*, are found in Japan. Analyses of the sex pheromones of all these species, conducted by our group, revealed substantial diversification among species. The evolution of the sex pheromone communication systems in *Ostrinia*, however, is not resolved at present, since our effort to clarify the phylogenetic relationships among *Ostrinia* species based on mitochondrial DNA sequences has encountered difficulty due to their unusual closeness. Fortunately, experimental cross hybridization between *Ostrinia* species is possible in several combinations, and offspring are viable. Analyses of the sex pheromones of F1, F2 and backcross progeny provide useful information on the genetic basis for the divergence of the sex pheromones. In this presentation, we summarize the present knowledge on the sex pheromone communication systems in *Ostrinia*, and discuss the evolution of the systems.

P-149 Mating sequence of *Brontispa longissima* (Coleoptera: Chrysomelidae) and Evidence for a Female Contact Sex Pheromone

Kei Kawazu, Ryoko Ichiki, Dung T. Dang and Satoshi Nakamura

Japan International Research Center for Agricultural Science, 1-1 Ohwashi, Tsukuba, 305-8686, Japan.

kkawazu@affrc.go.jp

The coconut hispine beetle, *Brontispa longissima* is one of the most serious insect pests of coconut in Southeast Asia. The beetle was accidentally introduced into Vietnam in 1999 and has been rapidly spreading to other countries in this area. Since chemical control cannot be applied due to its high costs and risks for the environment, we have been aiming to control the beetle using its pheromone. To clarify the existence of a pheromone in this species, we observed the mating behaviour and conducted a series of bioassays. After approaching a female, a male touched and held the female with his antennae and/or forelegs, mounted the female, extended its penis toward her abdominal tip, and finally copulated. Thus, we defined the male behaviour into 5 phases, “touch, hold, mount, penis extension, and copulation”.

Under the bioassays, males showed mating attempts of “penis extension” toward females that were killed by freezing at -20°C . No males showed “mount” behaviour to females washed with hexane. When the washed females were re-treated with the hexane extract from a female body or female elytra, males also showed “penis extension” toward those females. These results indicate the presence of a female sex pheromone that is perceptible by direct contact and plays an important role in mating of *B. longissima*.

Key words: *Brontispa longissima*, mating behaviour, contact sex pheromone, invasive insect pests

P-150 Temporal Interpretation of Spatial Odor Concentration Gradient in Mould Mites

Takeshi Kojima and Masayuki Sakuma

Laboratory of Insect Physiology, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan

kojima@kais.kyoto-u.ac.jp

Mould mites, *Tyrophagus putrescentiae* localized a food attractant odor source placed in a petri dish, where the only available directional information was an odor concentration gradient. In this circumstance, mites may use a temporal cue acquired walking along the gradient, instead of a spatial one perceived by instantaneous comparison. In order to identify the cue responsible for the localization, mites' orientation behaviour was analysed in a micro-locomotion compensator. A test mite was allowed to walk freely on a glass plate arena in the locomotion compensator, where odorous patches were arranged in an odorless background by supplying odor to the mite while it was in the patch. Mites were successfully confined in the attractant patches in this experimental set up, where the only cue the mites could perceive at the boundary was the abrupt change in odor concentration. In practice, many mites straying from patches launched a loop turn to return themselves to the odorous patches. More controlled experiments were conducted with respect to the sensori-motor context. Immediately after the cessation of the odor exposure, mites elicited a series of alternate loop turns gradually waning over the course of a minute, which allowed replicated re-entrance to the patch even if they failed to return. Since the cessation of the odor stimulus effectively functioned as a returning cue to the patch, mould mites were able to employ temporal odorous pattern to interpret the spatially distributed odor concentration gradient in a closed space such as in a petri dish.

Key words: mite, locomotion compensator, chemotaxis, concentration gradient, wavelet

P-151 Virtual Reality in Insect Olfactory Behavior

Masayuki Sakuma

Laboratory of Insect Physiology, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan
sakuma@kais.kyoto-u.ac.jp

Animals employ external cues to control their orientation behavior in a way that guides them towards a goal. A servosphere locomotion compensator apparatus, or Kramer's treadmill, frequently has been used for the analysis of the orientation behavior of ambulatory insects. The position of a test animal on the sphere is continually monitored with a remote sensor, and then by rotating the sphere, it returns to the top of the sphere automatically. Owing to this locomotion compensation system, the position of a freely walking test insect can be restricted in a small space, allowing us to control the cue presentation to the test insect precisely. When this apparatus is refined by recent computer technology and combined with actuators that generate external cues, a virtual sensory field can be created and the maneuvering of the animal in response to it can be investigated in a fully automated experiment. The aim was to conduct a virtual-reality experiment, by presenting odor to the test animal in exactly the way it would occur during actual maneuvering to a natural odor source. If the animal successfully reached a goal set on a virtual plane, it could be concluded that the animal employs the same tactics as those written in the computer program. Male silk moths and the German cockroaches were guided towards respective sex and aggregation pheromone sources set on the virtual plane.

Key words: servosphere, locomotion compensator, pheromone, virtual reality, orientation

P-152 The Nestmate Recognition and Aggressiveness in Uicolonial Ant *Formica yessensis*

Midori Kidokoro-Kobayashi^a, Misako Iwakura, Shingo Fujiwara^b, Seigo Higashi^b and Mamiko Ozaki^a

^a Department of Biology, Faculty of Science, Kobe University, Kobe, 657-8501, Japan

^b Course in Animal Ecology, Graduate School Of Environmental Science, Hokkaido University, N10, W5, Hokkaido, 060-0810, Japan
midori@sapphire.kobe-u.ac.jp

In most social insect species, individuals recognize nestmates from non-nestmates and aggressively reject the latter, which maintains the relatedness among nestmates high. *Formica yessensis*, which is a representative native unicolonial ant in Japan. They forms supercolony, in which workers can associate with each other among nests. Thus, the mechanism of the nestmate recognition of such a unicolonial ant might be something different. We sampled *F. yessensis* from six localities around Sapporo, *i.e.* Hoshioki (H), Shinkawa (S), Tarukawa (T), Ishikari (I), Oshoro (O) and Jozankei (J); H, S, T, and I were situated in a supercolony, O and J were located far from this supercolony. We investigated the genetic structure in each nests, the pattern of the cuticular hydrocarbons (CHCs), electrophysiological responses of antennal CHC-sensilla and aggressive behavior in the field. Comparing genetic structures of workers between nests, we found low relatedness in any comparisons. Discriminant analysis of CHCs demonstrated that the CHC patterns are clustered each nest but overlapped among nests. However, the electrophysiological response of CHC-sensilla in H showed higher responsiveness to the CHCs of O and J, rather than the CHCs of S, T, I, which belong to a single supercolony together with H. Consistently, the workers of H were aggressive toward the workers of O and J, but less aggressive toward the workers of S, T, I.

Key words: ant, cuticular hydrocarbons, electrophysiological response, genetic structure, supercolony

P-153 The Effect of Physiological Factors on Butterfly Territorial Status

Tsuyoshi Takeuchi

Department of Biofunctional Science and Technology, Graduate School of Biosphere Science, Hiroshima University, Kagamiyama, Higashihiroshima 739-8528, Japan
t-takeuchi@hiroshima-u.ac.jp

Males of many butterfly species compete for mating territories via noncontact aerial interactions. How butterflies settle their contests is a mystery because there are few physical attacks during their contests. In some odonate species, males of which compete over territories via aerial interactions like butterflies, it is known that males with heavier flight-muscle or those with larger lipid reserves tend to win. This indicates the importance of flight ability in aerial contests. *Chrysozephyrus smaragdinus* is a lycaenid butterfly, males of which exhibit a typical territorial behaviour. Using the butterfly, I compared several morphological and physiological traits of territorial owners to those of nonowners to investigate whether butterfly territorial status is correlated with these traits like odonate species. The differences in body size, flight-muscle ratio, and age between owners and nonowners were not significant. Owners had less lipid reserves than nonowners. Considering the fact that lipid mass decreased as males of *C. smaragdinus* got old (they consume more energy than they recover through their adult life), owners' less lipid reserves suggest that owners consume more energy for territorial defense, rather than suggest that leaner males are more dominant. This result indicates that owners can defend their territories in spite of their worse physiological condition. Factors other than physical ability, such as motivation, should be considered to understand the territorial dominance in butterflies.

Key words: butterflies, contest, lipid, territorial status

P-154 Visual Mate Location in Pheromone Mediated Flight of the Black Chafer, *Holotrichia loochooana loochooana* Males

Midori Fukaya

Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
fukaya@kais.kyoto-u.ac.jp

Function of female visual cues in pheromone mediated mate location of the black chafer, *Holotrichia loochooana loochooana* (Sawada) (Coleoptera: Scarabaeidae) males were examined in the field condition. Males directly landed on black lures significantly more frequently than white ones treated with the female pheromone, anthranilic acid, while they never approached on untreated lures regardless of the color. When an untreated black lure was placed 5cm from a white lure treated with the pheromone, males were landed significantly more frequently on the former one. Frequency of the landing onto the untreated black lure significantly decreased when the distance between the two lures was increased from 0 to 20 cm. These observations demonstrated that males of *H. l. loochooana* locate a female precisely and land on her by visual cues after reaching the vicinity (within 20 cm) by olfaction.

P-155 Ultrasounds: A Communication Channel Other than Pheromones in Moths

Takuma Takanashi^a, Ryo Nakano^b, Yukio Ishikawa^b, Annemarie Surlykke^c and Niels Skals^c

^a Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan

^b Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

^c Institute of Biology, University of Southern Denmark, DK-5230, Odense M, Denmark
takanasi@affrc.go.jp

Sexual communication in moths has been studied intensively. However, these studies concentrated almost exclusively on sex pheromone mediated communications with very few incorporating acoustic communication. Here we report on acoustic communication by male ultrasonic songs in three *Ostrinia* moths, *O. furnacalis*, *O. scapularis*, and *O. nubilalis*, which attract conspecific males by sex pheromones. Upon landing close to a pheromone-releasing female, males show a series of courtship behaviors involving emission of an ultrasonic song. The song of *O. furnacalis* was composed of chirps, *i.e.*, groups of pulses, with a broadband frequency of 25–100 kHz. Interestingly, the songs of the other two *Ostrinia* species differed from that of *O. furnacalis* in spectral and time structures. Electrophysiological recordings using the three species show that their hearing is broadly tuned to the frequencies of the songs. In *O. furnacalis*, the mating success of deaf females (hearing organ punctured) and muted males (sound-producing organ covered) was reduced compared to intact and sham-operated individuals. These findings on acoustic communication in *Ostrinia* are discussed in the context of moth communication through non-olfactory modalities. Reference: Nakano et al. (2006) *Naturwissenschaften* 93, 292–296.

Key words: acoustic communication, courtship song, hearing, sex pheromone, *Ostrinia furnacalis*

P-156 Topographic Organization of Olfactory and Contact Chemosensory Afferents in an Insect Brain

Hiroshi Nishino

Research Institute for Electronic Science, Hokkaido University, Sapporo 060-0812, Japan

As in vertebrates, chemosensory afferents in insects are grouped into functional modulli (e.g. glomeruli) based on their chemical identity in the first-order center. However, organization of chemosensory afferents based on their peripheral locations has been little understood. By staining individual or populations of chemosensory afferents in the antennae of the cockroach *Periplaneta americana*, the author shows that contact-chemosensory (taste) afferents and olfactory afferents project with different branching patterns to different neuropilar regions in the central nervous system. In brief, projection patterns of contact chemosensory afferents resembled those of mechanosensory afferents, in which axon terminals are orderly arranged based on three-dimensional positions in the antenna. Olfactory afferents converging onto individual glomeruli were loosely segregated based on their periphery origins. Possible functional roles of so-called somatotopic organization of chemosensory afferents on feeding or mating in insects are discussed.

Key words: antenna, receptive fields, sensory neurons, glomerulus, pheromone

P-157 The Interaction between Pheromone and Plant Odor Processing in the Moth Brain **Shigehiro Namiki^a and Ryohei Kanzaki^b**

^a Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8572, Ibaraki, Japan

^b Research Center of Advanced Science and Technology Komaba 4-6-1, Meguro 153-8904, Tokyo, Japan
namiki@brain.imi.i.u-tokyo.ac.jp

We investigated whether background application of host plant odor affects pheromonal processing in the insect brain. Host plant odors usually enhance the activity of pheromone orientation behavior in insects. Recently, electrophysiological studies show that modulatory effect of plant odor on pheromonal processing occurs at the sensor level. However, few studies report the physiology of the central brain neurons in response to the blend. In present study, we performed conventional intracellular recording from the neurons in the brain of male silkworm moth, *Bombyx mori*. We mainly tested the responses to (1) bombykol, the major pheromone component, (2) *cis*-3-hexen-1-ol, host plant volatile, and (3) the blend of these two chemicals. The cell type was identified by use of following intracellular staining with Lucifer Yellow. The response of bombykol-selective principal neurons in the first order olfactory center was enhanced by *cis*-3-hexen-1-ol and highly reproducible ($n=14$). Spontaneous activity of the neurons was not affected by exposure to host odor. Some of the neurons in the next processing stages also showed enhanced response to the blend ($n=5$). By contrast, the others showed more complex responses. The synergistic response was observed in third order olfactory neurons ($n=3$). Also, we observed the shift of response onset without firing rate changes ($n=2$). While the modulatory of blend odor is relatively simple in first relay station, processing in higher stages is seemed to be more complex.

Key words: pheromone, host plant, intracellular recording, moth, brain

P-158 Odor Responses of Cockroach's Descending Interneurons and Motor Neurons **Jun Inouchi^a and Junheon Kim^b**

^a Insect Interaction Research Unit, Division of Insect Sciences, National Institute of Agrobiological Sciences (NIAS), Ohwashi 1-2, Tsukuba, Ibaraki 305-8634, Japan

^b Division of Forest Insect Pests and Diseases, Korea Forest Research Institute (KFRI), 207 Cheongnyangni 2, Dongdaemun, Seoul 130-712, Republic of Korea
inouchi@affrc.go.jp

In insect central nervous systems, descending interneurons (DNs) carry the final information from the brain to thoracic motor systems to initiate specific patterns of behavior. Using extracellular recording method, we have studied neural activities of the DN and the motor neurons (MNs) in male cockroach (*P. americana*) to olfactory stimuli (1-Hexanol, diet, pheromone) to the antenna. In this study, recordings were made from the DN of both side connectives close to the thoracic ganglion and the MNs from both side nerves (nerves 5 and 6; N5 and N6) of the mesothoracic ganglion. MNs in N5 and N6 mainly innervate extensor and flexor muscles in the mesothoracic leg, respectively. Activities recorded from DN in the connective typically showed significant greater firing rates to olfactory stimulations of the antenna ipsilateral to the recording site. The increase of spike number of DN was dose dependent. Reciprocal activities of the MNs (presumed slow excitatory extensor MNs) and synchronized activities (presumed peripheral inhibitory neurons), synchronized with the DN activities, were recorded from both sides N5 to the stimulus of one side antenna. Synchronous MN activities were recorded from the N5 and the contralateral N6. In recordings from N6, activities of slow excitatory flexor MNs (presumed MN 5 and MN6) were seen to the stimuli of the contralateral antenna to the recording site. These MN activity patterns are similar to the initiation of spontaneous rhythmic leg movements in intact male cockroach. The results show that the males can make spatial comparisons between their two antennae, these males could be able maintain position themselves by the activities of DN and MNs to odor sources.

Key words: descending interneuron, motor neuron, odor responses, orientation, *P. americana*

P-159 Odor Concentration and Temporal Pattern Controlled Olfactory Stimulator for Insect Physiology

Koutaroh Okada and Masayuki Sakuma

Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa- Oiwaketho Sakyoku Kyoto 606-8502, Japan
kokada@kais.kyoto-u.ac.jp

The olfactory system of an insect brain codes for information of odorant quality and quantity using the temporal pattern of neural activity as well as the neuron's firing. Although odorant temporal pattern and accurate concentration information are important in olfactory stimulation and analysis, those factors are difficult to be controlled. In response to this problem, we developed an olfactory stimulator, which enabled us to control both odor temporal pattern and concentration. The stimulator is composed of two parts: one where a known concentration's gas is produced and the other is a small wind tunnel (section of measurement region; circle, $\phi = 25$ mm) of low turbulence flow with gentle wind speed. The working system is as follows. With a start switch of the stimulator, a constant quantity of odorant solution is vaporized with a hot wire in an airtight chamber. Next, the gas is filled in a Pitot tube by the plunger moving of a syringe. Then, the gas is discharged into the wind tunnel through a tip aperture of the Pitot tube. The discharge of gas is controlled by a solenoid valve. Finally, the gas flowed laminar with arbitrary velocities between 0.6–0.18 m/s and reached an insect antenna placed in the wind tunnel. Peripheral Interface Controller (PIC) controls hot wire current for heating up the odorant solution, plunger moving and solenoid valve. From now on, we are going to measure olfactory information processing time with known odorant concentration in the brain of cockroach (*Periplaneta americana*. L.), by measuring the time from onset of stimulation to starting odor-evoked typical behaviour monitored by muscle potential activity.

Key words: olfaction, olfactory stimulation, wind tunnel, PIC, processing time

P-160 Appetite Change by Odor Experience in the Blowfly, *Phormia regina*

Ken Nishida, Toru Maeda and Mamiko Ozaki

Department of Biology, Graduate School of Science, Kobe University, Rokkodaicho 1-1, Nada-ku, Kobe 657-8501, Japan.
071s316s@stu.kobe-u.ac.jp

It is known that daily experience of odors of foods influence on appetite in humans. When the blowflies, *P. regina* were fed on sucrose solution with odor of D-limonene, which is a general plant metabolite having oral toxicity for them, their appetite decreased. The sugar receptor cells in the taste sensilla of *P. regina* electrophysiologically respond to above a threshold concentration sucrose. We recorded the response of the taste sensilla to the sucrose solutions in the D-limonene odor-experienced or non-experienced flies. The magnitude of response defined as frequency of impulses was not significantly different between flies with and without odor experience. It was suggested that appetite decrease in the flies, which had experienced odor of D-limonene, was not caused by reduction of responsiveness of sugar receptor cell but at the central nervous system. We discovered that tyramine, a biogenic amine in the fly brain, significantly decreased in the D-limonene-experienced flies and that injection of tyramine recovered their appetite to the normal level or more. Then we made the antibody against tyramine receptor and carried out western blot analysis for the fly brain with this antibody.

Key words: appetite; D-limonene; odor experience; tyramine; tyramine receptor

P-161 Study on Appetite Regulation Mechanism in a novel *Drosophila melanogaster* Mutant
Haruka Okamoto^a, Azusa Nishimura^b and Mamiko Ozaki^b

^a Kyoto Institute of Technology, Matsugasaki-Hashimotocho, Sakyo, Kyoto 606-8585, Japan

^b Kobe University, Rokkodaicho 1-1, Nada, Kobe 657-8501, Japan

079s317s@stu.kobe-u.ac.jp

This research was aimed at understanding appetite regulation mechanism by using the Taiwan strain of *Drosophila melanogaster*, which shows the extraordinary feeding behavior. *D. melanogaster* extended the proboscis to sugar solution, if the concentration of sugar applied to the chemosensilla exceeds a feeding behavioral threshold. In the proboscis extension reflex test after 24 hr starvation, this strain showed 100 times higher feeding sensitivity to sucrose than MEL6, a wild type. We also measured feeding sensitivity to sucrose by two-choice test. Taiwan significantly tended to prefer 0.1 mM sucrose to water as longer starvation period, while MEL6 did not. Using the tip-recording method, electrophysiological response to sucrose was recorded from a single tarsal chemosensillum. Taiwan showed higher sensitivity to sucrose as longer starvation period up to 26 hr. These results suggested that Taiwan phenotype had the characteristic of “Starvation-dependent increase in behavioral and electrophysiological taste sensitivity to sucrose”. Furthermore, we searched for the responsible genes by means of genetical mapping with the whole chromosome substitution lines between Taiwan and MEL6. As a result, both MMT line, which carries the third chromosome of Taiwan, and the MTM line, which carries the second chromosome of Taiwan in the genetic background of MEL6, showed the Taiwan phenotype. This phenotype would be caused by multiple genes which have epistatic effects, and in future, we will identify the responsible genes.

Key words: appetite, *Drosophila melanogaster*, feeding behavior, starvation, taste

P-162 Effects of Calmodulin Antagonists on Taste Response and their Use in Analyzing the Role of Taste Information on Feeding Behavior of the Caterpillar, *Bombyx mori*

Kiyoshi Asaoka

National Institute of Agrobiological Sciences, Ohwashi 1-2, Tsukuba 305-8634, Japan

asaoka@affrc.co.jp

Two paired sensilla styloconica on the maxillary galea of Lepidopteran larvae are one of well-studied insect taste sensilla particularly with respect to their role in dietary selection. In order to find the possible involvement of proposed transduction molecules in insect taste cells, I am investigating the effect of some pharmacological agents on the taste responses of the three cells, a sugar cell, an inositol cell and a deterrent cell in either of the two sensilla of *Bombyx mori* using the tip recording method. In the present experiment, I use W-5 and W-7 that are Ca²⁺-calmodulin antagonists although the former is less specific. Depending on concentration, both W-5 and W-7 equally suppress the response to sucrose, inositol or strychnine with a short latency (less than 1 s). In the absence of taste stimuli, W-7 evokes vigorous spike activity (one and occasionally more types) in some sensilla after a long period of silence (several seconds or minutes). Such a response is prominent with increasing concentrations of W-7. Thus, breaking the quietness of the taste cell is a more possible effect of the calmodulin antagonist, W-7. In addition, the inhibitory effect of W-5 on taste responses could be used as a tool to find the role of specific stimuli perceived by the peripheral galeal sensilla on feeding behavior. Feeding behavior was analyzed by recording electromyograms from mandibular closer muscles in free-moving larvae presented with an artificial diet containing W-5. Results suggest that the inhibitory effects on taste signals are reflected obviously on the prolonged time taken for food sampling behavior or the intermittent time between continuous bites.

Key words: taste, feeding, calmodulin antagonist, galeal styloconic sensilla, caterpillar

P-163 Characteristic EAD Responses of Male Dark Winged Fungus Gnat, *Bradysia Paupera* (Diptera: Sciaridae) to Series of Female Born Cuticular Lipids

Yining Liu^a and Hiroshi Honda^b

^a School of Life Science, East China Normal University, North Zhongshan Rd. 3663 Shanghai, 200062, China

^b Institute of Agriculture and Forestry, University of Tsukuba, Ten-nodai 1-1-1, Tsukuba, Ibaraki, 305-8572, Japan

Since the male mushroom fly behaviorally attracted and showed matting dances to the crude female cuticular washes, we tested male's EAD response to the series of authentic chemical candidates identified from cuticular washes by GCMS. The result shows that male antenna responded sensitively to n-aldehydes with carbon number C7-C18 and n-hydrocarbons with carbon number C10-C14, but was no sensitive to 2-ketones from C16 to C21 which weighed about 44.2% of the total lipids. Behaviorally bioassay was also coincide with the evidence. Thus we suggested the sex pheromone emitted by female fly is likely structural close to the aldehyde with carbon chain number C10-C14, and the characteristic EAD responses of the fly might be a proper joint material for further molecular studies to elucidate the odor reception mechanism.

Key words: *Bradysia paupera*, EAD, cuticular lipids, sex pheromone

P-164 Effect of Different Sugars and Concentrations on Feeding Response and Longevity of the Larval Parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae)

K. Hoang Le^a and Keiji Takasu^b

^a Graduate School of Bioresource and Environmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

^b Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

lkhoang@brs.kyushu-u.ac.jp

Sugars are important food source for parasitic wasps to sustain their life and reproduction. However, previous studies have shown that feeding response and their effects on longevity and reproduction are different among different sugars. To evaluate importance of sugars on adult food of *Microplitis croceipes*, a larval parasitoid of *Helicoverpa/Heliopsis* spp., we examined their longevity given four sugars, fructose, sucrose, glucose as well as feeding response to those sugars. When given 2M of each sugars, wasps lived significantly longer period than wasps given only water, but the effect on longevity was different between maltose and the other sugars. Wasps given maltose survived for 8-11 days, while wasps given fructose, glucose or sucrose lived for 25-26 days for females, and 20-23 days for males, except 13 days for males given sucrose. When individual adults were given different concentrations of sugars, the threshold concentration required to elicit a positive response from at least 50% of wasps was 1/16-1/32M for fructose, sucrose and glucose except for 1/8M for females responding to glucose. The threshold for maltose for both sexes was 1/2M and significantly lower than those for other sugars tested. Feeding time increased with increasing concentration at or below the threshold concentrations. These results suggest that dose responses by wasps to sugars reflect the nutritional values of the sugars. However, when given maltose and fructose alternately, wasps preferred feeding on maltose than fructose at 1M, but fructose than maltose at 1/4M.

Key words: *Microplitis croceipes*, feeding, sugar concentration, longevity

P-165 Analysis of Odorant-Binding Proteins in Antennae of the Geometrid Species, which Produces Lepidopteran Type II Sex Pheromone Components

Hayaki Watanabe, Hiroko Tabunoki, Nami Miura, Ryoichi Sato, and Tetsu Ando*

Graduate School of Bio-Applications and Systems Engineering (BASE), Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

*antetsu@cc.tuat.ac.jp

Information on the olfactory system in antennae of Geometridae moths is very limited, and odorant-binding proteins (OBPs) working as transporters of lipophilic odors have not been identified. In the first investigation on this family of insects, we examined antennal OBPs of the Japanese giant looper, *Ascotis selenaria cretacea*. RT-PCR experiments using several pairs of degenerate primers designed from known cDNA sequences encoding lepidopteran OBPs successfully amplified partial sequences of two pheromone-binding proteins, named AscrPBP1 and AscrPBP2 in reference to their corresponding nucleotide sequence homologies with other PBPs. Using 5'- and 3'-rapid amplification of cDNA end strategies, a cDNA clone for AscrPBP1 encoding a protein of 141 amino acids was isolated. Western blotting with the antiserum against recombinant PBP1 overexpressed in *Escherichia coli* showed that the AscrPBP1 gene was more strongly expressed in male antennae than in female antennae. Furthermore, natural AscrPBP1 was isolated by immunoprecipitation with the antiserum, and its binding ability was evaluated by using synthetic sex pheromonal compounds with a C₁₉ chain. The result indicated that AscrPBP1 bound not only the pheromone components, 3,6,9-nonadecatriene and its 3,4-epoxy derivative, but also unnatural 6,7- and 9,10-epoxy derivatives. While no general odorant-binding proteins (GOBPs) were amplified in the RT-PCR experiments, two antisera prepared from GOBP1 and GOBP2 of *Bombyx mori* suggested the occurrence of at least two GOBPs in the *A. s. cretacea* antennae.

P-166 Isolation and Characterization of Intracellular Proteins that are Phosphorylated in Response To PBAN Stimulation

Atsushi Ohnishi and Shogo Matsumoto

Molecular Entomology Laboratory, RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan

aohnishi@riken.jp

Since pheromone biosynthesis activating neuropeptide (PBAN) acts directly on the pheromone gland (PG), the entire bombykol biosynthesis process regulated by PBAN occurs in the PG. Although we have observed an influx of extracellular calcium into the PG following PBAN stimulation, the precise mechanisms underlying the intracellular signal transduction cascade activated by PBAN remain largely unknown. Our recent results suggest that the PBAN-induced influx of calcium promotes the formation of a calcium/calmodulin complex, activation of calcineurin, and culminates in activation of the terminal reductive modification step, a PG-specific acyl-CoA reductase pgFAR, and the lipase that liberates bombykol precursor (C₁₆:2) from lipid droplets. Furthermore, we have cloned and characterized genes encoding calmodulin and the heterosubunits of calcineurin from the PG. Calcineurin is a serine/threonine protein phosphatase that is activated in the presence of calcium and calmodulin. These results suggest that a series of phosphorylation/dephosphorylation events are involved in the PBAN intracellular signal transduction cascade. To identify proteins that are phosphorylated in response to PBAN, we performed immunoblots with three types of antibodies against phosphorylated amino acids. To date, five immunoreactive bands have been detected.

Key words: *Bombyx mori*, bombykol, PBAN signaling, phosphorylation

P-167 Determination of the PBAN Receptor (PBANR) in the Japanese Giant Looper, *Ascotis selenaria cretacea*, Which Produces an Epoxyalkenyl Sex Pheromone

Takeshi Kawai^a Atsushi Ohnishi^b, Shogo Matsumoto^b and Tetsu Ando

^a Graduate School of BASE, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan

^b Molecular Entomology Laboratory, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan

kwi303@gmail.com

The biosynthesis of lepidopteran sex pheromones is regulated by a pheromone biosynthesis-activating neuropeptide (PBAN). Receptors of the peptide (PBANR) have been identified from insects secreting Type I pheromones such as *Bombyx mori* and *Helicoverpa zea*. The PBAN of a geometrid moth (*Ascotis selenaria cretacea*) secreting the Type II pheromone has a unique structure and mode of action. It has double FXPRL motifs and activates the pheromone gland to incorporate a biosynthetic precursor in hemolymph. In order to clarify the details of the activation process, we attempted to identify the PBANR of *A. s. cretacea*. A partial sequence of the putative Ascc-PBANR was amplified from cDNA of the pheromone gland with degenerate primers designed for the other PBANRs. Using a PCR-based cloning strategy, a cDNA clone encoding the predicted seven transmembrane domains was isolated; however, full-length cDNA was not identified at the 3-prime. An RT-PCR experiment with the mRNA of *A. s. cretacea* females confirmed the clone expression at the pheromone gland. Interestingly, the external-membrane region from the *N*-terminus to the first transmembrane domain has 64 amino acids. This sequence is longer than those of *B. mori* and *H. zea* by 26 and 28 amino acids, respectively, suggesting that it is a candidate for the binding site of Ascc-PBAN with a different structure from that of the PBANs of other species.

Key words: Lepidoptera, Geometridae, pheromone biosynthesis, G protein-coupled receptor

P-168 Identification of 11,14,17-Icosatrienoic and 13,16,19-Docosatrienoic Acids, Biosynthetic Intermediates of Lepidopteran Sex Pheromones Derived from Linolenic Acid

Kanae Matsuoka and Tetsu Ando

Graduate School of Bio-Applications and Systems Engineering (BASE), Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

antetsu@cc.tuat.ac.jp

Polyunsaturated hydrocarbons and their epoxy derivatives with a C₁₇-C₂₃ straight chain compose the second major group of lepidopteran sex pheromones. These Type II pheromones commonly include double bonds at the 3- and/or 6- and/or 9-positions, indicating their biosynthesis from dietary linoleic or linolenic acids via decarboxylation after chain elongation. Our previous studies with a geometrid species, *Ascotis selenaria cretacea*, revealed that the pheromonal hydrocarbon (C₁₉ 3,6,9-triene) was produced outside of a pheromone gland, transported to the pheromone gland after associating with lipophorin, and oxidized to the epoxide. In order to confirm the biosynthetic pathway, we analyzed fatty acids in an abdominal integument including oenocyte cells, where hydrocarbons in an epicuticle are produced. After purification using HPLC with an ODS column and methylation, the GC-MS analysis showed the occurrence of methyl 11,14,17-icosatrienoate in a lipid extraction of the *A. s. cretacea* females. In another experiment with an arctiid species, *Syntomoides imaon*, which secreted C₂₁ 3,6,9-trienes, methyl 13,16,19-docosatrienoate, in addition to the icosatrienoate, was detected. This constitutes first identification of novel acyl intermediates in Type II pheromone biosynthesis, and lack of the docosatrienoate in *A. s. cretacea* indicates that the biosynthesis is strictly regulated by a species-specific system of the chain elongation.

Key words: Lepidoptera, Geometridae, Arctiidae, biosynthetic intermediate, long chain fatty acid

P-169 Study on an Epoxygenase Working on the Type II Pheromone Biosynthesis

Takeshi Fujii^{a, b}, Masataka G Suzuki^b, Shogo Matsumoto^b, and Tetsu Ando^a

^a Graduate School of BASE, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

^b Molecular Entomology Laboratory, The institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama 351-0198, Japan

takeshi-aobadai@msj.biglobe.ne.jp

Many genes encoding a desaturase implicated in the sex pheromone biosynthesis of female moths are identified, whereas no gene encoding epoxygenase is reported. Virgin female of Japanese giant looper, *Ascotis selenaria cretacea*, produces epoxyalkene [(Z,Z)-6,9-cis-3,4-epoxynonadecadiene] as a main pheromone component from a triene precursor [(Z,Z,Z)-3,6,9-nonadecatrien] via enzymatic epoxidation in a pheromone gland. Decade ago, an epoxygenase from the plant had been characterized as a membrane protein containing non-heme iron. The plant epoxygenase shows sequence similarity to acetylenase and desaturase that have three histidine-rich motifs. Based on this finding, we assumed that the insect epoxygenase for the sex pheromone biosynthesis might contain non-heme iron similar to the insect desaturase. As a result of PCR using degenerate primers, five genes (*epoc1-5*) were isolated. RT-PCR expression analysis demonstrated that *epoc1* is specifically expressed in the pheromone gland. Although *in vitro* functional assay using insect cells (Sf9) failed to demonstrate that Epoc1 was epoxygenase, down-regulation of endogenous *epoc1* by RNAi reduced the pheromone titer to 40% of the control. Furthermore, when the deuterium labeled triene was injected into the abdomen of the female moths, the corresponding labeled epoxy pheromone was not detected in *epoc1* knock down individuals. These results indicate that *epoc1* plays some role in the pheromone gland.

Key words: geometrid moth, pheromone gland, sex pheromone biosynthesis, epoxygenase

P-170 A Novel Peptide, p4442, from *Bombyx mori* Larval Hemolymph Senses the Excess Sterol Diet

Shinji Nagata, Yukie Omori, Fumi Sakai and Hiromichi Nagasawa

Department of applied biological chemistry, Graduate school of agricultural and life sciences, The university of Tokyo. Yayoi, 1-1-1, Bunkyo-ku, Tokyo, 113-8657, Japan

anagashi@mail.ecc.u-tokyo.ac.jp

Dietary nutrients influence feeding behavior of all animal, including insects. In insects, intake of sterol compounds from their diet is required in compensation for their inability to synthesize sterol compounds *de novo*. In the present study, we investigated the insect larval response to the excess preferable (metabolizable) sterol in the diet using the silkworm, *Bombyx mori* larvae. In the analyses of MALDI-TOF MS of larval hemolymph fed on excess sterol-containing diet, the profile of mass spectrum covering the peptide molecular weight was changed. In particular, a novel peptide composed of 44 amino acids, designated p4442, was apparently increased in the larval hemolymph after feeding the excess sterol-containing diet compared with that in the larvae fed *ad libitum*. Molecular cloning of a cDNA encoding p4442 revealed that p4442 mRNA was predominantly synthesized in the fat body and secreted after cleavage of a signal sequence and C-terminal lysine residue. Northern hybridization revealed that p4442 mRNA transcription was expressed more throughout the feeding periods than during the quiescent periods before or after molting. Also, the resumed feeding after quiescent period improved mRNA expression level of p4442. Thus, examination of p4442 transcription levels may lead to monitoring the quality and quantity of sterols or some other compounds in the diet. To address this hypothesis, we now investigate the promoter region using a luciferase reporter assay, surveying the nutrient driving p4442 transcription *in vitro*.

Key words: *Bombyx mori*, hemolymph, peptide, sterols, feeding behavior

P-171 Molecular Cloning of Ecdysone Receptor and Ultraspiracle from the Scorpion *Liocheles australasiae*

Yoshiaki Nakagawa, Atsushi Sakai, Fumie Magata, Takehiko Ogura, Masahiro Miyashita, and Hisashi Miyagawa

Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
naka@kais.kyoto-u.ac.jp

Scorpions are members of Arthropoda, but they are classified to a different subphylum from that of insects and crustaceans. Although the mechanisms of molting and metamorphosis are intensively studied in insects, the molting mechanism is unknown in scorpions. In this study we cloned cDNAs for the ecdysone receptor (EcR) and ultraspiracle (USP) of Japanese scorpions *Liocheles australasiae* (LaEcR and LaUSP), and deduced amino acid sequences of the proteins. Total cDNA sequences of *LaEcR* and *LaUSP* were 2881bp and 1977bp long, respectively. The open reading frames of LaEcR and LaUSP encoded 560 and 414 amino acids, respectively. LaEcR was most homologous to the EcR of the lone star tick *Amblyomma americanum* followed by EcRs of Orthoptera and Coleoptera, and LaUSP was homologous to the USPs of ticks and the insects Orthoptera and Coleoptera. Both LaEcR and LaUSP were successfully prepared *in vitro* using the rabbit reticulocyte lysate. An ecdysone agonist, ponasterone A, specifically bound to LaEcR ($K_D=4.2$ nM), but did not bind to LaUSP. The binding affinity of ponasterone A to LaEcR was not enhanced in the presence of LaUSP which was different from the case of insects.

Key words: ecdysone receptor (EcR), scorpion, ultraspiracle (USP), cDNA cloning, ponasterone A

P-172 Spook and Spookier, Highly Related P450 Enzymes, Code for Stage-Specific Components of the Ecdysteroid Biosynthetic Pathway in *Drosophila melanogaster*

Hajime Ono^{a, c}, Kim F. Rewitz^b, James T. Warren^b, Lawrence I. Gilbert^b and Michael B. O'Connor^a

^a Department of Genetics, Cell Biology and Development, HHMI, University of Minnesota, USA

^b Department of Biology, University of North Carolina, USA

^c Present address: Graduate School of Agriculture, Kyoto University, Japan
onoono@kais.kyoto-u.ac.jp

20-hydroxyecdysone (20E), the molting hormone of insects, is required for embryogenesis, larval molting, metamorphosis and oogenesis. Recently, the genes coding for P450 enzymes which catalyze the final four steps in the ecdysteroid biosynthetic pathway have been identified. Of the Halloween P450s, only *spook* (*spo*) remains uncharacterized. *Spo* mutant embryos can be rescued by application of an artificial pulse of 20E and some of them can eclose as adult. However, in rescued *spo* mutant females, their ovaries fail to develop normally. *Spo* is expressed at early embryogenesis and midoogenesis but is not expressed at the larval stages. The fact that *Spo* activity is not required during larval stages suggests that some other P450 may act during post-embryonic stage. We identified a *spo* homolog, dubbed *spookier* (*spok*), localized in heterochromatin. In contrast to *spo*, *spok* is specifically expressed in the prothoracic gland after late embryogenesis. Loss of *spok* function mediated by RNAi leads to arrest of development at the first larval instar. This phenotype can be rescued by feeding the larva 20E, E or ketodiol but not 7dC, which suggests that *Spo* and *Spok* are likely to be components in the uncharacterized reaction step(s) between 7dC and ketodiol. In contrast to *Drosophila*, we have identified only one *spopo* homolog that is expressed throughout development in lepidopteran species. These studies suggest that an evolutionary split between Drosophilidae and Lepidoptera in regulation of the ecdysteroid biosynthetic pathway has occurred by gene duplication and divergence events.

Key words: ecdysteroid, biosynthesis, P450, paralogous genes, *Drosophila melanogaster*

P-173 Purification and Characterization of a Novel Short-Chain Insecticidal Toxin from the Venom of the Scorpion *Liocheles australasiae*

Nobuto Matsushita, Masahiro Miyashita, Atsushi Sakai, Yoshiaki Nakagawa and Hisashi Miyagawa

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
nmatsush@kais.kyoto-u.ac.jp

Scorpion venoms contain a variety of peptides, which are toxic to mammals, insects, and crustaceans. These toxic peptides can interact with ion channels with high affinity and selectivity. These biological features make scorpion toxins useful tools for probing the structures of different ion channels and evaluating their physiological contribution to cell and organ behavior. In this study, we report the purification and characterization of an insecticidal toxin (LaIT1) from the venom of the scorpion *Liocheles australasiae*, which inhabits in the western Pacific region including Japan and Australia. Edman sequencing and mass spectrometric analysis revealed that the toxin is composed of 36 amino acid residues with a molecular weight of 4200 Da, cross-linked by only two disulfide bridges. The pattern of this disulfide bonds was assigned by LC/MS analysis after enzymatic digestion. This toxin showed insect toxicity against crickets at a dose of 1.0 μg /insect, but no effect was observed against mice even after injection of 1.0 μg of LaIT1 by the intracerebroventricular route. LaIT1 shows no sequence homology to any other known toxins, suggesting that it belongs to a novel structural class of short-chain toxins.

Key words: scorpion venom; insecticidal peptide; disulfide bridge; neurotoxin; short-chain toxin

P-174 Family 18 Chitinase from a Microbial Source as a Potent Bioinsecticide on Eri Silkworm, *Samia ricini*

Khondkar Ehteshamul Kabir and Kotaro Konno

Laboratory of Insect-Plant Interaction, National Institute of Agrobiological Sciences, 1-2 Ohwashi, Tsukuba, Ibaraki 305-8634, Japan
kekabir@affrc.go.jp

A family 18 chitinase [poly{1,4-(*N*-acetyl- β -D-glucosaminide)} glycanohydrolase], (EC 3.2.1.14) from *Serratia marcescens* with an molecular mass of 67-kDa showed 60% mortality of Eri silkworm, *Samia ricini* larvae in response to oral ingestion of chitinase at concentration of 10U/ml in 72 hours, compared to 0% mortality, in control insects, treated with 10 mM K-phosphate buffer (pH 6.3). The same concentration, food consumption was inhibited to 100%. The chitinase when injected in the hemocoel at concentration of 10 U/ml gave 80% mortality in 96 hours compared to 0% mortality in controls. Microphotographic images clearly showed numerous dark spots appears all around the integument of chitinase ingested larvae, appears within 12 hours after chitinase ingestion. Stereoscopic microphotographic images indicated that the Peritrophic Membrane (PM) of the Eri silkworm 4th stage larvae ingested chitinases at concentration of 10 U/ml *in vivo* are completely degraded. Fluorescence images with FITC-CBD, of the chitin-rich-foregut, dissected from chitinase ingested larvae clearly indicate that chitin, which an important structural component of foregut, was digested by chitinase activity. This novel finding suggest that chitinase may preliminary attack the foregut chitin during ingestion and then it may sequentially attack chitinous PM through its chitinolytic activity. Our results open up the possibility to use chitinases as a bioinsecticidal protein that should have agronomic potential for insect control.

Key words: chitinase, *Serratia marcescens*, bioinsecticidal protein, *Samia ricini*, chitin degradation

P-175 Isolation and Characterizations of Glucosyltransferase Concerned with Flower Colour of Common Coral Tree (*Erythrina crista-galli* L.)

Tetsuya Arita, Susumu Teramoto and Kunijiro Yoshitama

Graduate School of Science and Technology, Kumamoto University, Kumamoto 860-8555, Japan

The genus *Erythrina* (Leguminosae) comprises 112 species, mostly arborescent and pantropical, but also extending into warm-temperate areas. Most of this species are pollinated by hummingbirds, although some are passerine pollinated. The common coral tree is a deciduous indigenous to southeast Brazil, Bolivia, Paraguay, Uruguay, and north Argentina. It spreads all over the world in frost-free climates and is the national flower of Argentina and Uruguay. It bears non-tubular, large, brilliant crimson flowers in June to October of ornamental, economic, and medicinal interest. The common coral tree is included in a paraphyletic assemblage of South American species that are basal in the genus. It was reported that not only birds but bees functionated as major pollinators. It was shown by the studies of our laboratory that cyanidin 3-glucoside and cyanidin 3-sophoroside (main pigment) was contained in petal and pelargonidin 3-glucoside in sepal.

There have been many reports of anthocyanidin 3-glucosyltransferase (3GT) in various plant species, but so far little is known of 3GT in woody plants, and also of anthocyanidin 3-glucoside-glucosyltransferase (3GGT).

Then, we describe in the study the purification and the characterization of some biochemical properties of 3GT and 3GGT concerning in the biosynthesis of the pollinator-attracting pigments.

Key words: *Erythrina crista-galli* L., glucosyltransferase, anthocyanin

P-176 Predominant Elemental Accumulation on the Mandibles of Various Termites

Wakako Ohmura^a, Hitoshi Imaseki^b, Takahiro Ishikawa^b, Hiroyuki Iso^b, Yoko Takametsu^c, Tsuyoshi Yoshimura^d and Youki Suzuki^a

^a Forestry and Forest Products Research Institute, P.O.Box 16, Tsukuba 305-8687, Japan

^b National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku Chiba 263-8555, Japan

^c Department of Biological and Environmental Sciences, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

^d Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji 611-0011, Japan
murasan@ffpri.affrc.go.jp

Chewing insects use their mandibles for cutting food into small fragments. Their mandibles commonly contain Zn and/or Mn, and sometimes Fe, with the accumulation of these metals acting to harden the cutting edges. Termites possess tough mandibles with which to cut wood and non-wood structures made of plastics, mortar, tiles and etc. In this study, termite mandibles of 12 species from 5 families were analyzed using micro-beam scanning PIXE, which is a power tool for detecting the elemental components. As a result, Mn was detected on the pigmentation area of the mandibles in all the termites except for *Mastotermes darwiniensis*. *M. darwiniensis* has no metal on the mandible, following that Mastotermitidae is the basal linkage among extant termites. On the other hand, Zn was only detected in kalotermitid termites, and was distributed in the mandibular cutting edges as a predominant metal. The coexistence of Zn and Cl was also observed. The fact of Zn existence in only kalotermitid termites' mandibles may support the theory that they have derived from ancestor termites independently to other families. Thus, elemental contents of termite mandibles might show significant information to solve the uncertainty of phylogenetic relation of the termites.

Key words: termite, mandible, micro-beam scanning PIXE, metal accumulation

P-177 Effects of Seven D-Fructose Analogs on the Growth of Lettuce and Cress Seedlings
Kozue Okada^a, Ken Izumori^b and Hisashi Kato-Noguchi^a

^a Faculty of Agriculture, Kagawa University, 2393 Miki Kagawa 761-0795, Japan

^b Rare Sugar Research Center, Kagawa University, 2393 Miki Kagawa 761-0795, Japan

Fructokinase is enzyme with a high specific affinity for D-fructose and acts as a sugar sensor which controls various metabolic and developmental processes in plants. It has become apparent that D-fructose is not only important energy source but also physiological signal repressing or activating plant genes involved in many essential processes including photosynthesis, respiration, starch and sucrose synthesis and degradation, and cell-cycle regulation. In order to elucidate the molecular basis of this fructose-sensor, effects of seven D-fructose analogs on plant growth was investigated. D-fructose significantly increased root and hypocotyl growth of lettuce and cress seedlings. However, D-psicose (the C-3 epimer of D-fructose) inhibited the root and hypocotyl growth of lettuce and cress at concentrations greater than 3 mM, and inhibitions were increased with increasing concentrations of D-psicose. These inhibitions were shown not to be osmotic effects, because when D-mannitol was substitute for D-psicose, no effect on the growth of these seedlings up to 100 mM. D-tagatose (the C-4 epimer of D-fructose), D-sorbose (the C-3 and C-4 diastereomer of D-fructose), L-fructose, L-psicose, L-tagatose and L-sorbose did not significantly effect the root and hypocotyl growth of lettuce and cress seedlings. D-talitol and allitol also did not significantly effect their growth up to 100 mM. These results suggest that only D-psicose may trigger a signal cascade resulting in the growth inhibition.

Key words: fructokinase, fructose, hekitol, ketohexose, growth inhibition, sugar sensor

P-178 Deposit Organ of Quercivorol, an Aggregation Pheromone of *Platypus quercivorus*
Tadakazu Nakashima^a and Nobuo Ogura^b

^a Forestry and Forest Products research Institute, Tsukuba 305-8687, Japan

^b Meiji University, Kawasaki 214-8571, Japan

tshima@ffpri.affrc.go.jp

Mass attacks and galleries elongation in the host oak trunks by the oak ambrosia beetle, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae), resulted in the heavy mortality of deciduous oaks, especially *Quercus crispula* Blume, in Japan. The newly isolated phytopathogenic fungus, *Raffaelea quercivora*, disorders the water flow in the trunk of attacked oaks and is vectored by *P. quercivorus*. The main component of the aggregation pheromone of the beetle was elucidated to be (1*S*,4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol, and named quercivorol.

Quercivorol was detected by GC-MS analysis in the volatiles from crushed newly emerged males, and from abdominal part, but not from head nor from thorax. The dissection of beetles showed that yellowish oily material was deposited in a hind-gut but no food materials were detected in an alimental canal. It is suggested that quercivorol was synthesized any organ (unidentified yet), deposited in hind-gut, impregnated to boring dust from anus, and a male beetle could feed on ambrosia fungi after females gallery elongation and fungi inoculation. Oak ambrosia beetle might have no need of the maturation feeding in a new gallery for the start of aggregation pheromone biosynthesis. The biosynthetic process of aggregation pheromone in *P. quercivorus* may be considerably different from that of well researched bark beetle's pheromone.

Key words: aggregation pheromone, *Platypus quercivorus*, biosynthesis, deposit organ

P-179 Antifeedants against *Locusta migratoria* from the Japanese Cedar, *Cryptomeria japonica*

Takehiro Kashiwagi^a, Bin Wu^b, Kyouko Iyota^b, Xiao Hui Chen^b, Shin-ich Tebayashi^b and Chul-Sa Kim^b

^a Japan Science and Technology Agency, Satellite Kochi, 185 Miyanokuti, Tosayamada, Kami, Kochi, 782-8502, Japan

^b Department of Bioresources Science, Faculty of Agriculture, Kochi University, B200 Monobe, Nankoku 783-8502, Japan

lachesis@cc.kochi-u.ac.jp

Locusta migratoria L. is native to semi-arid regions of equatorial Africa and a well-known serious pest to cereal in the world. Locust swarms of gregarious phase sometimes cause massive damage to crops in all continents except Antarctica.

We have recently found that a crude methanol extract of a Japanese cedar, *Cryptomeria japonica* (Taxodiaceae), which is well known as a peculiar plant of Japan, strongly inhibits *L. migratoria* from feeding.

Based on bioassay-guided fractionation, (1*S*,6*R*)-2,7(14),10-bisabolatrien-1-ol-4-one, (+)-7(14),10-bisaboladien-1-ol-4-one, ferruginol and (-)-cubebol were isolated and identified as antifeedants against this insect species. Of these isolated compounds, (1*S*,6*R*)-2,7(14),10-bisabolatrien-1-ol-4-one and (+)-7(14),10-bisaboladien-1-ol-4-one showed activity only when they were combined. Similarly, ferruginol and (-)-cubebol also did only when they were combined. Each compound alone showed no activity. The plural components are required to exhibit antifeeding activity of *C. japonica* against *L. migratoria*.

Key words: *Locusta migratoria*, *Cryptomeria japonica*, antifeedant, (1*S*,6*R*)-2,7(14),10-bisabolatrien-1-ol-4-one, (+)-7(14),10-bisaboladien-1-ol-4-one

P-180 Protective Effect of Di-O-caffeoylquinic Acid on Human-derived Neurotypic SH-SY5Y Cells against Alzheimer's Disease Amyloid-beta-induced Toxicity

Hiroko Isoda, Han Junkyu and Hideyuki Shigori

Life and Environmental Sciences, Univ. of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8572, Japan

isoda@sakura.cc.tsukuba.ac.jp

In the present report using in vitro assays involving the Alzheimer's disease model, we demonstrate the protective effect of Caffeoylquinic acids (CQA). CQAs inhibited the toxicity of amyloid- β on human neurotypic (SH-SY5Y) cells. Proteomics analysis on 3,5-di-CQA treated SH-SY-5Y cells showed that the significant expression of phosphoglycerate kinase1 (PGK1), which is related to oxidative stress response. Furthermore, Alzheimer's model mice (SAM P8) mice that got per oral administration of CQA recovered their memories of swimming training experiment during 28 days. These results suggest that CQAs may be relevant in the protection against neurodegeneration, particularly Alzheimer's disease.

Q-001 Discovery of a Bioactive Antifouling Compound Produced by a Deep-Sea Bacterium *Streptomyces* sp. and its Potential Mechanism against Larval Settlement of the Polychaete *Hydroides elegans*

Ying Xu, Honglei Li, Xiancui Li, Xiang Xiao and Peiyuan Qian

Department of Biology and Coastal Marine Laboratory, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong

Deep-sea microorganisms are a new source of bioactive compounds. Following up with our early work that screened 176 deep-sea bacterial strains for their antifouling activity, we cultured one bioactive *Streptomyces* strain UST040711-290 in large-scale and isolated one active compound from the bacterial spent broth medium using bioassay-guided fractionation. This compound inhibited the larval settlement of a major fouling tubeworm *Hydroides elegans* with an EC₅₀ value of 0.6 $\mu\text{g ml}^{-1}$ the effect was reversible and non-toxic. This *Streptomyces* strain had the highest yield of this bioactive compound when it was cultured at 30°C, pH 7 in a modified MGY medium with elevated nutrient concentrations. Laboratory investigation further showed that this bioactive compound down-regulated the expression level of genes *Ran GTPase binding protein (RBP)* in *H. elegans* larvae, suggesting it may inhibit larval settlement through affecting certain cell proliferation process.

Q-002 Effect of Different Allelopathic Extracts on Weeds and Wheat Crop

Imtiaz Khan^a, Gul Hassan^a, M. I. Khan^a and Meher Gul^b

^a Department of Weed Science, Faculty of Crop Protection Science, NWFP Agricultural University Peshawar-25130-Pakistan

^b G and G Officer, Kashmir Highway, Aabpara-Islamabad-Pakistan
imtiazagri@yahoo.com

Field studies were initiated at Malakandher Research Farm, NWFP Agricultural University, Peshawar-Pakistan during rabi 2003-04 to study the effect of different allelopathic extracts on weeds and wheat crop. The experiment was laid out in randomized complete block design with four replications. The treatments comprised of extracts from *Ammi visnaga* and *Convolvulus arvensis*, extracted either in ethanol or CHCl₃, each applied either in full or half strengths. An untreated check was also included in the study. The extracts were applied as post emergence of the crop as well as weeds. Ghaznavi-98 variety of wheat in plot size of 5 × 1.5 m² was planted in the third week of November 2003. The data were recorded on weed density m⁻², plant height (cm), spike length (cm), No. of grains spike⁻¹, 1000 grain weight (g), grain yield spike⁻¹ (g), biological yield (t ha⁻¹) and grain yield (t ha⁻¹). The major weeds infesting the experiment were *Avena fatua*, *Coronopus didymus*, *Euphorbia helioscopia*, *Fumaria indica*, *Convolvulus arvensis*, *Rumex dentatus*, *Chenopodium album*, *Poa annua*, *Medicago denticulata*, and *Vicia sativa*. The data for the individual traits were subjected to the analysis of variance technique and the means were separated by the unprotected LSD test. For controlling weeds CHCl₃ extract of *Convolvulus* at full dose and *Ammi* half dose proved to be the best in inhibiting weeds density, giving only 33.67 weeds m⁻² each as compared to 101.0 in the untreated check. The maximum grain yield of 1.153 t ha⁻¹ was recorded in *Convolvulus arvensis* extracted in CHCl₃ applied at the half dose and 1.120 t ha⁻¹ in *A. visnaga* also extracted in the same solvent applied at the full dose. While, the minimum grain yield (0.790 t ha⁻¹) was recorded in the untreated check plots. Further research is recommended to fine tune the findings.

Key words: weeds, allopathic extract, wheat crop

Q-003 Studies on Dormancy of Wild Onion Ecotypes in Combination with Chemical, Chemical Concentration and Temperature Regimes

Muhammad Ishfaq Khan, Gul Hassan and Imtiaz Khan

Department of Weed Science, NWFP Agricultural University Peshawar-Pakistan 25130

ishfaqws@yahoo.ca

Dormancy is the failure of seeds to germinate under favorable environmental conditions. It is an adaptive significance in weeds to persist in the agro-ecosystems. Several chemicals inducing germination in seeds have been identified. Hence, laboratory studies on wild onion (*Asphodelus tenuifolius*) seeds were undertaken at NWFP, Agricultural University Peshawar, Pakistan to investigate the dormancy breaking by using GA₃, KNO₃, Thiourea and Sodium Azide at 0 to 800 ppm exposed to 10, 20 and 30°C temperature regimes. Experiment was laid out in completely randomized design with a split-split-split plot arrangement. Temperatures were assigned to main plots, biotypes to sub-plots, while chemicals to sub-sub plots and the concentrations were assigned to sub-sub-sub plots. Each sub-sub-sub-plot comprised of single Petri-dish planted with 20 seeds of wild onion. The germination percentage data were subjected to ANOVA and the means were separated by LSD test. The data revealed temperatures, biotypes, chemicals, concentrations and their interactions significantly affecting germination except the interactions temperature × biotypes × concentration, biotypes × chemical × concentrations and the four way interaction among temperature × biotype × chemical × concentration. The highest germination was recorded at 20°C (47.41%), while on 1.09% germination was recorded at 30°C. Mianwali biotypes germinated the most (40.83%) as compared to 24.38 and 22.88% germination in Karak and Bhakkar biotypes. Mianwali when exposed to 20°C had the highest germination (69.13%). Among the chemicals the highest germination was recorded in KNO₃ and thiourea. Mianwali biotype when exposed to KNO₃ or GA₃ or thiourea out performed all other biotype × chemical interactions. The temperature effect over-rides the chemicals or biotype effects.

Key words: wild onion, ecotypes, chemical, temperature, concentrations

Special Photo Exhibition

The Color and Shape of a Flower through the Insect's Eye

Hiroshi Fukui,^{a, b} Junko Tsukioka,^a Junji Toyoda^{a, c} and Katsumi Goto^a

^a “team INSECT’s EYE”, addressed at the Garden of Medicinal Plants, Kyoto Pharmaceutical University, 39 Hayashi, Hino, Fushimi, Kyoto 601-1405

^b Professor Emeritus (Kagawa University)

^c Creative Office Yutaka

fukui-h@pe.kagawa-u.ac.jp

info@co-yutaka.com

A Small White (*Pieris rapae*, Monshirocho in Japanese) is capable of recognizing its male or female mate using UV-rays, suggesting that insects utilize UV-rays to identify objects. Most flowers depend on insects for pollination and have pollen grains located strategically for easy removal and transportation from flower to flower. Many insect pollinators can easily locate a flower to feed on the nectar. Each pollen grain must protect its DNA within from the harmful UV-rays. One method of protection could be to emit fluorescence, when irradiated by UV-rays, from the surface of the pollen and/or anther. Therefore, the color and shape of the flower would appear different in the insect especially under UV-rays.



under visible light



under UV light

Hibiscus mutabilis, cotton rosemallow, Fuyoh in Japanese

The photographs of the flowers (shown below are those of *Hibiscus mutabilis*, cotton rosemallow, Fuyoh in Japanese) were taken under illumination of visible and UV (ca 360 nm in the dark) lights. The pollen and/or the anther emit fluorescence only under the UV light. Some insects may utilize this phenomenon to locate and feed on the floral nectar. The arrangement of petals may act as a kind of parabola disc to converge/concentrate sunlight on top of the floral filaments. The compounds on the surface of the pollen and/or anther responsible for the emission of fluorescence remain to be elucidated.